UNDERSTANDING THE ROLE OF PENTRAXIN 3 IN SENESCENCE AND FIBROGENESIS IN ADIPOSE TISSUE AND LIVER DURING AGING

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HSIN-CHING LEE

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ADVISOR: XIAOLI CHEN

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Abstract

The inflammaging is defined as chronic and low-grade inflammation when aging. The pathogenesis of age-related diseases largely results from the development of inflammaging. Interestingly, patients with obesity share largely similar phenotypes with aging and both obesity and aging are accompanied by progressive inflammation in adipose tissue. Pentraxin 3 (PTX3) is a soluble pattern recognition receptor and plays an important role in the innate immune system. Moreover, PTX3 can be induced by different inflammatory stimuli in adipocytes. The change levels of plasma PTX3 has been observed in patients with metabolic diseases or aging individuals, however, investigating the role of PTX3 in adipose tissue and liver during inflammation and aging is still needed since the mechanism is not completely understood. My research is focused on understanding the role of PTX3 in senescence and fibrogenesis in adipose tissue and liver during aging. In the first project, we investigated the effect of PTX3 deficiency on cellular senescence and fibrogenesis of stromal vascular (SV) cells of brown adipose tissue (BAT) and inguinal (ING) white adipose tissue (Ing-WAT) from old female mice, and we found out that PTX3 deficiency results in increased senescence and fibrogenesis in SV cells of subcutaneous adipose tissue. In the second project, we determined the direct role of PTX3 in senescence and fibrogenesis by investigating the

reversal effect of recombinant mouse PTX3 (rmPTX3) on senescence and fibrogenesis in SV cells. Unfortunately, we didn't see the reversal effect of recombinant mouse PTX3 (rmPTX3) on senescence and fibrogenesis in SV cells. In the third project, we examined whether PTX3 deficiency impacts senescence and lipogenesis in liver *in vivo*, and we demonstrated that PTX3 deficiency itself significantly downregulated gene expression in lipogenesis, lipolysis, and mitochondria function in liver. Also, PTX3 deficiency attenuated fibrogenesis and gluconeogenesis in liver. In summary, my master research reveals that PTX3 deficiency results in increased senescence and fibrogenesis in SV cells of subcutaneous adipose tissue in old female mice and PTX3 deficiency attenuates lipid transport and oxidation, fibrogenesis, and gluconeogenesis in the liver.

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CHAPTER 1

Literature Review

Literature Review

The prevalence of overweight and obesity has increased around the world since the 1980s [1]. Obesity has become a worldwide health issue and it increases risks not only for chronic metabolic diseases such as type-2 diabetes (T2DM), but also for cancer, cardiovascular diseases, autoimmune disorders such as rheumatoid arthritis and osteoarthritis (OA), and other neurological disorders (cognitive impairment and dementia) [2]. According to WHO (https://www.who.int/newsroom/fact-sheets/detail/ageing-and-health), the population over 60 years old is expected to double between 2015 and 2050, pointing out that aging has turned into a universal biological problem and phenomenon [3]. Aging is a complex phenomenon. As we are in an aging society, it becomes increasingly important to understand why aging leads to higher susceptibility to chronic lethal diseases and has recently increased morbidity, disability, and frailty. Most age-related diseases are a result of chronic inflammation, a common characteristic of both obesity and aging; Aging results in such a state termed as inflammaging. Inflammaging is thought to be a sterile, progressive, and low-grade inflammation during aging, which is associated with increased insulin resistance, oxidative stress, adipose tissue redistribution, and multiple organ system alterations [4]. In addition, recent investigations have demonstrated that there is the interconnection between immune and metabolic responses during aging process; this relationship is defined as metainflammaging [4]. Metainflammation is characterized by elevated circulating lipopolysaccharide/endotoxin levels inside the body [5]. Nutrient excess or overnutrition expanded adipocytes leading to excessive body fat accumulation. Moreover, adipose tissue inflammation has been associated with metabolic stress and hypoxia when adipocytes expanded in size [6]. Hypertrophic adipocytes are characterized by increased inflammatory cytokine secretion and decreased anti-inflammatory adipokines such as leptin and adiponectin secretion [7,8]. Interestingly, obesity and aging share similar phenotypes and pathophysiological alterations. For example, adipose tissue becomes dysfunctional and undergoes chronic and sterile inflammation, elevated lipotoxicity and lipogenesis, decreased mitochondria function, increased cellular senescence, and reduced brown adipose tissue function [9,10]. Hence, in order to completely understand the pathogenesis of obesity, metabolic disorders, and aging, it is of importance to understand the roles of adipose tissue and cytokines/adipokines during inflammation and metabolic homeostasis.

I. Adipose Tissue Inflammation in Obesity

The Types and Functions of Adipose Tissue

Adipose tissues are present in discrete depots throughout the body [11]. There are two major types of adipose tissues: white adipose tissue and brown adipose tissue [12]. White adipose tissue plays a critical role in whole-body energy metabolism. It not only stores excess energy, but also secretes hormones, regulates glucose and lipid metabolism, controls appetite and immunity [13,14]. Subcutaneous adipose tissues (SAT) including abdominal, gluteal, and femoral. SAT store approximately 80% of total body fat inside our body. In the meanwhile, intraabdominal fat depots are located within internal organs. For example, visceral adipose tissues (VAT) are associated with digestive organs and intra- and retro-peritoneal depots represent 5~20% of total body fat in humans [14]. Also, VAT is more associated with macrophage infiltration, adipocyte death, and adipose tissue remodeling in obesity condition [15]. In addition, white adipocytes derive from Myf5- progenitor cells [16,17] with the characters of unilocular lipid droplets and low density of mitochondria [18]. In contrast, brown adipose tissue (BAT), found in cervical, supraclavicular, and paravertebral regions, can produce heat through thermogenesis and it has been indicated as the primary thermoregulatory tissue during non-shivering cold exposure [19]. Moreover, brown adipocytes originate from Myf5+ precursor cells [16,17] and the mature brown adipocytes contain multilocular lipid droplets, enriched mitochondria, and expression of UCP1 [18]. Some studies have pointed out that mature white adipocytes may directly convert into brown-like adipocytes or beige adipocytes in response to chronic cold exposure environment [20,21,22]. Intriguingly, while they derive from different cellular lineages, brown and beige adipocytes share similar biochemical characteristics. In addition, adipose tissue is a complex tissue composed mainly of mature adipocytes and stromal vascular fraction (SVF) cells and can regulate metabolic, hormonal, and immune processes by releasing multiple bioactive compounds. Stromal vascular cells include preadipocytes, immune cells, blood cells, endothelial cells, and macrophages [23]. Since each adipose depots composed of distinct cell subpopulation, the cell composition alters during aging process and chronic cold exposure condition [24,25]. Moreover, adipose tissue is considered as an endocrine organ because of its secretory capability. Adipose tissue can secrete a variety of signaling mediators such as adipokines. Through controlling these mediators, adipose tissue is able to communicate with other organs to maintain systemic homeostasis [13,26].

Inflammation and Dysfunction of Adipose Tissue in Obesity

Adipose tissue becomes dysfunctional during the development of obesity. In obesity, adipose tissue experiences excessive fat storage and altered cell composition

[15]. For example, obesity leads to the expansion of visceral adipose tissue but the reduction of both brown adipose tissue and subcutaneous white adipose tissue, as well as hypertrophy or hyperplasia of adipocytes [14,27]. Moreover, elevated free fatty acids (FFA) and dysregulated adipokine production could contribute to adipose tissue inflammation and lipid accumulation leading to systemic metabolic complications [13,14].

Obesity not only causes increased adipocyte hypertrophy and altered adipokines secretion, but also leads to immune response dysregulation of immune response [28,29]. Elevated FFAs induces insulin secretion, decreases insulin sensitivity in muscle and liver due to impaired GLUT4 translocation, and causes endothelial dysfunction like increased insulin resistance by impairing GLUT4 translocation [29]. In addition, proinflammatory cytokines is increased in obesity patient and led to systemic inflammation. The ability to induce anti-inflammatory adipokines such as leptin and adiponectin is suppressed during obesity. Study has suggested that the macrophage polarization not only affects the profile of cytokines composition in adipose tissue, but also plays an important role in the development of during obese pathologies [30]. Usually, activated macrophages can be divided into two subsets: classically activated M1 macrophages (mainly involved in pro-inflammatory responses) and alternatively activated M2 macrophages (mostly engaged in anti-inflammatory responses). Studies have demonstrated that adaptation to thermogenesis is more associated with M2 macrophages cells and M1 macrophages induce obesity-associated inflammation and increase insulin resistance while M2 macrophages protect against inflammation and strengthen insulin sensitivity [31,32]. In the nutshell, adipocyte hypertrophy has been associated with an impact on insulin resistance secretion, substrate utilization, adipose tissue dysfunction, and systemic inflammation. Therefore, adipocyte hypertrophy has been considered as it plays a critical role in increasing metabolic risk factor for type 2 diabetes, cardiovascular diseases, and ectopic lipid deposition.

II. The Inflammation of Adipose Tissue During Aging

Inflammaging in Adipose Tissue

The function of immune system declines gradually after midlife. Due to this reason, the elderly is more susceptible to infection as well as metabolic diseases, such as type 2 diabetes, hypertension, cancer, and autoimmune disorders. Studies have shown that the elderly, especially people with inferior physical fitness or frailty, have a strong connection with a phenomenon called inflammaging. Inflammaging is a chronic and low-grade inflammation with increased pro-inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor α (TNFα), and senescence-associated secretory phenotype

(SASP) in adipose tissue [33]. During aging, the body loses brown adipose tissue and subcutaneous white adipose tissue, but gains visceral white adipose tissue [15]. Increased visceral white adipose tissue has been closely associated with metabolic disorders and chronic low-grade inflammation. Researchers have been attempting to understand what the sources of the inflammatory cytokines are in adipose tissue. After decades of research, it becomes clear that both immune cells and non-immune cells, such as adipocytes and senescent cells, are able to release the inflammatory factors contributing to adipose tissue inflammation. Among immune cells, macrophages are viewed as the main inflammatory cytokine producers. In a study, macrophage polarization has shown to not only affect thermogenesis, but also impair lipolysis in an NLRP3 inflammasome-dependent pathway in adipose tissue [34]. A recent study indicated that aged-B cells could injure lipolysis through modulating the pathways of NLRP3 inflammasome and IL-1β release [35]. This study suggests that besides macrophages, B-cells could affect lipid metabolism and adipocyte thermogenesis. In addition, repeated and progressive pathogenic infections such as cytomegalovirus (CMV) infection, Epstein-Barr virus, and gut microbiota dysbiosis could contribute to inflammaging in old individuals [36,37]. Molecules coming from cell debris or being placed in the wrong location from the decayed cells and organs can be other exogenous triggers of inflammation. As the ability of our immune system to clear these substances declines with age, inflammation occurs progressively.

Source of inflammaging

Genetic Susceptibility

Genetic variants could affect blood levels of inflammatory cytokines [38]. Studies have demonstrated that some common variants of the *IL1RN* gene are associated with the plasma concentrations of *IL-1β* and *IFNγ* [39]. In addition, those variants could determine the pathophysiology of human infections [40] and may increase the risk of insulin resistance and inflammation [41,42,43]. Moreover, a study indicated that the single nucleotide polymorphism (SNP) rs2069837 in *IL6* was highly related to longevity, suggesting that there is a connection between *IL6* and mortality in old individuals [44]. Studies have also showed that some variants in the *IL6R* gene increases the risk of many diseases such as rheumatoid arthritis, type 1 diabetes, and depression [45]. This information indicates that the variability of genes could influence the concentrations of several inflammatory markers in plasma.

MicroRNAs (miRNAs) are non-coding, single-stranded RNAs with 17-25 nucleotides that play an important role in modulating protein expression and regulating mRNA stability or translational ability [46,47]. Studies have suggested that age-related changes in miRNAs increase the development of inflammaging [48,49]. For instance, some miRNAs could repress inflammation through reducing the gene expression of Toll-like receptors (TLR) signaling pathway components [50]. In the nucleus, microRNA is transcribed as pri-miRNA and turns into pre-miRNA by *Drosha*; pre-miRNA is then transported to the cytoplasm and processed by *Dicer* and *Ago2* into a mature, single-strand miRNA [48,49]. It is believed that most of the circulating or secreted miRNAs are released by vesicle-independent mechanisms in combination with *Ago2* [51] and the levels of microRNA change in obesity, inflammation, and cellular senescence [52,53]. The alteration of several miRNAs has been observed during the initiation of inflammation [50]. This information suggests that miRNAs may regulate the processes of inflammation and aging.

Cellular Senescence

Cellular senescence was first described as the irreversible arrest of normal human cells in the *in vitro* culture [54]. Senescence is a cellular response to injury or stress condition via telomere erosion or prolonged DNA damage [55,56,57]. Oxidative stress, telomere erosion, DNA harmful agents, and senescence-associated secretory phenotype

(SASP) can accelerate the senescence progression [58]. During aging, senescent cells accumulate and secrete pro-inflammatory cytokines or so called SASP such as interleukins (*IL-1 α, IL-1β, and IL-6*), metalloproteinases (interstitial collagenase (*MMP1*), stromelysin 1 (*MMP3*), collagenase 3 (*MMP13*)), chemokines (*IL-8*), and other extracellular matrix molecules in many tissues and organs [59] . Moreover, senescent cells are accompanied by increased expression of senescence-associated β-galactosidase (*SA-β-gal*), *p16INK4a*, *p21*, and *p53* [60,61,62] as well as elevated SASP secretion [63,64]. The elevated SASP contributes to the increased risk of developing age-associated diseases such as type 2 diabetes, cancer, and hypertension. The senescent stimuli can induce senescence through multiple signaling pathways, including *p38/MAPK* signaling, *NFκB/p65* signaling, *TGFβ/SMAD* signaling, *mTOR* signaling, and *Wnt* signaling [64,65]. In the normal condition, our body can eliminate the senescent cells via immune response, and they would not have significant impact on the proliferation and metabolism of neighbouring healthy cells. However, once the immune cells become dysfunctional as a result of obesity or aging, they lose the ability to clear senescent cells. Therefore, senescent cells accumulate in neighbouring cells and facilitate the development of cellular senescence in the tissues. Ultimately, senescent cells induce inflammation, causing tissue dysfunction in aged tissues [66,67].

Nevertheless, there are some therapies available to clear senescent cells. For example, the activation of mammalian target of rapamycin (*mTOR*) pathway has been demonstrated to promote SASP. Hence, by using rapamycin to inhibit *mTOR* pathway, we could decrease the harmful senescent cells [68]. Furthermore, both toll-like receptors and the downstream transcription factor, *NFκB*, play a critical role in SASP secretion [69,70]. As a result, compounds such as metformin and Bay11-7082 could modulate *NFκB* signaling pathway, therefore, ameliorate inflammaging and cellular senescence [71,72]. In addition, through strengthening mitochondria function and increasing autophagy, metformin can also attenuate the inflammaging of T-helper 17 cells in diabetes [72]. Besides the above-mentioned therapies, senescent cells can also be eliminated by inhibiting anti-apoptotic protein expression, enhancing immunosurveillance of immune cells, and reducing SASP.

<u>Self-debris accumulates inside the body</u>

Self-debris is a common phenomenon inside the body, which occurs when cells are injured releasing waste products generated from cellular and metabolic process. In the normal condition, extracellular debris can be recognized by the immune system through different specific receptors and broken down by engulfment in phagocytic vesicles [73]. However, the production of damaged cells and organelles increases with age, which triggers inflammaging. Molecules that are released by stressed cells during severe infection or ischemia are named danger-associated molecular patterns (DAMPs), including free radicals from oxidative stress and metabolites (e.g. ATP, oxidized LDL, cardiolipin, and wasted nucleosides). If these molecules could not be cleared in time, they would accumulate and increase the risk of inflammaging [74]. The imbalance between the production and the elimination of these debris' molecules could trigger inflammatory responses. For example, since the accumulation of DAMPs activates the NOD-like receptors (particularly *NLRP3* inflammasome), caspase1-dependent secretion of different types of pro-inflammatory cytokines such as *IL-1β* and *IL-18* increases. Studies have shown that the obstruction of the *NLRP3* inflammasome reduces multiple age-related diseases such as insulin resistance and inflammation, and increases longevity [75], which could in turn reduce the risk of inflammaging.

<u>Immune system</u>

Studies have suggested that the dysfunction of immune cells can also lead to inflammaging. The normal function of immune system is very critical, as it protects the body from being infected by viruses and bacteria and activate the downstream pathways to help recover from wounds. However, the immune system becomes impaired with aging and the chronic infections can also progressively alter immune functions, leading to the elevation of the inflammatory markers in plasma. Human CMV and HIV infections are permanent diseases which is known to accelerate the immunosenescence. Although partial studies showed that human CMV infection in older individuals is linked to increased cardiovascular disease (CVD) and inflammaging, decreased immune response, and elevated mortality of elderly [76,77,78], whether there is a direct connection between CMV infections and CVD and mortality is still controversial [79]. In addition, there is a repeated and chronic inflammation in HIV patients; the risk of developing CVD and frailty in patients with HIV is higher than average individuals [80,81,82]. The exhaustion of memory *CD41+* T cells is known as the main reason for the cause of increased gut epithelium permeability, which allows microbial pathogens to translocate into the blood circulation, resulting in chronic inflammation in HIV patients [83]. Moreover, since HIV patients are suffered from antiretroviral- associated lipodystrophy and visceral obesity, they may further elevate the risk of insulin resistance and inflammation [84].

The Change of Lipid Metabolism During Aging

Aging not only alters adipose tissue function, distribution, and composition, but also has an effect on lipid metabolism [85]. Studies have demonstrated that the levels of both triglyceride and lipoproteins were increased during aging [85,86]. Because of impaired mitochondria function and lower levels of catecholamines, thermogenic capacity of brown adipose tissue decreases during aging [87,88]. Interestingly, several studies have indicated that the blockade of the *NLRP3* inflammasome could maintain the mechanism of catecholamine-induced lipolysis in adipose tissue macrophages during aging [89], and aged adipose tissue B cells could weaken lipolysis and damage the homeostasis of body temperature when the body is exposed to the cold environment in the *NLRP3*- and *IL1*-dependent manner. In other words, the clearance of aged adipose tissue B cells could rebuild the cold-exposure tolerance and ability of lipolysis [90]. Studies also found that aging reduces the capability of cold-stimulated beige adipocyte activation in both mice and humans [91,92]. Furthermore, recent studies have demonstrated that enhancing thermogenesis in adipose tissue can efficiently improve metabolic capability and ameliorate inflammation [93].

Our previous studies indicated that lipopolysaccharide (LPS)-induced *NFκB/p65* signaling reduced adipogenesis resulting in premature senescence in adipose stromal-vascular cells [94]. Both SASP and pro-inflammatory cytokines could activate the

NFκB/p65 signaling pathway, thereby inducing cellular senescence [95]. *TGFβ* is known to promote cellular senescence through activating *p16*, *p21*, and *NFκB* signaling pathways. Through suppressing *TGFβ/Smad3* signaling pathway, the brown adipose tissue-specific gene expression can be promoted in white adipose tissue [96]. The *mTORC1* plays an important role in promoting cell growth and balancing lipid metabolism [97]. The dysregulation of *mTORC1* is associated with a great range of human diseases from metabolic disorders to cancers [98]. Rapamycin, a *mTORC1* inhibitor could delay aging by controlling transcriptional activity of *NFκB*. Moreover, rapamycin can enhance longevity in mice, and rapamycin-based therapies might be beneficial for treating aging-related diseases [99]. Lipophagy, one type of selective autophagy degrades lipid droplets, which is an important mechanism to maintain lipid homeostasis [100]. Studies have suggested that lipophagy is dysfunctional during aging contributing to metabolic disorders [101]. Moreover, *Wnt* signaling is known to be involved in metabolic disorders [102] and plays a role in regulating energy balance and cellular homeostasis. Atypical modulation of canonical and non-canonical *Wnt* system disrupts the metabolic processes resulting in adipose tissue expansion and metainflammation in obesity [103]. The non-canonical *Wnt* signaling pathway promotes adipogenesis and induces adipose tissue inflammation [104]. In addition, the activation of *p38/MAPK* signaling pathway promotes senescence and aging through regulating the oxidative stress [105], and the inhibitor of *p38/MAPK* could block the beginning of cardiovascular diseases as well as senescence and aging [106]. In summary, during aging, the composition and formation of adipose tissue changes, the rate of adipogenesis increases, and cellular senescence elevates. All of the changes have an impact on lipid metabolism leading to adipose tissue dysfunction.

III. The Role of PTX3 in Homeostasis of Inflammation and Metabolism

Expression and Regulation of PTX3 Under Inflammation

PTX3 is a soluble pattern recognition receptor that involves in the humoral innate immune system [107]. When encountering inflammatory signals or microbial recognition, PTX3 can be immediately produced by several cell types such as innate immune cells, fibroblasts, and adipocytes [108]. Inflammatory stimuli such as *TNFα*, *IL1β*, or toll-like receptors signaling can induce the secretion of PTX3 [109,110] and PTX3 acts as a modulator of inflammation through regulating the activation of complement and enhancing pathogen elimination by apoptotic cells [111]. The regulation of PTX3 expression is related to several signaling pathways and involves many types of cells. For instance, *IL1* receptors and MyD88-dependent TLR/NFκB signaling pathway could stimulate PTX3 expression in immune and stromal cells [112,113] through JNK and ERK1/2 MAPK pathways. *TNF* could promote PTX3 expression in human airway smooth muscle cells (HASMC) [114]. Moreover, glucocorticoids could modulate PTX3 expression in HASMC via mechanisms in a GC receptor and ERK-dependent manner [115].

Our previous studies have demonstrated that proinflammatory mediators and lipopolysaccharides (LPS) could stimulate PTX3 expression in different adipose depots. Compared to stromal vascular cells (SV cells), adipocytes secrete more PTX3 in response to LPS stimulation [116,117]. We have also found that in the LPS-induced condition, adipose tissue showed higher PTX3 expression than other tissues or organs, and PTX3 deficiency reinforces the inflammation stimulated by LPS in adipose tissue in mice. PTX3 deficiency led to decreased white fat mass and impaired adipogenesis in mice, indicating PTX3 may play an important role in lipid metabolism [116]. Moreover, compared to stimuli such as norepinephrine and palmitic acid, LPS stimulation induced the highest levels of PTX3 expression and secretion in adipocytes. Additionally, we have identified that adipocytes and preadipocytes but not macrophages are the main cell types that secrete PTX3 in response to LPS stimulation [118].

Many studies have suggested that the change of plasma PTX3 levels could influence the inflammation homeostasis. One study showed that PTX3 overexpression could protect mice from the infection of low-dose Gram-negative bacteria. However, PTX3 overexpression mice showed increased lethality rate under high-dose gram-negative bacteria [119]. The study indicated that PTX3 overexpression has a dual role in controlling inflammation. In addition, PTX3 deficiency has been shown to elevate vascular inflammation and atherosclerosis in mice [120]. Moreover, PTX3 deficiency was related to the worsened idiopathic pulmonary fibrosis (IPF), the reduced removal of fibrin, and the enhanced collagen deposition [112]. In a nutshell, these results indicate that PTX3 deficiency strengthens persistent inflammation and inflammatory responses. Furthermore, studies have indicated that PTX3 can be viewed as a useful therapeutic target for patients with autoimmune diseases [121]. PTX3 may be an early biomarker for diagnosing thyroid associated ophthalmopathy [122] and assessing the severity of sepsis or septic-shock-associated acute kidney injury (SSAKI) [123,124]. Interestingly, a recent study suggested that individuals with COVID-19 showed a higher level of PTX3 in their plasma and it might be viewed as a convincing prognostic indicator of short-term mortality in COVID-19 patients [125].

The Roles of PTX3 in Metabolic Diseases and Aging

PTX3 plays a vital role in the beginning of obesity by accelerating inflammation and elevating inflammatory response [126]. The lower concentrations of PTX3 in plasma may also contribute to inflammaging [127]. Studies have demonstrated that there is a negative correlation between PTX3 and glucose-stimulated insulin secretion, and obese people showed increased PTX3 gene expression in the visceral adipose tissue [128]. PTX3 could also act as a biomarker for healthy metabolic response [127] as well as a predictor of osteoporosis (OP) and osteoarthritis (OA) bone-related phenotypes [129]. Moreover, as a biomarker for cardiovascular disease (CVD), PTX3 is engaged in the pathogenesis of CVD-associated inflammation [130,131]. The alterations of PTX3 and *adropin* may be able to predict the renal damage to different degrees in patients with type 2 diabetes [132]. One study suggested that a high-fat diet could increase the serum PTX3 levels in mice and rats [126,133]. However, the other showed that there was a lower level of PTX3 in epididymal adipose tissue and plasma in obese mice with diabetes compared to non-obese mice [134]. In addition, our unpublished data demonstrated that mice with high-fat diet had decreased levels of PTX3 expression in adipose tissue. A recent study indicated that PTX3 in plasma was positively related to the severity of metabolic syndrome [135]. In conclusion, the changes in plasma PTX3 levels in metabolic diseases or aging indicates that PTX3 plays a critical role in metabolic health and inflammation. In addition, as an anti-inflammatory protein, PTX3 not only promotes inflammation in obesity and aging, but also affects lipid and glucose metabolism. However, further investigations are needed to understand the molecular mechanism by which PTX3 modulates senescence and inflammation in adipose tissue during aging and obesity.

References

1. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. *Jama.* 2016;315(21):2284-8.

2. Wang YC, McPherson K, Marsh T, et al. 2011. Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet.* 2011;378(9793):815-825.

3. Melzer D, Pilling LC, Ferrucci L. The genetics of human ageing. *Nat Rev Genet.* 2020;21(2):88-101.

4. Gregor MF, Hotamisligil GS. Inflammatory Mechanisms in Obesity. *Annu Rev Immunol.* 2011;29(1):415-445.

5. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie.* 2016;124:11-20.

6. Halberg N, Khan T, et al. Hypoxia-inducible factor 1α induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol.* 2009;29(16):4467-4483.

7. Skurk T, Alberti-Huber C, Herder C, and Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab.* 2007;92(3):1023-33.

8. Meyer LK, Ciaraldi TP, Henry RR, et al. Adipose tissue depot and cell size dependency of adiponectin synthesis and secretion in human obesity. *Adipocyte*. 2013;2(4):217-226.

9. Palmer AK, Kirkland JL. Aging and adipose tissue: potential interventions for diabetes and regenerative medicine. *Exp Gerontol.* 2016;86(C):97-105.

10. Chung KW. Advances in Understanding of the Role of Lipid Metabolism in Aging. *Cells.* 2021;10(4):880.

11. Shen W, Wang Z, Punyanita M, Lei J, Sinav A, Kral JG, Imielinska C, Ross R,

Heymsfield SB. Adipose tissue quantification by imaging methods: a proposed classification. *Obes.Res.* 2003;11:5-16.

12. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol.* 2016;7(Suppl 2):30.

13. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Archives Medical Sci Ams.* 2013;2(2):191-200.

14. Lee MJ, Wu Y, Fried SK. Adipose Tissue Heterogeneity: Implication of depot differences in adipose tissue for Obesity Complications. *Mol Aspects Med.* 2013;34(1):1-11.

15. Strissel KJ, Stancheva Z, Miyoshi H, et al. Adipocyte Death, Adipose Tissue Remodeling, and Obesity Complications. *Diabetes.* 2007;56(12):2910-2918.

16. Sidossis L, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. *J Clin Invest.* 2015;125(2):478-486.

17. Giralt M, Villarroya F. White, Brown, Beige/Brite: Different Adipose Cells for Different Functions? *Endocrinology.* 2013;154(9):2992-3000.

18. Sanchez-Gurmaches J, Hung C-M, Guertin DA. Emerging Complexities in Adipocyte Origins and Identity. *Trends Cell Biol.* 2016;26(5):313-326.

19. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev.* 2004;84(1):277-359.

20. Barbatelli G, Murano I, et al. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am J Physiol Endocrinol Metab.* 2010;298(6):E1244-53.

21. Rosenwald M, Perdikari A, Rülicke T, Wolfrum C. Bidirectional interconversion of brite and white adipocytes. *Nat Cell Biol.* 2013;15(6):659-667.

22. Himms-Hagen J, Melnyk A, Zingaretti MC, Ceresi E, Barbatelli G. Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am J Physiol Cell Physiol.* 2000;279(3):C670-681.

23. Lenz M, Arts ICW, Peeters RLM, Kok TM de, Ertaylan G. Adipose tissue in health and disease through the lens of its building blocks. *Sci Rep-uk.* 2020;10(1):10433.

24. Burl RB, Ramseyer VD, Rondini EA, Pique-Regi R, Lee Y-H, Granneman JG. Deconstructing Adipogenesis Induced by β3-Adrenergic Receptor Activation with Single-Cell Expression Profiling. *Cell Metab.* 2018;28(2):300-309.e4.

25. Bank HV, Kirsh C, Simcox J. Aging Adipose: Depot Location Dictates Age-Associated Expansion and Dysfunction. Ageing Res Rev. 2021;67:101259.

26. Arner P, Kulyté A. MicroRNA regulatory networks in human adipose tissue and obesity. *Nat Rev Endocrinol.* 2015;11(5):276-288.

27. Ghaben AL, Scherer PE. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol.* 2019;20(4):242-258.

28. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol.* 2010;314(1):1-16.

29. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest.* 2011;121(6):2094-2101.

30. Chylikova J, Dvorackova J, Tauber Z, Kamarad V. M1/M2 macrophage polarization in human obese adipose tissue. *Biomed Pap.* 2018;162(2):79-82.

31. Castoldi A, Naffah de Souza C, Camara NO, Moraes-Vieira PM. The macrophage switch in obesity development. *Front Immunol*. 2016;5(6):637.

32. Covarrubias AJ, Aksoylar HI, Horng T. Control of macrophage metabolism and activation by mTOR and Akt signaling. *Semin Immunol.* 2015;27(4):286-296.

33. Mau T, Yung R. Adipose tissue inflammation in aging. *Exp Gerontol.* 2018;105(J. Gerontol. A Biol. Sci. Med. Sci. 71 2016):27-31.

34. Camell CD, Sander J, Spadaro O, et al. Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature.* 2017;550(7674):119-123.

35. Camell CD, Günther P, Lee A, et al. Aging Induces an Nlrp3 Inflammasome-Dependent Expansion of Adipose B Cells That Impairs Metabolic Homeostasis. *Cell Metab.* Published online 2019.

36. Vaiserman AM, Koliada AK, Marotta F. Gut microbiota: a player in aging and a target for anti-aging intervention. *Ageing Res Rev.* 2017;35:36-45.

37. Fransen F, Van Beek AA, Borghuis T, et al. Aged gut microbiota contributes to systemical inflammaging after transfer to germ-free mice. *Front Immunol.* 2017;8:1385.

38. Smith AJP, Humphries SE. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev.* 2009;20(1):43-59.

39. Rafiq S, et al. Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. *Genes Immun.* 2007;8(4):344-351.

40. Carrol ED, Payton A, et al. The IL1RN promoter rs4251961 correlates with IL-1 receptor antagonist concentrations in human infection and is differentially regulated by GATA-1. *J. Immunol.* 2011;186(4):2329-2335.

41. Herder C, Nuotio ML, et al. Genetic determinants of circulating interleukin-1 receptor antagonist levels and their association with glycemic traits. *Diabetes.* 2014;63(12):4343-4359.

42. Uresti-Rivera EE, García-Jacobo RE, et al. The presence of the 1068 G>A variant of P2X7 receptors is associated to an increase in IL-1Ra levels, insulin secretion

and pancreatic β-cell function but not with glycemic control in type 2 diabetes patients. *Gene.* 2018;652:1-6.

43. Hajmrle C, Smith N, et al. Interleukin-1 signaling contributes to acute islet compensation. *JCI Insight.* 2016;1(4):e86055.

44. Zeng Y, Nie C, et al. Novel loci and pathways significantly associated with longevity. *Sci Rep.* 2016;6:21243.

45. Khandaker GM, Zammit S, Burgess S, Lewis G, Jones PB. Association between a functional interleukin 6 receptor genetic variant and risk of depression and psychosis in a population-based birth cohort. *Brain Behav Immun.* 2018;69:264-272.

46. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem.* 2010;79:351-379.

47. Hruštincová A, Votavová H, Merkerová MD. Circulating MicroRNAs: Methodological Aspects in Detection of These Biomarkers. *Folia biologica.* 2015;61(6):203-218.

48. Olivieri F, Bonafe M, et al. Age- and glycemia- related miR-126-3p levels in plasma and endothelial cells. *Aging (Albany NY).* 2014;6:771-787.

49. Olivieri F, Albertini MC, et al. DNA damage response (DDR) and senescence: shuttled inflamma-miRNAs on the stage of inflamm-aging. *Oncotarget.* 2015;6(34):35509-35521.

50. O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol.* 2011;11(3):163-175.

51. Arroyo JD, Chevillet JR, the EKP of, 2011. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *National Acad Sciences.* 2011;108:5003-5008.

52. Dluzen DF, Hooten NN, Evans MK. Extracellular RNA in aging. Wiley

Interdiscip Rev Rna. 2016;8(2):1-23.

53. Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z. miRNA in Plasma Exosome is Stable under Different Storage Conditions. *Molecules.* 2014;19(2):1568-1575.

54. Hayflick L. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res.* 1965;37(3):614-636. doi:10.1016/0014-4827(65)90211-9

55. Fagagna F d'Adda di, Reaper PM, Clay-Farrace L, et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature.* 2003;426(6963):194-198.

56. Rodier F, Coppé J-P, Patil CK, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol.* 2009;11(8):973-979.

57. Herbig U, Jobling WA, Chen BPC, Chen DJ, Sedivy JM. Telomere Shortening Triggers Senescence of Human Cells through a Pathway Involving ATM, p53, and p21CIP1, but Not p16INK4a. *Mol Cell.* 2004;14(4):501-513.

58. Yaswen P, Stampfer MR. Molecular changes accompanying senescence and immortalization of cultured human mammary epithelial cells. *Int J Biochem Cell Biology.* 2002;34(11):1382-1394.

59. Hernandez-Segura A, et al. Unmasking transcriptional heterogeneity in senescent cells. *Curr Biol.* 2017;27(11):2652-2660.e4.

60. Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC. Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc National Acad Sci.* 1996;93(24):13742-13747.

61. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras Provokes Premature Cell Senescence Associated with Accumulation of p53 and p16INK4a. *Cell.* 1997;88(5):593-602.

62. Stein GH, Drullinger LF, Soulard A, Dulić V. Differential Roles for Cyclin-

Dependent Kinase Inhibitors p21 and p16 in the Mechanisms of Senescence and Differentiation in Human Fibroblasts. *Mol Cell Biol.* 1999;19(3):2109-2117.

63. Coppé J-P, Patil CK, Rodier F, et al. Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the p53 Tumor Suppressor. *Plos Biol.* 2008;6(12):e301 16.

64. Coppé J-P, Patil CK, Rodier F, et al. A Human-Like Senescence-Associated Secretory Phenotype Is Conserved in Mouse Cells Dependent on Physiological Oxygen. *Plos One.* 2010;5(2):e9188.

65. Wei W, Ji S. Cellular senescence: Molecular mechanisms and pathogenicity. *J Cell Physiol.* 2018;233(12):9121-9135.

66. Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Bio.* 2014;15(7):482-496.

67. Prata LGPL, Ovsyannikova IG, Tchkonia T, Kirkland JL. Senescent cell clearance by the immune system: Emerging therapeutic opportunities. *Semin Immunol.* 2019;40:101275.

68. Herranz N, Gallage S, Mellone M, et al. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat Cell Biol.* 2015;17(9):1205-1217.

69. Micco RD, Krizhanovsky V, Baker D, Fagagna F d'Adda di. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Bio.* 2021;22(2):75-95.

70. Tchkonia T, Zhu Y, Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest.* 2013;123(3):966-72.

71. Xiong W, Sun KY, et al. Metformin alleviates inflammation through suppressing FASN-dependent palmitoylation of Akt. *Cell Death Dis.* 2021;12(10):934.

72. Bharath LP, Agrawal M, McCambridge G, et al. Metformin Enhances Autophagy and Normalizes Mitochondrial Function to Alleviate Aging-Associated Inflammation. *Cell Metab.* 2020;32(1):44-55.e6.

73. Medina CB, Ravichandran KS. Do not let death do us part: 'find- me' signals in communication between dying cells and the phagocytes. *Cell Death Differ.* 2016;23(6):979-989.

74. Goldberg EL, Dixit VD. Drivers of age- related inflammation and strategies for healthspan extension. *Immunol Rev.* 2015;265(1):63-74.

75. Youm YH, Grant RW, et al. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab.* 2013;18(4):519-532.

76. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow- up. *Am J Epidemiol.* 2010;172(4):363-371.

77. Aiello AE, Chiu YL, Frasca D. How does cytomegalovirus factor into diseases of aging and vaccine responses, and by what mechanisms? *GeroScience.* 2017;39(3):261-271.

78. Spyridopoulos I, Martin-Ruiz C, et al. CMV seropositivity and T- cell senescence predict increased cardiovascular mortality in octogenarians: results from the Newcastle 85+ study. *Aging Cell.* 2016;15(2):389-392.

79. Goldeck D, Pawelec G, et al. No strong correlations between serum cytokine levels, CMV serostatus and hand- grip strength in older subjects in the Berlin BASE- II cohort. *Biogerontology*. 2016;17(1):189-198.

80. Nasi M, Biasi SD, et al. Ageing and inflammation in patients with HIV infection. *Clin Exp Immunol.* 2017;187(1):44-52.

81. Brothers TD, Kirkland S, et al. Frailty in people aging with human immunodeficiency virus (HIV) infection. *J Infect Dis.* 2014;210(8):1170-1179.

82. Stein JH, Hsue PY. Inflammation, immune activation, and CVD risk in individuals with HIV infection. *JAMA*. 2012;308(4):405-406.

83. Brenchley JM, Price DA, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med.* 2006;12(12):1365-1371.

84. Grunfeld C, Rimland D, et al. Association of upper trunk and visceral adipose tissue volume with insulin resistance in control and HIV- infected subjects in the FRAM study. *J Acquir Immune Def Syndr.* 2007;46(3):283-290.

85. Spitler KM, Davies BSJ. Aging and plasma triglyceride metabolism. *J Lipid Res.* 2020;61(8):1161-1167.

86. Hamilton MT, Areiqat E, Hamilton DG, Bey L. Plasma Triglyceride Metabolism in Humans and Rats during Aging and Physical Inactivity. *Int J Sport Nutr Exe.* 2001;11(s1):S97-S102.

87. Camell CD, Sander J, Spadaro O, et al. Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature.* 2017;550(7674):119-123.

88. Mennes E, Dungan CM, Frendo-Cumbo S, Williamson DL, Wright DC. Aging-Associated Reductions in Lipolytic and Mitochondrial Proteins in Mouse Adipose Tissue Are Not Rescued by Metformin Treatment. *Journals Gerontology Ser.* 2014;69(9):1060-1068.

89. Camell CD, Sander J, Spadaro O, et al. Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature.* 2017;550(7674):119-123.

90. Camell CD, Günther P, Lee A, et al. Aging Induces an Nlrp3 Inflammasome-Dependent Expansion of Adipose B Cells That Impairs Metabolic Homeostasis. *Cell Metab.* Published online 2019.

91. Berry DC, Jiang Y, Arpke RW, et al. Cellular Aging Contributes to Failure of

Cold-Induced Beige Adipocyte Formation in Old Mice and Humans. *Cell Metab.* 2017;25(1):1-35.

92. Shin W, Okamatsu-Ogura Y, Machida K, Tsubota A, Nio-Kobayashi J, Kimura K. Impaired adrenergic agonist-dependent beige adipocyte induction in aged mice. *Obesity.* 2017;25(2):417-423.

93. Bartke A, Brannan S, Hascup E, Hascup K, Darcy J. Energy Metabolism and Aging. *World J Men's Heal.* 2020;38(2):222.

94. Zhao M, Chen X. Effect of lipopolysaccharides on adipogenic potential and premature senescence of adipocyte progenitors. *Am J Physiol-endoc M.* 2015;309(4):E334-E344.

95. Wei W, Ji S. Cellular senescence: Molecular mechanisms and pathogenicity. *J Cell Physiol.* 2018;233(12):9121-9135.

96. Li Z-Y, Chen Z-L, Zhang T, Wei C, Shi W-Y. TGF-β and NF-κB signaling pathway crosstalk potentiates corneal epithelial senescence through an RNA stress response. *Aging.* 2016;8(10):2337-2354.

97. Ricoult SJH, Manning BD. The multifaceted role of mTORC1 in the control of lipid metabolism. *Embo Rep.* 2013;14(3):242-251.

98. Rabanal-Ruiz Y, Otten EG, Korolchuk VI. mTORC1 as the main gateway to autophagy. *Essays Biochem.* 2017;61(6):565-584.

99. Apelo SIA, Lamming DW. Rapamycin: An InhibiTOR of Aging Emerges From the Soil of Easter Island. *J Gerontol A Biol Sci Med Sci.* 2016;71(7):841-9.

100. Shin DW. Lipophagy: Molecular Mechanisms and Implications in Metabolic Disorders. *Mol Cells.* 2020;43(8):686-693.

101. Hansen M, Rubinsztein DC, Walker DW. Autophagy as a promoter of longevity: insights from model organisms. *Nat Rev Mol Cell Bio.* 2018;19(9):579-593.

102. Yamauchi M, Sugimoto T. Wnt signaling and bone metabolic diseases. *Clin Calcium.* 2019;29(3):329-336.

103. Aamir K, Khan HU, et al. Wnt signaling mediates TLR pathway and promote unrestrained adipogenesis and metaflammation: Therapeutic targets for obesity and type 2 diabetes. *Pharmacol Res.* 2020;152:104602.

104. Fuster JJ, Zuriaga MA, Ngo DT-M, et al. Noncanonical Wnt Signaling Promotes Obesity-Induced Adipose Tissue Inflammation and Metabolic Dysfunction Independent of Adipose Tissue Expansion. *Diabetes.* 2014;64(4):1235-1248.

105. Papaconstantinou J. The Role of Signaling Pathways of Inflammation and Oxidative Stress in Development of Senescence and Aging Phenotypes in Cardiovascular Disease. *Cells.* 2019;8(11):1383.

106. Hongo A, Okumura N, et al. The Effect of a p38 Mitogen-Activated Protein Kinase Inhibitor on Cellular Senescence of Cultivated Human Corneal Endothelial Cells. *Invest Ophthalmol Vis Sci.* 2017;58(9):3325-3334.

107. Alberti L, Gilardini L, Zulian A, et al. Expression of long pentraxin PTX3 in human adipose tissue and its relation with cardiovascular risk factors. *Atherosclerosis.* 2009;202(2):455-460.

108. Erreni M, Manfredi AA, Garlanda C, Mantovani A, Rovere-Querini P. The long pentraxin PTX3: A prototypical sensor of tissue injury and a regulator of homeostasis. *Immunol Rev.* 2017;280(1):112-125.

109. Lee GW, Lee TH, Vilcek J. TSG-14, a tumor necrosis factor- and IL-1-inducible protein, is a novel member of the pentaxin family of acute phase proteins. *Journal of immunology (Baltimore, Md : 1950).* 1993;150(5):1804-1812.

110. Basile A, Sica A, d'Aniello E, et al. Characterization of the promoter for the human long pentraxin PTX3. Role of NF-kappaB in tumor necrosis factor-alpha and interleukin-1beta regulation. *Journal of Biological Chemistry.* 1997;272(13):8172-8178.

111. Porte R, Davoudian S, et al. The Long Pentraxin PTX3 as a Humoral Innate Immunity Functional Player and Biomarker of Infections and Sepsis. *Front Immunol.* 2019;10:794.

112. Doni A, Mantovani A, Bottazzi B, Russo RC. PTX3 Regulation of Inflammation, Hemostatic Response, Tissue Repair, and Resolution of Fibrosis Favors a Role in Limiting Idiopathic Pulmonary Fibrosis. *Front Immunol.* 2021;12:676702.

113. Lin Q, Fang D, Fang J, et al. Impaired Wound Healing with Defective Expression of Chemokines and Recruitment of Myeloid Cells in TLR3-Deficient Mice. *J Immunol.* 2011;186(6):3710-3717.

114. Zhang J, Koussih L, et al. TNF up-regulates Pentraxin3 expression in human airway smooth muscle cells via JNK and ERK1/2 MAPK pathways. *Allergy Asthma Clin Immunol.* 2015;11:37.

115. Zhang J, Koussih L, et al. Glucocorticoids regulate pentraxin-3 expression in human airway smooth muscle cells. *PLoS One.* 2019;14(8):e0220772.

116. Guo H, Qiu X, Deis J, Lin T-Y, Chen X. Pentraxin 3 deficiency exacerbates lipopolysaccharide-induced inflammation in adipose tissue. *Int J Obesity.* 2019;44(2):1-14.

117. Lin T, Guo H, Chen X. Pentraxin 3 Regulates miR-21 Expression and Secretion in Brown Adipocytes During Lipopolysaccharide-Induced Inflammation. *Obesity.* 2020;28(2):323-332.

118. Lin T-Y, Guo H, Chen X. Unraveling mechanisms of pentraxin 3 secretion in adipocytes during inflammation. *J Mol Endocrinol.* 2021;67(3):55-69.

119. Soares AC, Souza DG, Pinho V, et al. Dual function of the long pentraxin PTX3 in resistance against pulmonary infection with Klebsiella pneumoniae in transgenic mice. *Microbes Infect.* 2006;8(5):1321-1329.

120. Norata GD, Marchesi P, Venu VKP, et al. Deficiency of the Long Pentraxin PTX3 Promotes Vascular Inflammation and Atherosclerosis. *Circulation.*

2009;120(8):699-708.

121. Wu Q, Cao F, Tao J, Li X, Zheng SG, Pan H-F. Pentraxin 3: A promising therapeutic target for autoimmune diseases. *Autoimmun Rev.* 2020;19(12):102584.

122. Mou P, Chen Z, et al. PTX3: A Potential Biomarker in Thyroid Associated Ophthalmopathy. *Biomed Res Int*. 2018;2018:5961974.

123. Tian R, Wang X, Pan T, et al. Plasma PTX3, MCP1 and Ang2 are early biomarkers to evaluate the severity of sepsis and septic shock. *Scand J Immunol.* 2019;90(6):e12823.

124. Tang Y, Yang X, et al. Bioinformatic analysis identifies potential biomarkers and therapeutic targets of septic-shock-associated acute kidney injury. *Hereditas.* 2021;158(1):13.

125. Brunetta E, Folci M, Bottazzi B, et al. Macrophage expression and prognostic significance of the long pentraxin PTX3 in COVID-19. *Nat Immunol.* 2021;22(1):19-24.

126. Bonacina F, Moregola A, Porte R, et al. Pentraxin 3 deficiency protects from the metabolic inflammation associated to diet-induced obesity. *Cardiovasc Res.* 2019;115(13):1867.

127. Slusher AL, Huang C-J. Association of pentraxin 3 with insulin resistance and glucose response following maximal aerobic exercise in obese and normal-mass individuals. *Can J Physiol Pharm.* 2016;94(7):734-738.

128. Osorio-Conles O, Guitart M, Chacón MR, et al. Plasma PTX3 protein levels inversely correlate with insulin secretion and obesity, whereas visceral adipose tissue PTX3 gene expression is increased in obesity. *Am J Physiol-endoc M.* 2011;301(6):E1254-E1261.

129. Visconti VV, Greggi C, et al. The long pentraxin PTX3: a novel serum marker to improve the prediction of osteoporosis and osteoarthritis bone-related phenotypes. *J Orthop Surg Res.* 2021;16(1):288.

130. Maekawa Y, Nagai T, Anzai A. Pentraxins: CRP and PTX3 and cardiovascular disease. *Inflamm Allergy Drug Targets.* 2011;10(4):229-35.

131. Waluś-Miarka M, Trojak A, et al. Correlates of pentraxin 3 serum concentration in men and women with type 2 diabetes. *Innate Immun*. 2020;26(5):351-357.

132. Li B, Tian X, et al. Pentraxin-3 and adropin as inflammatory markers of early renal damage in type 2 diabetes patients. *Int Urol Nephrol.* 2020;52(11):2145-2152.

133. Sawczyn T, Stygar D, et al. Effect of Ileal Transposition (IT) on Angiopoietin-Like Protein-8 (ANGPTL8) and Pentraxin (PTX3) Plasma Level in Sprague-Dawley Rats Fed High-Fat Diet (HFD). *Int J Endocrinol.* 2021;2021:6699923.

134. Miyaki A, Choi Y, Maeda S. Pentraxin 3 Production in the Adipose Tissue and the Skeletal Muscle in Diabetic-Obese Mice. *Am J Medical Sci.* 2014;347(3):228-233.

135. Zlibut A, Bocsan I, Pop R, et al. Role of pentraxin-3 in risk assessment of patients with metabolic syndrome. *Physiology Int.* 2019;106(3):283-293.

CHAPTER 2

The Role of Pentraxin 3 in Senescence and Fibrogenesis in Adipose Tissue and Liver During Aging

Summary

Pentraxin 3 (PTX3) plays a vital role in the beginning of obesity by accelerating inflammation and elevating inflammatory response. Both obesity and aging change the concentration of PTX3 in plasma; PTX3 deficiency induces chronic low-grade inflammation and cellular senescence. Aging not only alters adipose tissue function, distribution, and composition, but also affects lipid metabolism. Our previous studies have identified that adipose tissue is the primary tissue that secretes and expresses PTX3 in response to lipopolysaccharide (LPS) stimulation. Moreover, we indicated that PTX3 deficiency led to lipid accumulation in the liver. In this study, we investigate the effect of PTX3 deficiency on senescence and fibrogenesis in adipose tissue and liver during aging. In the *in vitro* studies, we demonstrated that PTX3 deficiency results in increased senescence and fibrogenesis in stromal vascular (SV) cells of subcutaneous adipose tissue in old female mice. In the *in vivo* studies, we found that PTX3 deficiency slightly promoted senescence and inflammation in liver of old female mice but attenuated lipid oxidative metabolism, fibrogenesis, and gluconeogenesis in liver of old female mice. In addition, PTX3 deficiency reduced the response to CL316,243, a β3-adrenergic receptor agonist, treatment in lipid and mitochondrial metabolism in the liver. In summary, PTX3 deficiency leads to elevated senescence and fibrogenesis in SV cells

of subcutaneous adipose tissue in old female mice. Moreover, PTX3 deficiency reduces lipogenesis, lipolysis, fatty acid oxidation, fibrogenesis, and gluconeogenesis in the liver of female mice during aging. Further investigations are needed to understand the molecular mechanism for the role of PTX3 in modulating senescence in adipose tissue and liver during aging.

Introduction

Aging is a complex process that can be affected by environmental and genetic factors, as well as regulated by epigenetic activities. During aging, adipose tissues undergo redistribution from subcutaneous to visceral depot; the body experiences the loss of brown adipose tissue (BAT) and subcutaneous white adipose tissue (sWAT) but the buildup of visceral adipose tissue (vWAT) [1,2]. Most age-related diseases are due to an increasing accumulation of vWAT, which is closely associated with a chronic low-grade inflammation, metabolic dysregulation, and multi-organ damage inside our body. The phenomenon of aging with chronic low-grade inflammation is termed "inflammaging" [3]. Inflammaging is a progressive and dynamic progress and considered an adaptive outcome of a protective response in the body. Moreover, inflammaging is accompanied by increased senescence-associated secretory phenotype (SASP). In adipose tissue, SASP is associated with cellular senescence and defects in various biological processes, such as insulin resistance and adipocyte hypertrophy [4]. Interestingly, people with obesity show similar phenotypes observed in the normal aging process, such as the hypertrophy of adipocytes and alterations of immune cell composition [5,6,7]. Aging also induces adipose tissue dysfunction, metabolic syndrome, increased pro-inflammatory cytokine secretion, increased plasma triglyceride and lipoprotein levels [8,9]. Studies have indicated that aging has a major impact on energy metabolism, and increasing energy expenditure can improve metabolic health. For example, activation of BAT and conversion of WAT to beige adipose tissue can increase thermogenesis, prevent obesity, and improve metabolic health [10]. However, due to the decline in the number of adipose progenitor cells with aging, aged mice lose the ability of forming beige adipocytes in WAT declines in mice [11]. In addition, the increasing cellular senescence with aging restrains the potential to form cold-induced beige adipocytes [12].

Cellular senescence was first described in the 1960s as the limited proliferation of human cells following long-term cultivation [13]. Senescent cells will accumulate with age and cellular senescence can regulate the embryogenesis and serve as a checkpoint that prevents the proliferation of tumor cells. Moreover, senescent cells can expand and display a flatten morphology [14], activate senescence-associated secretory phenotype (SASP) secretion [15,16], change mitochondrial functions [17] leading to increased production of reactive oxygen species (ROS). Excessive ROS production could cause lipid damage and promote DNA damage response (DDR) [18], leading to inherent resistance to apoptosis [19]. The activity of senescence-associated β- galactosidase (SA-β-gal) is a common method for assessing cellular senescence [20,21]. Both telomere shortening and proliferating cells attacked by oxidative stress are the two major known mechanisms for the induction of cellular senescence [22]. In addition to telomere erosion, immune cells and their regulated immune responses play an important role in the development of cellular senescence [23]. In the normal physiological condition, senescent cells can be cleaned by immune cells. However, the immune system loses the ability to fully clear senescent cells with aging. Therefore, senescent cells accumulate in the tissues, which increases SASP secretion leading to the further impairment of immune system function and the enhancement of senescence [24,25]. Ultimately, the tissues and cells lose the ability to regenerate, becoming dysfunctional, and the multiple senescence-associated chronic disorders occur.

Pentraxin 3 (PTX3) is a soluble pattern recognition receptor and acts as a regulator of the immune system [26]. Numerous types of cells can produce PTX3 under different stimuli, including monocytes/macrophage, fibroblasts, vascular endothelial cells, dendritic cells, and adipocytes [27,28]. In addition, researchers found that *TNFα* and *IL1β* as well as the activation of TLR4/NFκB signaling pathway can stimulate the vascular endothelial cells to secrete the PTX3 [29,30]. Studies have revealed that PTX3 plays an important role in the progress of obesity and during aging process by triggering inflammation and secretion of proinflammatory cytokines [31,32]. Furthermore, PTX3 has been associated with the metabolic syndrome [33] and our previous works indicate that PTX3 deficiency increased the LPS-induced inflammation of adipose tissue in mice [34]. In previous studies, we also demonstrated that PTX3 deficiency led to increased senescence and inflammation in BAT, ING-WAT, and GON-WAT SV cells isolated from old female mice, and PTX3 deficiency increased senescence and inflammation in brown and white adipose tissue as well as elevated lipid transport and oxidation in white adipose tissue of old female mice. Therefore, in this study, we aimed to investigate the effect of PTX3 recombinant on aging, senescence and fibrogenesis. We also determined whether PTX3 deficiency affects liver metabolism in old female mice similar to what it affects in brown/white adipose tissue senescence and inflammation.

Materials and Methods

Animal and Treatments

As described previously [35], PTX3 deficiency (PTX3 KO) mice were kindly provided by Professor Martin M Matzuk (Baylor College of Medicine, Houston, Texas). C57BL/6 mice were from The Jackson Laboratory (Bar Harbor, ME). Male PTX3 knockout mice were backcrossed to C57BL/6 mice for 10 generations before mice were used for the experiments. For the experiments, we used the heterozygous mating method to generate wild-type (WT) and PTX3 KO mice. All animal experiments were conducted with the approval of the University of Minnesota Animal Care and Use Committee and followed by the National Institutes of Health guidelines for laboratory animal care. Mice were housed in a 12h light/dark cycle with food and water available *ad libitum*.

The experimental procedure was modified from previous studies [36,37]. Both WT and PTX3 KO female mice (22-24 months old) were treated with either CL316243 (CL, Cayman 17499; 0.5 mg/kg body weight) or saline as control (C) via intraperitoneal injection for four days. Liver and adipose depots were collected for further analysis after 24 hours of the last injection.

Primary Stromal-Vascular Cell Culture and Treatment

Stromal-vascular cells (SV cells) were isolated from interscapular brown adipose tissue (BAT) and inguinal white adipose tissue (ING) of WT and PTX3 KO female mice. Both BAT and ING SV cells were isolated and cultured as mentioned previously [38]. In the 75T flask, SV cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Corning 10-017-CV) with 100 units/ml penicillin-streptomycin (Invitrogen) and 20% fetal bovine serum (FBS, Atlanta Biological) for BAT SV cells or 10% FBS for ING

SV cells. The cells were seeded at a density of $1x10^5$ per well in 6-well plate; when reaching 70-80% confluence (usually at day 6), SV cells were replaced with fresh medium (1.5 ml/well, C) or treated with rmPTX3 (100 ng/ml) for 6 days (Figure 1). In the control group, fresh medium (1.5 ml/well) was replaced with the new medium at day 9 and day 12 for the cells; the cells and culture media were harvested at day 12. In the rmPTX3 group, the cells were treated with 100 ng/ml rmPTX3 (1.5 ml/well) for 3 days and replaced with fresh rmPTX3 medium (1.5 ml/well) for additional 3 days; at day 12, the cells and culture media were collected.

RNA Isolation and Relative Quantitative RT-PCR

By following the manufacturer's instruction, we used TRI reagent (SigmaAldrich T9424) to extract the total RNA from tissues or cells. As for mRNA detection, the cDNA was synthesized from the total RNA (1 μg) by using high-capacity cDNA reverse transcription kit (Applied BiosystemsTM), followed by PCR using PowerUp^{TM} SYBRTM Green Master Mix (Applied BiosystemsTM). The quantitative amplification was meaured by a QuantStudio 3 real-time PCR system (Applied BiosystemsTM). Actb (β-actin) mRNA was viewed as an internal control. All the primer sequences are shown in Table 1 and to quantify the results, the ΔΔCt method [38] was utilized.

Statistical Analysis

Data are shown as means ± s.e.m. Analysis was performed using one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test to compare group means among multiple groups. When comparing group's means between two groups, we used an unpaired two-tailed Student's *t*-test. All analysis was conducted with Prism 9 (GraphPad Software, LLC) and p-value < 0.05 was considered as significant.

Results

PTX3 Deficiency Results in Increased Senescence and Fibrogenesis in Stromal Vascular (SV) Cells Isolated from Subcutaneous Adipose Tissue of Old Female Mice

Our previous studies have shown that the gene expression of proinflammatory cytokines was increased in adipose tissue from Ptx3 knockout mice (KO) treated with lipopolysaccharides (LPS) or fed with a high-fat diet [34]. Moreover, the adipocyte differentiation capacity of stromal vascular (SV) cells isolated from Ptx3 KO mice was decreased [34]. During aging, the adipokine secretion inside our body changes which further causes the adipose tissue dysfunction, leading to systemic metabolic disturbances [9,39]. In addition, aging impairs adipogenesis resulting from the increased cellular senescence of adipocyte progenitors. As a consequence, we investigated the impact of PTX3 deficiency on cellular senescence of stromal vascular (SV) cells of brown adipose tissue (BAT) and inguinal (ING) white adipose tissue (Ing-WAT) from old female mice. First of all, we determined how PTX3 deficiency affects senescence and fibrogenesis of SV cells and whether decreased adipogenesis observed in Ptx3 KO mice results from senescence of adipose progenitor cells within SV cells. The heatmaps showed the effect of PTX3 deficiency on the mRNA expression of senescence and fibrogenesis markers in SV cells from BAT (Figure 2A and 2B) and ING (Ing-WAT) (Figure 3A to 3B). We demonstrated that the gene expression of senescence markers *p16* and *p21* was significantly decreased in Ptx3 KO SV cells from BAT adipose tissue (Figure 2A). While the gene expression of senescence marker *p16* was significantly increased in Ptx3 KO ING SV cells (Figure 3A). Furthermore, the gene expression of senescence-associated secretory phenotype (SASP) and fibrogenesis markers *Tgfβ, Col1a1*, and *Timp2* was significantly downregulated in Ptx3 KO BAT SV cells (Figure 2B). On the contrary, the gene expression of fibrogenesis markers *Mmp2* and *Itga5* was significantly upregulated in Ptx3 KO ING SV cells (Figure 3B). In summary, our results suggest that PTX3 deficiency results in increased senescence and fibrogenesis in SV cells of subcutaneous adipose tissue.

Effect of PTX3 Recombinant on Aging, Senescence and Fibrogenesis

Next, we determine the direct role of PTX3 in senescence and fibrogenesis by investigate the reversal effect of recombinant mouse PTX3 (rmPTX3) on senescence and fibrogenesis in SV cells. We treat both wild type (WT) and Ptx3 KO SV cells isolated from old female mice as previously described. Figure 1A shows the experiment procedure. Briefly, after reaching 70-80% confluence, the control group (C) was replaced with the fresh medium every three days, and rmPTX3 group was treated with 100 ng/ml rmPTX3 (1.5 ml/well) for 3 days and then replaced with fresh rmPTX3 medium (1.5 ml/well) for another 3 days. After 6 days of the treatment, the cells were collected for the analysis of gene expression. As shown in Figure 4A, the rmPTX3 treatment was able to significantly reduce the mRNA expression of *p21* but had no effect on the expression of other two aging markers *p16* and *p53* in WT BAT-derived SV cells. However, in the Ptx3-deficient condition, rmPTX3 treatment was unable to reverse the expression of three aging markers *p16, p21*, and *p53*. Instead, rmPTX3 treatment led to a trend towards an increase in *p53* expression in BAT-derived Ptx3 KO SV cells. In terms of fibrogenesis, rmPTX3 treatment was able to significantly reduce the expression of *Timp2*, as well as led to a trend towards a decrease in the expression of *MMP2* and *Itga5* genes in BAT-derived WT SV cells (Figure 4B). Interestingly, rmPTX3 treatment had a differential effect on the expression of fibrogenesis genes in Ptx3 KO BAT-derived SV cells, leading to upregulation of *MMP2* and *Itga5* expression. Moreover, rmPTX3 treatment was able to upregulate the expression of *Pdgfra*, a marker of mesenchymal stem cells in both WT and Ptx3 KO SV cells. *Pdgfra* is known to be required for adipose tissue development [40]. Increased *Pdgfra* expression by rmPTX3 suggests that rmPTX3 is able to increase or reverse impaired adipogenesis in Ptx3 KO BAT. In ING SV cells, rmPTX3 had no effect on the expression of aging markers in WT and Ptx3 KO SV cells except for the upregulation of *p53* expression by rmPTX3 in WT SV cells (Figure 5A). rmPTX3 treatment reduced the expression of *Timp2* in WT SV cells, as well as decreased the expression of *Cd9* and *Itga5* in Ptx3 KO SV cells. However, rmPTX3 was unable to change the expression of other fibrogenesis genes examined such as *MMP2, Pdgfra*, and *Col6a3* in both WT and Ptx3 KO SV cells (Figure 5B).

PTX3 Deficiency Results in Increased Senescence and Inflammation in Liver in Old Female Mice

It has been indicated that aging leads to the change of lipid metabolism and the failure of beige adipocyte formation in white adipose tissue [9,12,41]. In addition, we have observed that PTX3 deficiency resulted in increased senescence and inflammation in SV cells of ING from old female mice. β3-adrenergic agonist, CL316,243, has been reported to stimulate the formation of new beige adipocytes (beige adipogenesis) [42,43]. Our previous study has demonstrated that both WT and PTX3 KO mice had a reduction in body weight after the 4-day treatment of CL316,243. Moreover, PTX3 deficiency resulted in lower serum levels of FFA and TG, lipid accumulation in the liver, and an increased senescence and inflammation in white adipose tissue. Therefore, we determined whether PTX3 deficiency impacts senescence and lipogenesis in liver *in vivo* as well. In this study, old WT and Ptx3 KO female mice (22-24 months of age) were treated with saline (C) or CL316,243 (CL) (daily IP injection of 0.5 mg/body weight) for 4 days. First of all, we examined the gene expression of senescence and SASP in liver of WT and PTX3 KO mice treated with saline (C) or CL. The results showed that PTX3 deficiency slightly increased the gene expression of senescence marker *p16* in liver under both saline- and CL-treated conditions, but elevated *p53* gene expression in mice only under the saline treatment (Figure 6A). With regards to SASP markers, PTX3 deficiency showed a trend towards an increase in the gene expression of *Tnfα, MIP1α, Mcp1*, and *Il18* in liver under both saline- and CL-treated conditions

(Figure 6B). However, none of these changes researched a statistical significance level. These results suggest that PTX3 deficiency has a minimal effect on liver senescence and inflammation under both saline- and CL-treated conditions.

PTX3 Deficiency Attenuates Lipid Oxidative Metabolism in Liver in Old Female Mice

Senescence has been associated with alternation of lipid metabolism and CL plays an important role in lipid catabolism [12,44,45]. Our previous studies have demonstrated that PTX3 deficiency accelerates lipid transport and oxidation in white adipose tissue, but decreases activity of lipogenesis and fatty acid transport in BAT (unpublished data). Therefore, in this study, we looked into whether PTX3 deficiency affects lipid metabolism and expression of genes involved in lipogenesis, lipolysis, fatty acids transport, and mitochondria oxidation in liver of old female mice in response to saline and CL treatment. Under the non-treated (saline) condition, genes controlling lipogenesis, lipolysis, and mitochondria oxidation were downregulated in Ptx3 KO mice; five of nine examined genes such as *Pparγ, Srebp1c, SCD1, ATGL*, and *Cox4*, were significantly downregulated and two of them *Pparα* and *Cpt1c* showed a trend towards decrease (Figure 7A and 7B). This data indicates a decreased activity of lipogenesis and fatty acid transport in liver under the non-treated condition. Upon CL treatment, the gene expression of *Scd1, Pparα, Cox4*, and *Cox8b* was significantly downregulated in WT mice. But no significant changes in the expression of these genes by CL treatment were found in Ptx3 KO mice except for only a trend towards decreased expression of *Cox4* and *Cox8b*, suggesting that Ptx3 KO mice have a decreased response to CL treatment in the activity of lipogenesis and fatty acid transport in liver (Figure 7A and 7B). However, upon CL treatment, Ptx3 KO mice showed slightly increased expression of lipogenesis genes *Pparγ, Srebp1c*, and *SCD1* (Figure 7A), but decreased expression of genes involved in lipolysis and mitochondria oxidation compared to WT mice (Figure 7B). In summary, we have demonstrated that PTX3 deficiency itself significantly downregulates gene expression in lipogenesis, lipolysis, and mitochondria function in liver. Moreover, PTX3 deficiency reduces the response to CL treatment in lipid and mitochondrial metabolism in the liver. These results suggest that PTX3 deficiency attenuates lipid transport and oxidation in liver.

PTX3 Deficiency Attenuates Fibrogenesis and Gluconeogenesis in Liver in Old Female Mice

In liver, CL treatment significantly attenuated *Ifnγ*, *α-SMA*, and *G6Pase* gene expression in WT mice, but this effect was reduced in Ptx3 KO mice (Figure 8A and

8B). In addition, four of the genes controlling fibrogenesis and glucogenesis, *Ifnγ, α-SMA, G6Pase*, and *PEPCK*, were downregulated in Ptx3 KO mice with saline treatment with the exception of inflammation gene *Cxcl10* showed a significant increase in Ptx3 KO mice (Figure 8A and 8B). The results indicate that PTX3 deficiency leads to a decreased activity of fibrogenesis and glucogenesis, but an increased inflammation in liver of mice.

Discussion

Our previous studies have revealed that PTX3 deficiency augmented inflammation induced by LPS in adipose tissue in mice [34]. According to our unpublished work, the gene expression data indicated that PTX3 deficiency not only resulted in increased senescence and inflammation in brown and white adipose tissue, but also enhanced lipid transport and oxidation in white adipose tissue of old female mice. In this study, we further investigated whether PTX3 deficiency increases senescence, inflammation, and fibrogenesis in SV cells of BAT and ING-WAT from old female mice. In addition, we observed that PTX3 deficiency increased the expression of genes involved in lipid metabolism in white adipose tissue, especially subcutaneous white adipose tissue of old female mice. Since impaired lipid metabolism in adipose tissues alters lipid concentrations in blood, which subsequently affect lipid metabolism in the liver, we also investigated the effect of PTX3 deficiency on lipid metabolism in the liver aged female mice. We demonstrated that PTX3 deficiency resulted in increased senescence and fibrogenesis in stromal vascular (SV) cells of ING-WAT from old female mice. However, when determining the direct role of PTX3 in senescence and fibrogenesis, we didn't see the reversal effect of recombinant mouse PTX3 (rmPTX3) on senescence and fibrogenesis in SV cells. The *in vivo* experiments revealed that PTX3 deficiency slightly promoted senescence and inflammation in liver of old female mice but attenuated lipid oxidative metabolism, fibrogenesis, and gluconeogenesis in liver of old female mice.

In the *in vitro* study, we investigated whether and how PTX3 deficiency induces senescence and fibrogenesis in SV cells isolated from BAT and ING-WAT of old female mice. We added fresh media to the SV cell culture every two days without removing old media after cells reached 70-80% confluence for six days. The rationale for this protocol is that as cells are reaching confluence, increased cell-cell contacts can accelerate replicative senescence caused by the accumulation of reactive oxidative species (ROS) [46] and rmPTX3 intervals for six days started after cells reaching 70-80% confluence. When compared to WT BAT-derived SV cells, the gene expression of senescence markers (*p16, p21, Tgfβ, and Col1a1*) and fibrogenesis marker (*Timp2*) were significantly decreased in Ptx3 KO BAT SV cells. However, the gene expression of fibrogenesis markers *Cd9* and *Itga5* showed significantly increased in Ptx3 KO BAT SV cells compared to WT cells under the rmPTX3 treatment while the gene expression of *Timp2* and *Pdgfrα* was significantly reduced in Ptx3 KO cells. Unlike BAT SV cells, Ptx3 KO ING SV cells had significantly elevated gene expression of *p16*. These results indicate that different adipose depots showed different mechanism for the development of senescence or aging. In addition, the Ptx3 KO ING SV cells upregulated the gene expression of fibrogenesis markers *MMP2* and *Itga5*, suggesting that PTX3 deficiency can induce senescence and inflammation in ING SV cells. In terms of the rmPTX3 treatment, the results showed that the mRNA expression of *p21* and *Timp2* were significantly decreased but had no effect on the expression of others markers in WT BAT-derived SV cells. However, in the Ptx3-deficient condition, rmPTX3 treatment was unable to reverse the expression of three aging markers *p16, p21,* and *p53*. On the contrary, the rmPTX3 treatment upregulates the expression of *MMP2* and *Itga5* in Ptx3 KO BAT-derived SV cells. Surprisingly, rmPTX3 treatment was able to upregulate the expression of *Pdgfra*, a marker of mesenchymal stem cells in both WT and Ptx3 KO SV cells. *Pdgfra* is known to be required for adipose tissue development [40], suggesting that rmPTX3 might be able to increase or reverse impaired adipogenesis in Ptx3 KO BAT. When it comes to ING SV cells, we only see the upregulation of *p53* expression by rmPTX3 in WT SV cells and rmPTX3 treatment reduced the expression of *Timp2* in WT SV cells, as well as decreased the expression of *Cd9* and *Itga5* in Ptx3 KO SV cells. However, rmPTX3 was unable to change the expression of other fibrogenesis genes examined such as *MMP2, Pdgfra, and Col6a3* in both WT and Ptx3 KO SV cells. Therefore, we conclude that there is no reversal effect of recombinant mouse PTX3 (rmPTX3) on senescence and fibrogenesis in ING SV cells.

In the *in vivo* study, we used mice at the age of 22-24 months since it is similar to about 73-80 years old in humans [47]. The mice at the age of 22-24 months are appropriate for the aging study. Previous studies have shown that aging led to the failure of beige adipocyte formation under cold exposure in both mice and humans [11,12], and CL316,243 (CL), a β3-adrenergic receptor agonist has been reported to induce the formation of new beige adipocytes. Therefore, we administrated CL316,243 (CL) to WT and Ptx3 KO mice via intraperitoneal injection to investigate whether and how PTX3 deficiency interferes with CL effect on senescence, lipid metabolism, and thermogenesis in liver from old female mice. The dose and duration of CL were based on and modified from previous studies [36,37], but most of the previous studies conducted similar experiments in young or male mice. It has been reported that the circulating free fatty acid and triglyceride levels increase during aging [8,48]. Our previous studies have demonstrated that CL significantly reduces serum levels of fatty acids and triacylglycerol. However, this effect of CL was diminished in Ptx3 KO mice. In this study, our result is consistent with the previous work that PTX3 deficiency reduces the response to CL treatment in lipid and mitochondrial metabolism in the liver. Although there is one study revealed that intermittent cold exposure could ameliorate liver steatosis [49] and the other study indicated that CL-induced lipolysis could increase hepatic triglyceride level [50], we observed slightly increased lipid accumulation in the liver in Ptx3 KO mice. We have also demonstrated that PTX3 deficiency itself significantly downregulates gene expression in lipogenesis, lipolysis, and mitochondria function in liver, suggesting that PTX3 deficiency attenuates lipid transport and oxidation in liver. Moreover, PTX3 deficiency attenuates fibrogenesis and gluconeogenesis in liver as well. All the data together indicates that PTX3 plays an important role in regulating age-related alteration of lipid metabolism. Nevertheless, how PTX3 deficiency influences lipid metabolism in the liver needs further investigations.

In summary, the results from the present study demonstrate that PTX3 deficiency results in increased senescence and fibrogenesis in SV cells of subcutaneous adipose tissue in old female mice. Moreover, PTX3 deficiency attenuates lipid transport and oxidation, fibrogenesis, and gluconeogenesis in the liver. However, in comparison to our previous studies, the results showed PTX3 deficiency has distinct results in different tissues. Our results suggest that PTX3 deficiency displayed more significant effect on adipose tissue than liver in terms of senescence. Therefore, further investigations of the regulatory mechanisms for PTX3 role in metabolism in liver are still needed.

Figures and Tables

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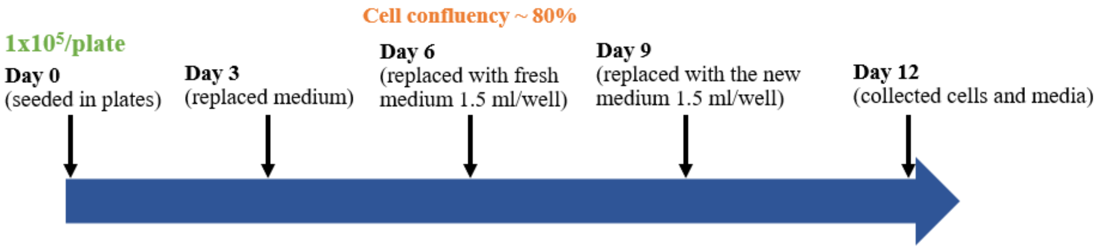

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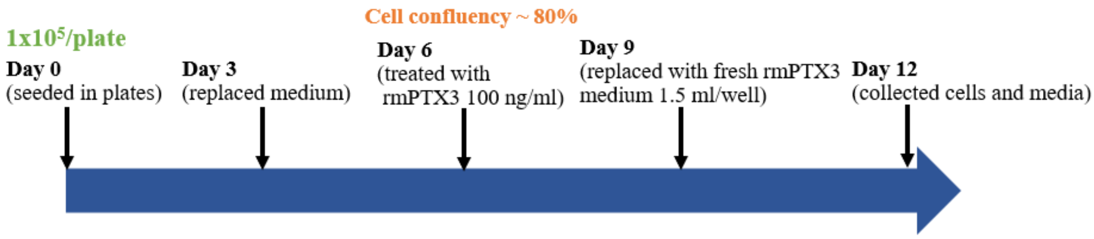

Figure 1. Cellular senescence models in stromal vascular (SV) cells. The cells were seeded at a density of $1x10^5$ per well in 6-well plate; when reaching 70-80% confluence (usually at day 6), SV cells were replaced with (A) fresh medium (1.5 ml/well, C) or (B) treated with rmPTX3 (100 ng/ml) for 6 days. In the control group (C), fresh medium (1.5 ml/well) was replaced with the new medium at day 9 and day 12 for the cells; the cells and culture media were harvested at day 12. In the rmPTX3 group, the cells were treated with 100 ng/ml rmPTX3 (1.5 ml/well) for 3 days and replaced with fresh rmPTX3 medium (1.5 ml/well) for additional 3 days; at day 12, the cells and culture media were collected.

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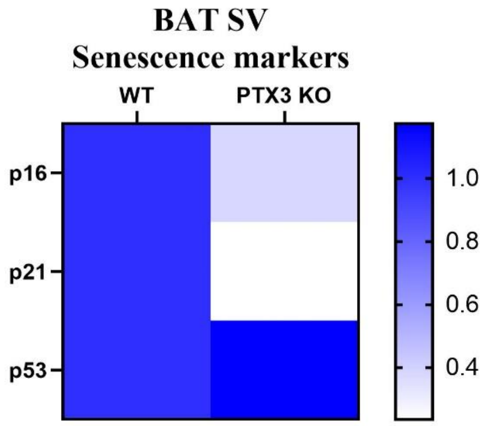

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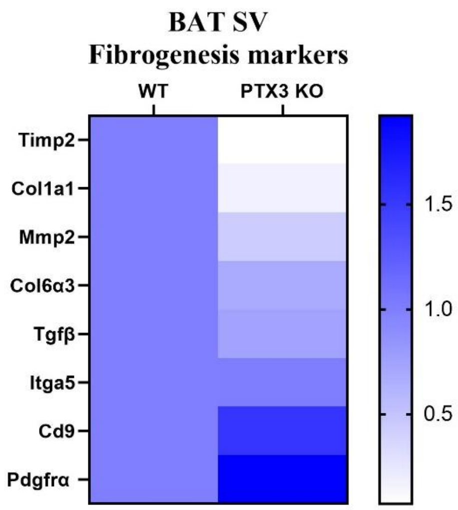

Figure 2. Heatmaps of (A) senescence and (B) fibrogenesis markers BAT SV cells from old female mice. Results are n = 3 in each group. The experiments were repeated two to three times (triplicate each time) yielding similar results.

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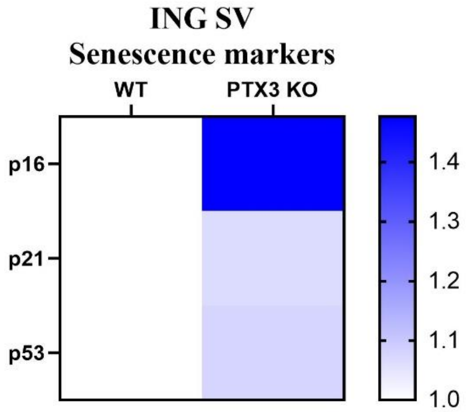

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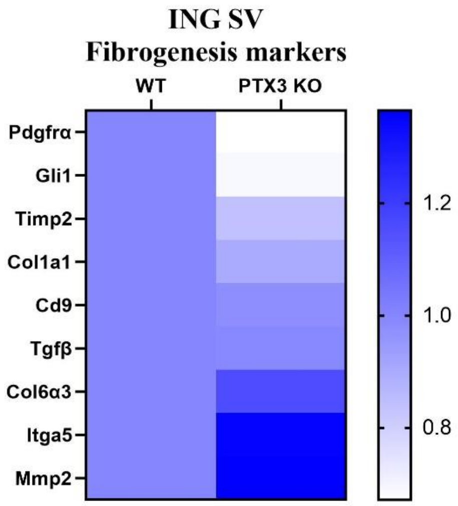

Figure 3. Heatmaps of (A) senescence and (B) fibrogenesis markers of ING SV cells from old female mice. Results are n = 3 in each group. The experiments were repeated two to three times (triplicate each time) yielding similar results.

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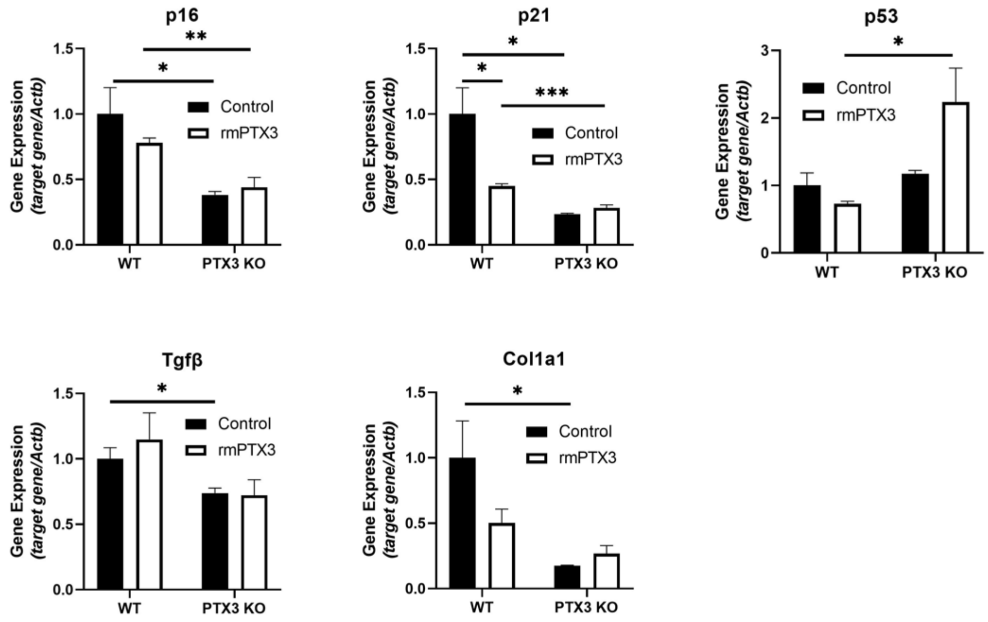

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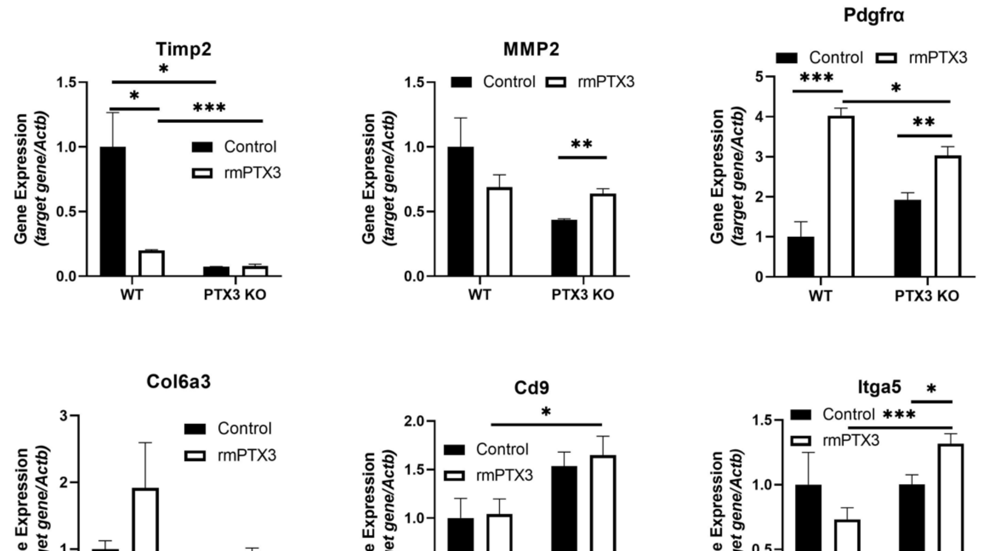

Figure 4. Gene expression of (A) senescence and senescence-associated secretory phenotype (SASP) and (B) fibrogenesis in the BAT SV cells from old female mice. Results presented as mean ± SE; n = 3 in each group. $^*P < 0.05$.

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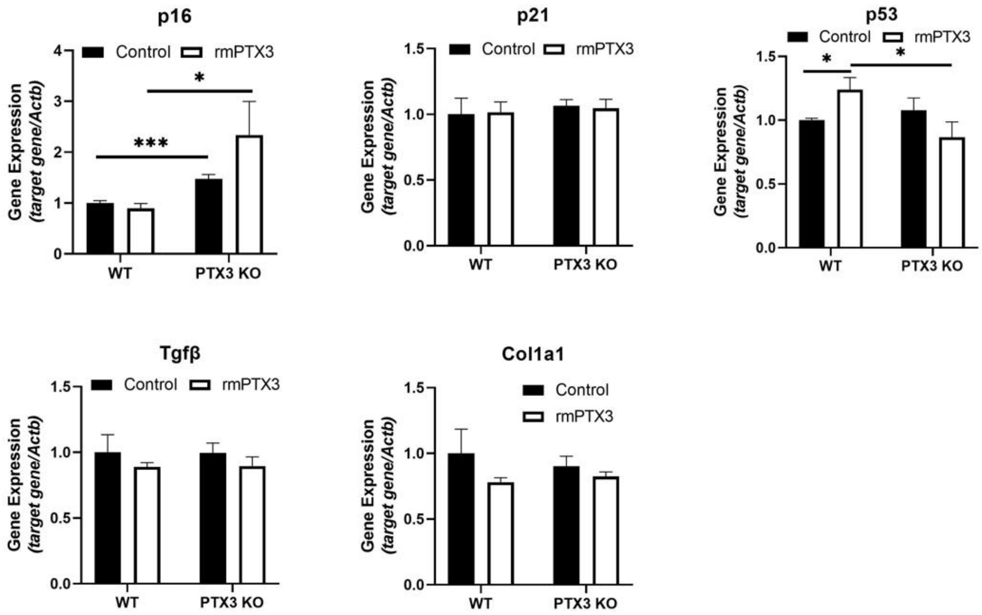

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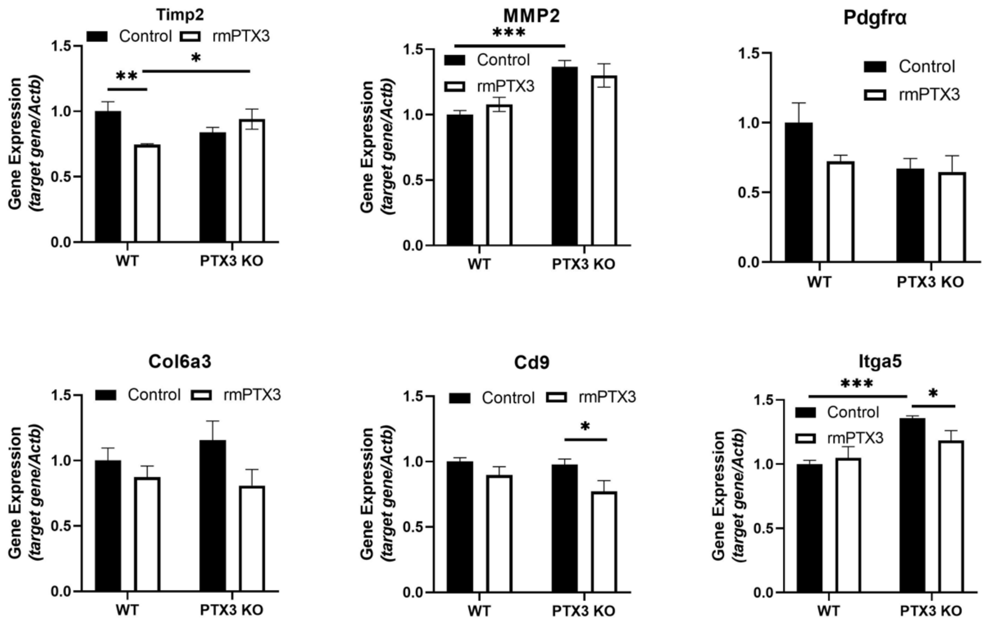

Figure 5. Gene expression of (A) senescence and senescence-associated secretory phenotype (SASP) and (B) fibrogenesis in the ING SV cells from old female mice. Results presented as mean ± SE; n = 3 in each group. *P < 0.05.

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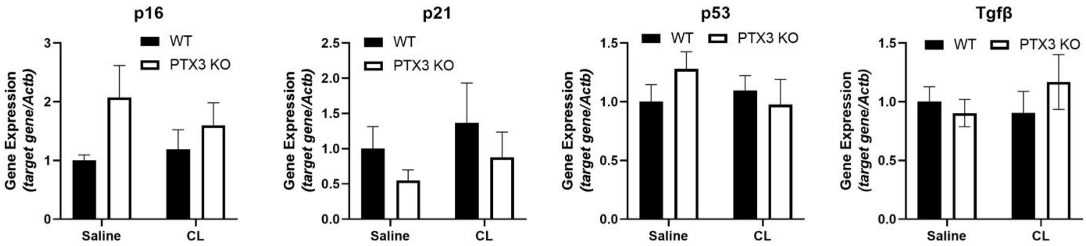

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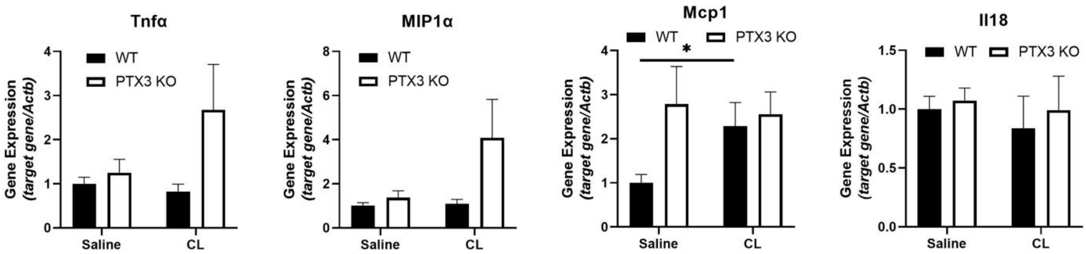

Figure 6. (A) Senescence and (B) senescence-associated secretory phenotype (SASP) gene expression of liver from old female mice treated with saline (C) or CL316243 (CL) for 4 days. Results presented as mean ± SE; n = 6 in each group. *P < 0.05.

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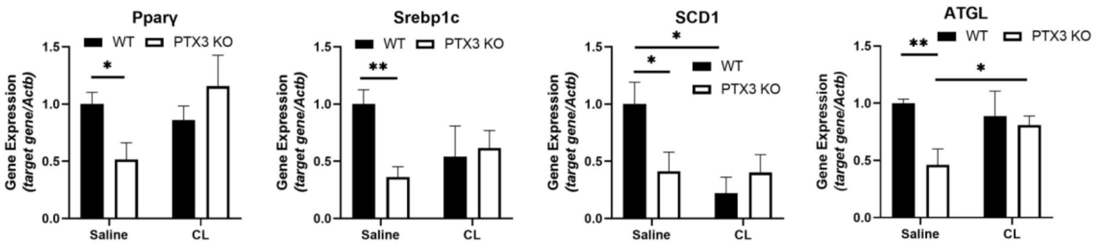

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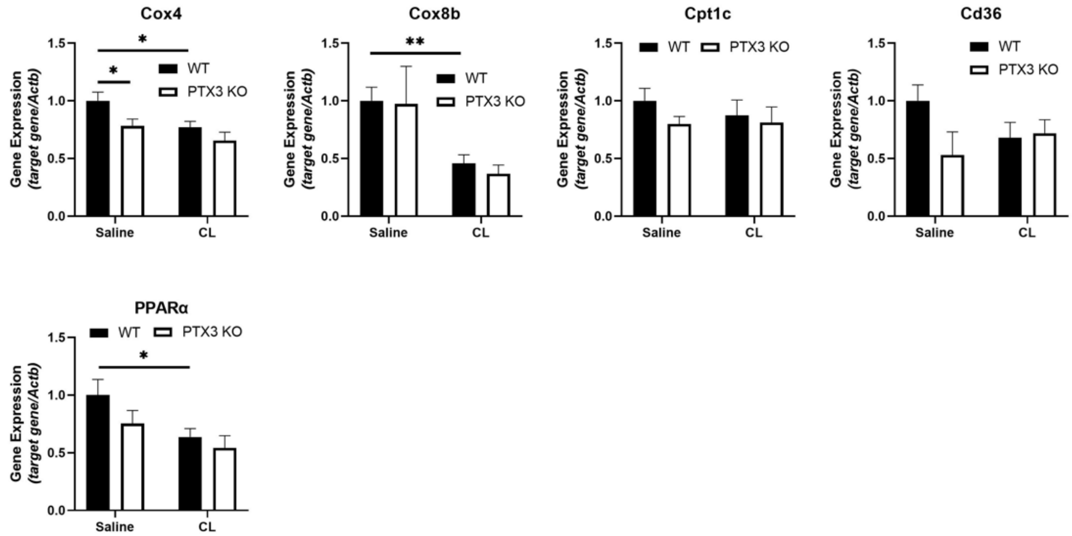

Figure 7. (A) Lipogenesis and lipolysis and (B) fatty acids transport and mitochondria oxidation gene expression of liver from old female mice treated with saline (C) or CL316243 (CL) for 4 days. Results presented as mean ± SE; n = 6 in each group. *P < 0.05.

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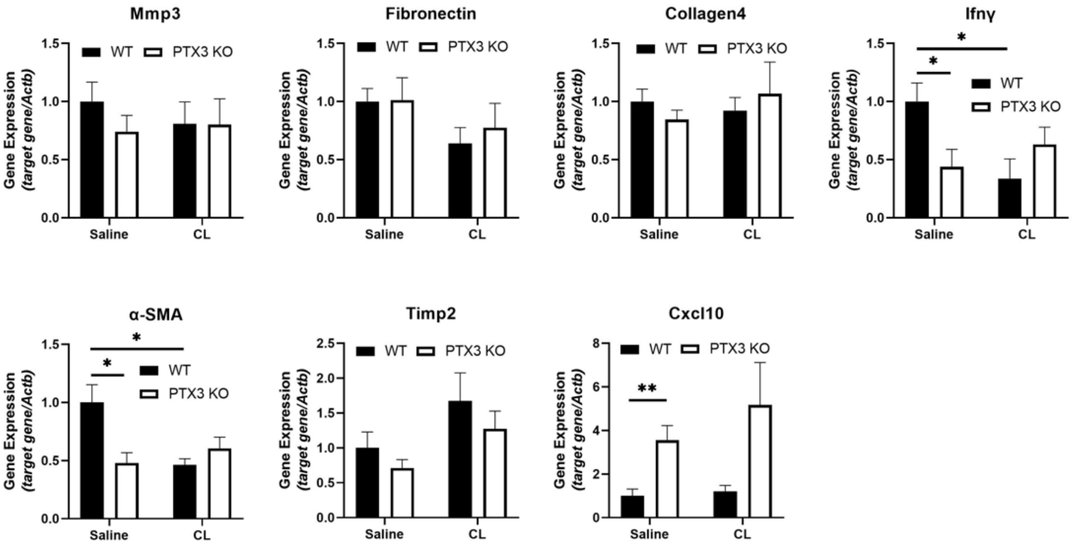

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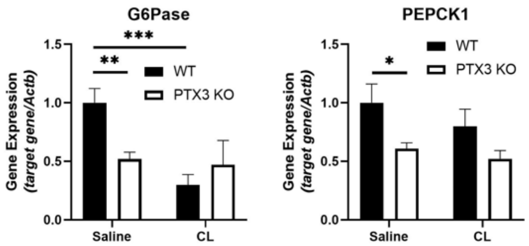

Figure 8. (A) Fibrogenesis and inflammation and (B) gluconeogenesis (GNG) gene expression of liver from old female mice treated with saline (C) or CL316243 (CL) for 4 days. Results presented as mean ± SE; n = 6 in each group. *P < 0.05.

Table 1. Primer Sequences for Real-Time RT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
Actb	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA
Adipoq	GCAGAGATGGCACTCCTGGA	CCCTTCAGCTCCTGTCATTCC
Cd36	TCTTGGCTACAGCAAGGCCAGATA	AGCTATGCAGCATGGAACATGACG
Col1a1	CAACCTGGACGCCATCAAG	CAGACGGCTGAGTAGGGAACA
Cox4	ATGTCACGATGCTGTCTGCC	GTGCCCCTGTTCATCTCGGC
Cox8b	AGGAGTGCGACCCCGAGAATC	GGCTAAGACCCATCCTGCTGG
Cpt1b	TCTAGGCAATGCCGTTCAC	GAGCACATGGGCACCATAC
Dgat	CTCTGCCACAGCATTGAGAC	TGCTACGACGAGTTCTTGAG
Fasn	CTGGACTCGCTCATGGGTG	CATTTCCTGAAGTTTCCGCAG
Il1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Il6	ACAGGCCTGACATCTTCTGC	TCTGACATGGCAGCCATTGT
Lpl	TGAGAAAGGGCTCTGCCTGA	GGGCATCTGAGAGCGAGTCTT
Mcp1	CTTCTGGGCCTGCTGTTCA	GAGTAGCAGCAGGTGAGTGGG
p16	CGTACCCCGATTCAGGTGAT	TTGAGCAGAAGAGCTGCTACGT
p21	GGCAGACCAGCCTGACAGAT	TTCAGGGTTTTCTCTTGCAGAAG
p53	CCAGGGCAACTATGGCTTC	CTCCGTCATGTGCTGTGACTT
Pgc1α	ACCGTAAATCTGCGGGATGATGGA	AGTCAGTTTCGTTCGACCTGCGTA
Pparγ	CAAGAATACCAAAGTGCGATCAA	GAGCAGGGTCTTTTCAGAATAATAAG
Serpine1	TTCCAAGGCATCCAGAAGCAGAGA	ACAGCAGCCGGAAATGACACATTG
Srebp1c	CTTTCCTGGCTTGTCCTTTGGGA	GCTGGAAGGCAAAGGAACAACTGA
Tnfα	ATGGCCTCCCTCTCATCAGT	CTTGGTGGTTTGCTACGACG
Tgfβ	CTGCTGACCCCCACTGATAC	AGCCCTGTATTCCGTCTCCT
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Vegfa	CACGACAGAAGGAGAGCAGAAGT	TTCGCTGGTAGACATCCATGAA

References

1. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. *Mol Aspects Med.* 2013;34(1):1-11.

2. Schosserer M, Grillari J, Wolfrum C, Scheideler M. Age-Induced Changes in White, Brite, and Brown Adipose Depots: A Mini-Review. *Gerontology.* 2018;64(3):229-236.

3. Mau T, Yung R. Adipose tissue inflammation in aging. *Exp Gerontol.* 2018;105(J. Gerontol. A Biol. Sci. Med. Sci. 71 2016):27-31

4. Borodkina, A. V., Deryabin, P. I., Giukova, A. A. & Nikolsky, N. N. 'Social life' of senescent sells: what is SASP and why study it? *Acta Naturae.* 10, 4–14 (2018).

5. Bai Y, Sun Q. Macrophage recruitment in obese adipose tissue. *Obes Rev.* 2015;16(2):127-136.

6. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest.* 2011;121(6):2094-2101.

7. Pérez LM, Pareja-Galeano H, Sanchis-Gomar F, Emanuele E, Lucia A, Gálvez BG. 'Adipaging': ageing and obesity share biological hallmarks related to a dysfunctional adipose tissue. *J Physiology.* 2016;594(12):3187-3207.

8. Spitler KM, Davies BSJ. Aging and plasma triglyceride metabolism. *J Lipid Res.* 2020;61(8):1161-1167.

9. Chung KW. Advances in Understanding of the Role of Lipid Metabolism in Aging. *Cells.* 2021;10(4):880.

10. Bartke A, Brannan S, Hascup E, Hascup K, Darcy J. Energy Metabolism and Aging. *World J Men's Heal.* 2020;38(2):222.

11. Shin W, Okamatsu-Ogura Y, Machida K, Tsubota A, Nio-Kobayashi J, Kimura K. Impaired adrenergic agonist-dependent beige adipocyte induction in aged mice.

Obesity. 2017;25(2):417-423.

12. Berry DC, Jiang Y, Arpke RW, et al. Cellular Aging Contributes to Failure of Cold-Induced Beige Adipocyte Formation in Old Mice and Humans. *Cell Metab.* 2017;25(1):1-35.

13. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res.* 1961;25(3):585-621.

14. Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of Cellular Senescence. *Trends in Cell Biology.* 2018;28(6):436-453.

15. Coppé JP, Patil CK, Rodier F, et al. A Human-Like Senescence-Associated Secretory Phenotype Is Conserved in Mouse Cells Dependent on Physiological Oxygen. *Plos One.* 2010;5(2):e9188.

16. Kuilman T, Michaloglou C, et al. Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network. *Cell.* 2008;133(6):1019-1031.

17. Kaplon J, Zheng L, et al. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature.* 2013,498:109-112.

18. Passos JF, Saretzki G, et al. Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence. *Plos Biology.* 2007;5(5):e110.

19. Sagiv A, et al. Granule exocytosis mediates immune surveillance of senescent cells. *Oncogene.* 2013,32:1971-1977.

20. Lee BY, Han JA, et al. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. *Aging Cell.* 2006,5:187-195.

21. Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 1995;92(20):9363-9367.

22. Herbig U, Jobling WA, Chen BPC, Chen DJ, Sedivy JM. Telomere Shortening Triggers Senescence of Human Cells through a Pathway Involving ATM, p53, and p21CIP1, but Not p16INK4a. *Mol Cell.* 2004;14(4):501-513.

23. Calcinotto A, Kohli J, et al. Cellular Senescence: Aging, Cancer, and Injury. *Physiol Rev.* 2019;99(2):1047-1078.

24. Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Bio.* 2014;15(7):482 496.

25. Prata LGPL, Ovsyannikova IG, Tchkonia T, Kirkland JL. Senescent cell clearance by the immune system: Emerging therapeutic opportunities. *Semin Immunol.* 2019;40:101275.

26. Garlanda C, Bottazzi B, et al. Pentraxins and atherosclerosis: the role of PTX3. *Curr Pharm Des.* 2011;17(1):38-46.

27. Chen W, Zhuang YS, Yang CX, et al. The Protective Role of the Long Pentraxin PTX3 in Spontaneously Hypertensive Rats with Heart Failure. *Cardiovasc Toxicol.* 2021;21(10):808-819.

28. Alberti L, Gilardini L, Zulian A, et al. Expression of long pentraxin PTX3 in human adipose tissue and its relation with cardiovascular risk factors. *Atherosclerosis.* 2009;202(2):455-460.

29. Woo, JM, Kwon MY, Shin DY, et al. Human retinal pigment epithelial cells express the long pentraxin PTX3. *Molecular Vision.* 2013;19:303–310.

30. Basile A, Sica A, d'Aniello E, et al. Characterization of the promoter for the human long pentraxin PTX3. Role of NF-kappaB in tumor necrosis factor-alpha and interleukin-1beta regulation. *Journal of Biological Chemistry.* 1997;272(13):8172-8178.

31. Bonacina F, Moregola A, Porte R, et al. Pentraxin 3 deficiency protects from the metabolic inflammation associated to diet-induced obesity. *Cardiovasc Res.* 2019;115(13):1867.

32. Slusher AL, Huang CJ, Acevedo EO. The Potential Role of Aerobic Exercise-Induced Pentraxin 3 on Obesity-Related Inflammation and Metabolic Dysregulation. *Mediators Inflamm.* 2017;2017,1092738.

33. Zlibut A, Bocsan I, Pop R, et al. Role of pentraxin-3 in risk assessment of patients with metabolic syndrome. *Physiology Int.* 2019;106(3):283-293.

34. Guo H, Qiu X, Deis J, Lin TY, Chen X. Pentraxin 3 deficiency exacerbates lipopolysaccharide-induced inflammation in adipose tissue. *Int J Obesity.* 2019;44(2):1-14.

35. Varani S, Elvin JA, Yan C, et al. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. *Mol Endocrinol.* 2002;16(6):1154-1167.

36. Burl RB, Ramseyer VD, Rondini EA, Pique-Regi R, Lee YH, Granneman JG. Deconstructing Adipogenesis Induced by β3-Adrenergic Receptor Activation with Single-Cell Expression Profiling. *Cell Metab.* 2018;28(2):300 309.e4.

37. Granneman JG, Burnazi M, Zhu Z, Schwamb LA. White adipose tissue contributes to UCP1-independent thermogenesis. *Am J Physiol-endoc M.* 2003;285(6):E1230-E1236.

38. Guo H, Bazuine M, Jin D, Huang MM, Cushman SW, Chen X. Evidence for the Regulatory Role of Lipocalin 2 in High-Fat Diet-Induced Adipose Tissue Remodeling in Male Mice. *Endocrinology.* 2013;154(10):3525-3538.

39. Mancuso P, Bouchard B. The Impact of Aging on Adipose Function and Adipokine Synthesis. *Front Endocrinol.* 2019;10:137.

40. Shin S, Pang Y, et al. Dynamic control of adipose tissue development and adult tissue homeostasis by platelet-derived growth factor receptor alpha. *Elife.* 2020;9:e56189.

41. Johnson AA, Stolzing A. The role of lipid metabolism in aging, lifespan

regulation, and age-related disease. *Aging Cell.* 2019;18(6):e13048.

42. Koh YJ, Park BH, Park JH, et al. Activation of PPARγ induces profound multilocularization of adipocytes in adult mouse white adipose tissues. *Exp Mol Medicine.* 2009;41(12):880-16.

43. Merlin J, Sato M, Chia LY, et al. Rosiglitazone and a β3-Adrenoceptor Agonist Are Both Required for Functional Browning of White Adipocytes in Culture. *Front Endocrinol.* 2018;9:277-17.

44. Millner A, Atilla-Gokcumen GE. Lipid Players of Cellular Senescence. Metabolites. 2020;10(9):339.

45. Klaus S, Seivert A, Boeuf S. Effect of the β3-adrenergic agonist Cl316,243 on functional differentiation of white and brown adipocytes in primary cell culture. Biochimica Et Biophysica Acta Bba - Mol Cell Res. 2001;1539(1-2):85-92.

46. Ho JH, Chen YF, Ma WH, Tseng TC, Chen MH, Lee OK. Cell Contact Accelerates Replicative Senescence of Human Mesenchymal Stem Cells Independent of Telomere Shortening and p53 Activation: Roles of Ras and Oxidative Stress. *Cell Transplant.* 2011;20(8):1209-1220.

47. Dutta S, Sengupta P. Men and mice: Relating their ages. *Life Sci*. 2016;152:244-248.

48. Hamilton MT, Areiqat E, Hamilton DG, Bey L. Plasma Triglyceride Metabolism in Humans and Rats during Aging and Physical Inactivity. *Int J Sport Nutr Exe.* 2001;11(s1):S97-S102.

49. Mitchell A, Alawi K, Aubdool A, et al. Intermittent cold exposure ameliorates fatty liver disease. *Endocr Abstr.* Published online 2019.

50. Heine M, Fischer AW, Schlein C, et al. Lipolysis Triggers a Systemic Insulin Response Essential for Efficient Energy Replenishment of Activated Brown Adipose Tissue in Mice. *Cell Metab.* 2018;28(4):644 655.e4.

Bibliography

1. Flegal, K. M., Kruszon-Moran, D., Carroll, M. D., Fryar, C. D. & Ogden, C. L. Trends in obesity among adults in the United States, 2005 to 2014. *JAMA - Journal of the American Medical Association* **315**, 2284–2291 (2016).
2. Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L. & Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* **378**, 815–825 (2011).
3. Melzer, D., Pilling, L. C. & Ferrucci, L. The genetics of human ageing. *Nat Rev Genet* **21**, 88–101 (2020).
4. Gregor, M. F. & Hotamisligil, G. S. Inflammatory mechanisms in obesity. *Annu Rev Immunol* **29**, 415–445 (2011).
5. Boutagy, N. E., McMillan, R. P., Frisard, M. I. & Hulver, M. W. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie* **124**, 11–20 (2016).
6. Halberg, N. *et al.* Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol* **29**, 4467–4483 (2009).
7. Skurk, T., Alberti-Huber, C., Herder, C. & Hauner, H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab* **92**, 1023–1033 (2007).
8. Meyer, L. K., Ciaraldi, T. P., Henry, R. R., Wittgrove, A. C. & Phillips, S. A. Adipose tissue depot and cell size dependency of adiponectin synthesis and secretion in human obesity. *Adipocyte* **2**, 217–226 (2013).
9. Palmer, A. K. & Kirkland, J. L. Aging and adipose tissue: potential interventions for diabetes and regenerative medicine. *Exp Gerontol* **86**, 97–105 (2016).
10. Chung, K. W. Advances in Understanding of the Role of Lipid Metabolism in Aging.

Cells **10**, (2021).

11. Shen, W. *et al.* Adipose tissue quantification by imaging methods: a proposed classification. *Obes Res* **11**, 5–16 (2003).
12. Choe, S. S., Huh, J. Y., Hwang, I. J., Kim, J. I. & Kim, J. B. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne)* **7**, (2016).
13. Coelho, M., Oliveira, T. & Fernandes, R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci* **9**, 191–200 (2013).
14. Lee, M. J., Wu, Y. & Fried, S. K. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. *Mol Aspects Med* **34**, 1–11 (2013).
15. Strissel, K. J. *et al.* Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes* **56**, 2910–2918 (2007).
16. Sidossis, L. & Kajimura, S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. *J Clin Invest* **125**, 478–486 (2015).
17. Giralt, M. & Villarroya, F. White, brown, beige/brite: different adipose cells for different functions? *Endocrinology* **154**, 2992–3000 (2013).
18. Sanchez-Gurmaches, J., Hung, C. M. & Guertin, D. A. Emerging Complexities in Adipocyte Origins and Identity. *Trends Cell Biol* **26**, 313–326 (2016).
19. Cannon, B. & Nedergaard, J. Brown adipose tissue: function and physiological significance. *Physiol Rev* **84**, 277–359 (2004).
20. Barbatelli, G. *et al.* The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am J Physiol Endocrinol Metab* **298**, (2010).

21. Rosenwald, M., Perdikari, A., Rülicke, T. & Wolfrum, C. Bi-directional interconversion of brite and white adipocytes. *Nat Cell Biol* **15**, 659–667 (2013).

22. Himms-Hagen, J. *et al.* Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am J Physiol Cell Physiol* **279**, (2000).

23. Lenz, M., Arts, I. C. W., Peeters, R. L. M., de Kok, T. M. & Ertaylan, G. Adipose tissue in health and disease through the lens of its building blocks. *Scientific Reports 2020 10:1* **10**, 1–14 (2020).

24. Burl, R. B. *et al.* Deconstructing Adipogenesis Induced by β3-Adrenergic Receptor Activation with Single-Cell Expression Profiling. *Cell Metab* **28**, 300-309.e4 (2018).

25. von Bank, H., Kirsh, C. & Simcox, J. Aging adipose: Depot location dictates age-associated expansion and dysfunction. *Ageing Res Rev* **67**, (2021).

26. Arner, P. & Kulyté, A. MicroRNA regulatory networks in human adipose tissue and obesity. *Nat Rev Endocrinol* **11**, 276–288 (2015).

27. Ghaben, A. L. & Scherer, P. E. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol* **20**, 242–258 (2019).

28. Unamuno, X. *et al.* Adipokine dysregulation and adipose tissue inflammation in human obesity. *Eur J Clin Invest* **48**, (2018).

29. Sun, K., Kusminski, C. M. & Scherer, P. E. Adipose tissue remodeling and obesity. *J Clin Invest* **121**, 2094–2101 (2011).

30. Chylikova, J., Dvorackova, J., Tauber, Z. & Kamarad, V. M1/M2 macrophage polarization in human obese adipose tissue. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia* **162**, 79–82 (2018).

31. Castoldi, A., de Souza, C. N., Saraiva Câmara, N. O. & Moraes-Vieira, P. M. The Macrophage Switch in Obesity Development. *Frontiers in Immunology* **6**, 1 (2015).

32. Covarrubias, A. J., Aksoylar, H. I. & Horng, T. Control of macrophage metabolism and activation by mTOR and Akt signaling. *Semin Immunol* **27**, 286–296 (2015).
33. Mau, T. & Yung, R. Adipose tissue inflammation in aging. *Exp Gerontol* **105**, 27–31 (2018).
34. Camell, C. D. *et al.* Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature* **550**, 119–123 (2017).
35. Camell, C. D. *et al.* Aging Induces an Nlrp3 Inflammasome-Dependent Expansion of Adipose B Cells That Impairs Metabolic Homeostasis. *Cell Metab* **30**, 1024-1039.e6 (2019).
36. Vaiserman, A. M., Koliada, A. K. & Marotta, F. Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res Rev* **35**, 36–45 (2017).
37. Fransen, F. *et al.* Aged Gut Microbiota Contributes to Systemical Inflammaging after Transfer to Germ-Free Mice. *Front Immunol* **8**, (2017).
38. Smith, A. J. P. & Humphries, S. E. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev* **20**, 43–59 (2009).
39. Rafiq, S. *et al.* Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. *Genes Immun* **8**, 344–351 (2007).
40. Carrol, E. D. *et al.* The IL1RN promoter rs4251961 correlates with IL-1 receptor antagonist concentrations in human infection and is differentially regulated by GATA-1. *J Immunol* **186**, 2329–2335 (2011).
41. Herder, C. *et al.* Genetic determinants of circulating interleukin-1 receptor antagonist levels and their association with glycemic traits. *Diabetes* **63**, 4343–4359 (2014).

42. Uresti-Rivera, E. E. *et al.* The presence of the 1068 G>A variant of P2X7 receptors is associated to an increase in IL-1Ra levels, insulin secretion and pancreatic β-cell function but not with glycemic control in type 2 diabetes patients. *Gene* **652**, 1–6 (2018).

43. Hajmrle, C. *et al.* Interleukin-1 signaling contributes to acute islet compensation. *JCI Insight* **1**, (2016).

44. Zeng, Y. *et al.* Novel loci and pathways significantly associated with longevity. *Scientific Reports 2016 6:1* **6**, 1–13 (2016).

45. Khandaker, G. M., Zammit, S., Burgess, S., Lewis, G. & Jones, P. B. Association between a functional interleukin 6 receptor genetic variant and risk of depression and psychosis in a population-based birth cohort. *Brain Behav Immun* **69**, 264–272 (2018).

46. Fabian, M. R., Sonenberg, N. & Filipowicz, W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* **79**, 351–379 (2010).

47. Circulating MicroRNAs: Methodological Aspects in Detection of These Biomarkers - PubMed. https://pubmed.ncbi.nlm.nih.gov/26789142/.

48. Olivieri, F. *et al.* Age- and glycemia-related miR-126-3p levels in plasma and endothelial cells. *Aging* **6**, 771–787 (2014).

49. Olivieri, F. *et al.* DNA damage response (DDR) and senescence: shuttled inflamma-miRNAs on the stage of inflamm-aging. *Oncotarget* **6**, 35509–35521 (2015).

50. O’Neill, L. A., Sheedy, F. J. & McCoy, C. E. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* **11**, 163–175 (2011).

51. Arroyo, J. D. *et al.* Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* **108**,

5003–5008 (2011).

52. Dluzen, D. F., Noren Hooten, N. & Evans, M. K. Extracellular RNA in aging. *Wiley Interdiscip Rev RNA* **8**, (2017).

53. Ge, Q. *et al.* miRNA in plasma exosome is stable under different storage conditions. *Molecules* **19**, 1568–1575 (2014).

54. Hayflick, L. THE LIMITED IN VITRO LIFETIME OF HUMAN DIPLOID CELL STRAINS. *Exp Cell Res* **37**, 614–636 (1965).

55. D'Adda Di Fagagna, F. *et al.* A DNA damage checkpoint response in telomere-initiated senescence. *Nature* **426**, 194–198 (2003).

56. Rodier, F. *et al.* Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nature Cell Biology 2009 11:8* **11**, 973–979 (2009).

57. Herbig, U., Jobling, W. A., Chen, B. P. C., Chen, D. J. & Sedivy, J. M. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell* **14**, 501–513 (2004).

58. Yaswen, P. & Stampfer, M. R. Molecular changes accompanying senescence and immortalization of cultured human mammary epithelial cells. *Int J Biochem Cell Biol* **34**, 1382–1394 (2002).

59. Hernandez-Segura, A. *et al.* Unmasking Transcriptional Heterogeneity in Senescent Cells. *Curr Biol* **27**, 2652-2660.e4 (2017).

60. Alcorta, D. A. *et al.* Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci U S A* **93**, 13742 (1996).

61. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. & Lowe, S. W. Oncogenic ras Provokes Premature Cell Senescence Associated with Accumulation of p53 and

p16INK4a. *Cell* **88**, 593–602 (1997).

62. Stein, G. H., Drullinger, L. F., Soulard, A. & Dulić, V. Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. *Mol Cell Biol* **19**, 2109–2117 (1999).

63. Coppé, J. P. *et al.* Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* **6**, (2008).

64. Coppé, J. P. *et al.* A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS One* **5**, (2010).

65. Wei, W. & Ji, S. Cellular senescence: Molecular mechanisms and pathogenicity. *J Cell Physiol* **233**, 9121–9135 (2018).

66. Muñoz-Espín, D. & Serrano, M. Cellular senescence: from physiology to pathology. *Nature Reviews Molecular Cell Biology 2014 15:7* **15**, 482–496 (2014).

67. Prata, L. G. P. L., Ovsyannikova, I. G., Tchkonia, T. & Kirkland, J. L. Senescent cell clearance by the immune system: Emerging therapeutic opportunities. *Semin Immunol* **40**, (2018).

68. Herranz, N. *et al.* mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat Cell Biol* **17**, 1205–1217 (2015).

69. di Micco, R., Krizhanovsky, V., Baker, D. & d'Adda di Fagagna, F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol* **22**, 75–95 (2021).

70. Tchkonia, T., Zhu, Y., van Deursen, J., Campisi, J. & Kirkland, J. L. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest* **123**, 966–972 (2013).

71. Xiong, W. *et al.* Metformin alleviates inflammation through suppressing FASN-dependent palmitoylation of Akt. *Cell Death & Disease 2021 12:10* **12**, 1–15 (2021).

72. Bharath, L. P. *et al.* Metformin Enhances Autophagy and Normalizes Mitochondrial Function to Alleviate Aging-Associated Inflammation. *Cell Metab* **32**, 44-55.e6 (2020).

73. Medina, C. B. & Ravichandran, K. S. Do not let death do us part: 'find-me' signals in communication between dying cells and the phagocytes. *Cell Death & Differentiation 2016 23:6* **23**, 979–989 (2016).

74. Goldberg, E. L. & Dixit, V. D. Drivers of age-related inflammation and strategies for healthspan extension. *Immunol Rev* **265**, 63–74 (2015).

75. Youm, Y. H. *et al.* Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab* **18**, 519–532 (2013).

76. Roberts, E. T., Haan, M. N., Dowd, J. B. & Aiello, A. E. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. *Am J Epidemiol* **172**, 363–371 (2010).

77. Aiello, A. E., Chiu, Y. L. & Frasca, D. How does cytomegalovirus factor into diseases of aging and vaccine responses, and by what mechanisms? *Geroscience* **39**, 261 (2017).

78. Spyridopoulos, I. *et al.* CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: results from the Newcastle 85+ study. *Aging Cell* **15**, 389–392 (2016).

79. Goldeck, D. *et al.* No strong correlations between serum cytokine levels, CMV serostatus and hand-grip strength in older subjects in the Berlin BASE-II cohort. *Biogerontology* **17**, 189–198 (2016).

80. Nasi, M. *et al.* Ageing and inflammation in patients with HIV infection. *Clinical and Experimental Immunology* **187**, 44 (2017).

81. Brothers, T. D. *et al.* Frailty in people aging with human immunodeficiency virus (HIV) infection. *J Infect Dis* **210**, 1170–1179 (2014).

82. Stein, J. H. & Hsue, P. Y. Inflammation, Immune Activation, and CVD Risk in Individuals With HIV Infection. *JAMA* **308**, 405–406 (2012).

83. Brenchley, J. M. *et al.* Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* **12**, 1365–1371 (2006).

84. Grunfeld, C. *et al.* Association of upper trunk and visceral adipose tissue volume with insulin resistance in control and HIV-infected subjects in the FRAM study. *J Acquir Immune Defic Syndr* **46**, 283–290 (2007).

85. Spitler, K. M. & Davies, B. S. J. Aging and plasma triglyceride metabolism. *J Lipid Res* **61**, 1161–1167 (2020).

86. Hamilton, M. T., Areiqat, E., Hamilton, D. G. & Bey, L. Plasma triglyceride metabolism in humans and rats during aging and physical inactivity. *Int J Sport Nutr Exerc Metab* **11 Suppl**, (2001).

87. Camell, C. D. *et al.* Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature* **550**, 119–123 (2017).

88. Mennes, E., Dungan, C. M., Frendo-Cumbo, S., Williamson, D. L. & Wright, D. C. Aging-associated reductions in lipolytic and mitochondrial proteins in mouse adipose tissue are not rescued by metformin treatment. *J Gerontol A Biol Sci Med Sci* **69**, 1060–1068 (2014).

89. Camell, C. D. *et al.* Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature* **550**, 119–123 (2017).

90. Camell, C. D. *et al.* Aging Induces an Nlrp3 Inflammasome-Dependent Expansion of Adipose B Cells That Impairs Metabolic Homeostasis. *Cell Metab* **30**, 1024-1039.e6 (2019).

91. Berry, D. C. *et al.* Cellular Aging Contributes to Failure of Cold-Induced Beige Adipocyte Formation in Old Mice and Humans. *Cell Metab* **25**, 166–181 (2017).

92. Shin, W. *et al.* Impaired adrenergic agonist-dependent beige adipocyte induction in aged mice. *Obesity (Silver Spring)* **25**, 417–423 (2017).

93. Bartke, A., Brannan, S., Hascup, E., Hascup, K. & Darcy, J. Energy Metabolism and Aging. *The World Journal of Men's Health* **39**, 222 (2021).

94. Zhao, M. & Chen, X. Effect of lipopolysaccharides on adipogenic potential and premature senescence of adipocyte progenitors. *Am J Physiol Endocrinol Metab* **309**, E334–E344 (2015).

95. Wei, W. & Ji, S. Cellular senescence: Molecular mechanisms and pathogenicity. *J Cell Physiol* **233**, 9121–9135 (2018).

96. Li, Z. Y., Chen, Z. L., Zhang, T., Wei, C. & Shi, W. Y. TGF-β and NF-κB signaling pathway crosstalk potentiates corneal epithelial senescence through an RNA stress response. *Aging* **8**, 2337–2354 (2016).

97. Ricoult, S. J. H. & Manning, B. D. The multifaceted role of mTORC1 in the control of lipid metabolism. *EMBO Rep* **14**, 242–251 (2013).

98. Rabanal-Ruiz, Y., Otten, E. G. & Korolchuk, V. I. mTORC1 as the main gateway to autophagy. *Essays Biochem* **61**, 565–584 (2017).

99. Arriola Apelo, S. I. & Lamming, D. W. Rapamycin: An InhibiTOR of Aging Emerges From the Soil of Easter Island. *J Gerontol A Biol Sci Med Sci* **71**, 841–849 (2016).

100. Shin, D. W. Lipophagy: Molecular Mechanisms and Implications in Metabolic

Disorders. *Mol Cells* **43**, 686–693 (2020).

101. Hansen, M., Rubinsztein, D. C. & Walker, D. W. Autophagy as a promoter of longevity: insights from model organisms. *Nature Reviews Molecular Cell Biology 2018 19:9* **19**, 579–593 (2018).

102. Yamauchi, M. & Sugimoto, T. [Wnt signaling and bone metabolic diseases]. *Clin Calcium* **29**, 329–336 (2019).

103. Aamir, K., Khan, H. U., Sethi, G., Hossain, M. A. & Arya, A. Wnt signaling mediates TLR pathway and promote unrestrained adipogenesis and metaflammation: Therapeutic targets for obesity and type 2 diabetes. *Pharmacol Res* **152**, (2020).

104. Fuster, J. J. *et al.* Noncanonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion. *Diabetes* **64**, 1235–1248 (2015).

105. Papaconstantinou, J. The Role of Signaling Pathways of Inflammation and Oxidative Stress in Development of Senescence and Aging Phenotypes in Cardiovascular Disease. *Cells* **8**, (2019).

106. Hongo, A., Okumura, N., Nakahara, M., Kay, E. P. & Koizumi, N. The Effect of a p38 Mitogen-Activated Protein Kinase Inhibitor on Cellular Senescence of Cultivated Human Corneal Endothelial Cells. *Invest Ophthalmol Vis Sci* **58**, 3325–3334 (2017).

107. Alberti, L. *et al.* Expression of long pentraxin PTX3 in human adipose tissue and its relation with cardiovascular risk factors. *Atherosclerosis* **202**, 455–460 (2009).

108. Erreni, M., Manfredi, A. A., Garlanda, C., Mantovani, A. & Rovere-Querini, P. The long pentraxin PTX3: A prototypical sensor of tissue injury and a regulator of homeostasis. *Immunol Rev* **280**, 112–125 (2017).

109. TSG-14, a tumor necrosis factor- and IL-1-inducible protein, is a novel member of the

pentaxin family of acute phase proteins - PubMed. https://pubmed.ncbi.nlm.nih.gov/7679696/.

110. Basile, A. *et al.* Characterization of the promoter for the human long pentraxin PTX3. Role of NF-kappaB in tumor necrosis factor-alpha and interleukin-1beta regulation. *J Biol Chem* **272**, 8172–8178 (1997).

111. Porte, R. *et al.* The Long Pentraxin PTX3 as a Humoral Innate Immunity Functional Player and Biomarker of Infections and Sepsis. *Frontiers in Immunology* **10**, 794 (2019).

112. Doni, A., Mantovani, A., Bottazzi, B. & Russo, R. C. PTX3 Regulation of Inflammation, Hemostatic Response, Tissue Repair, and Resolution of Fibrosis Favors a Role in Limiting Idiopathic Pulmonary Fibrosis. *Front Immunol* **12**, (2021).

113. Lin, Q. *et al.* Impaired wound healing with defective expression of chemokines and recruitment of myeloid cells in TLR3-deficient mice. *J Immunol* **186**, 3710–3717 (2011).

114. Zhang, J. *et al.* TNF up-regulates Pentraxin3 expression in human airway smooth muscle cells via JNK and ERK1/2 MAPK pathways. *Allergy Asthma Clin Immunol* **11**, (2015).

115. Zhang, J. *et al.* Glucocorticoids regulate pentraxin-3 expression in human airway smooth muscle cells. *PLoS One* **14**, (2019).

116. Guo, H., Qiu, X., Deis, J., Lin, T. Y. & Chen, X. Pentraxin 3 deficiency exacerbates lipopolysaccharide-induced inflammation in adipose tissue. *Int J Obes (Lond)* **44**, 525–538 (2020).

117. Lin, T., Guo, H. & Chen, X. Pentraxin 3 Regulates miR-21 Expression and Secretion in Brown Adipocytes During Lipopolysaccharide-Induced Inflammation. *Obesity*

(Silver Spring) **28**, 323–332 (2020).

118. Lin, T. Y., Guo, H. & Chen, X. Unraveling mechanisms of pentraxin 3 secretion in adipocytes during inflammation. *J Mol Endocrinol* **67**, 55–69 (2021).
119. Soares, A. C. *et al.* Dual function of the long pentraxin PTX3 in resistance against pulmonary infection with Klebsiella pneumoniae in transgenic mice. *Microbes Infect* **8**, 1321–1329 (2006).
120. Norata, G. D. *et al.* Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. *Circulation* **120**, 699–708 (2009).
121. Wu, Q. *et al.* Pentraxin 3: A promising therapeutic target for autoimmune diseases. *Autoimmun Rev* **19**, (2020).
122. Mou, P., Chen, Z., Jiang, L., Cheng, J. & Wei, R. PTX3: A Potential Biomarker in Thyroid Associated Ophthalmopathy. *Biomed Res Int* **2018**, (2018).
123. Tian, R. *et al.* Plasma PTX3, MCP1 and Ang2 are early biomarkers to evaluate the severity of sepsis and septic shock. *Scandinavian Journal of Immunology* **90**, (2019).
124. Tang, Y. *et al.* Bioinformatic analysis identifies potential biomarkers and therapeutic targets of septic-shock-associated acute kidney injury. *Hereditas* **158**, (2021).
125. Brunetta, E. *et al.* Macrophage expression and prognostic significance of the long pentraxin PTX3 in COVID-19. *Nature Immunology 2020 22:1* **22**, 19–24 (2020).
126. Bonacina, F. *et al.* Pentraxin 3 deficiency protects from the metabolic inflammation associated to diet-induced obesity. *Cardiovascular Research* **115**, 1861–1872 (2019).
127. Slusher, A. L. & Huang, C. J. Association of pentraxin 3 with insulin resistance and glucose response following maximal aerobic exercise in obese and normal-mass individuals. *Can J Physiol Pharmacol* **94**, 734–738 (2016).
128. Osorio-Conles, O. *et al.* Plasma PTX3 protein levels inversely correlate with insulin

secretion and obesity, whereas visceral adipose tissue PTX3 gene expression is increased in obesity. *Am J Physiol Endocrinol Metab* **301**, (2011).

129. Visconti, V. V. *et al.* The long pentraxin PTX3: a novel serum marker to improve the prediction of osteoporosis and osteoarthritis bone-related phenotypes. *J Orthop Surg Res* **16**, (2021).

130. Maekawa, Y., Nagai, T. & Anzai, A. Pentraxins: CRP and PTX3 and cardiovascular disease. *Inflamm Allergy Drug Targets* **10**, 229–235 (2011).

131. Waluś-Miarka, M. *et al.* Correlates of pentraxin 3 serum concentration in men and women with type 2 diabetes. *Innate Immun* **26**, 351–357 (2020).

132. Li, B. *et al.* Pentraxin-3 and adropin as inflammatory markers of early renal damage in type 2 diabetes patients. *Int Urol Nephrol* **52**, 2145–2152 (2020).

133. Sawczyn, T. *et al.* Effect of Ileal Transposition (IT) on Angiopoietin-Like Protein-8 (ANGPTL8) and Pentraxin (PTX3) Plasma Level in Sprague-Dawley Rats Fed High-Fat Diet (HFD). *International Journal of Endocrinology* **2021**, (2021).

134. Miyaki, A., Choi, Y. & Maeda, S. Pentraxin 3 production in the adipose tissue and the skeletal muscle in diabetic-obese mice. *Am J Med Sci* **347**, 228–233 (2014).

135. Zlibut, A. *et al.* Role of pentraxin-3 in risk assessment of patients with metabolic syndrome. *Physiol Int* **106**, 283–293 (2019).

136. Schosserer, M., Grillari, J., Wolfrum, C. & Scheideler, M. Age-Induced Changes in White, Brite, and Brown Adipose Depots: A Mini-Review. *Gerontology* **64**, 229–236 (2018).

137. Borodkina, A. v., Deryabin, P. I., Giukova, A. A. & Nikolsky, N. N. “Social Life” of Senescent Cells: What Is SASP and Why Study It? *Acta Naturae* **10**, 4 (2018).

138. Bai, Y. & Sun, Q. Macrophage recruitment in obese adipose tissue. *Obes Rev* **16**, 127–

136 (2015).

139. Pérez, L. M. *et al.* 'Adipaging': ageing and obesity share biological hallmarks related to a dysfunctional adipose tissue. *The Journal of Physiology* **594**, 3187 (2016).

140. Hayflick, L. & Moorhead, P. S. The serial cultivation of human diploid cell strains. *Experimental Cell Research* **25**, 585–621 (1961).

141. Hernandez-Segura, A., Nehme, J. & Demaria, M. Hallmarks of Cellular Senescence. *Trends Cell Biol* **28**, 436–453 (2018).

142. Kuilman, T. *et al.* Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* **133**, 1019–1031 (2008).

143. Kaplon, J. *et al.* A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature 2013 498:7452* **498**, 109–112 (2013).

144. Passos, J. F. *et al.* Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence. *PLOS Biology* **5**, e110 (2007).

145. Sagiv, A. *et al.* Granule exocytosis mediates immune surveillance of senescent cells. *Oncogene 2013 32:15* **32**, 1971–1977 (2012).

146. Lee, B. Y. *et al.* Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. *Aging Cell* **5**, 187–195 (2006).

147. Dimri, G. P. *et al.* A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* **92**, 9363–9367 (1995).

148. Calcinotto, A. *et al.* Cellular Senescence: Aging, Cancer, and Injury. *Physiol Rev* **99**, 1047–1078 (2019).

149. Garlanda, C. *et al.* Pentraxins and atherosclerosis: the role of PTX3. *Curr Pharm Des* **17**, 38–46 (2011).

150. Chen, W. *et al.* The Protective Role of the Long Pentraxin PTX3 in Spontaneously

Hypertensive Rats with Heart Failure. *Cardiovasc Toxicol* **21**, 808–819 (2021).

151. Human retinal pigment epithelial cells express the long pentraxin PTX3 - PubMed. https://pubmed.ncbi.nlm.nih.gov/23401658/.
152. Slusher, A. L., Huang, C. J. & Acevedo, E. O. The Potential Role of Aerobic Exercise-Induced Pentraxin 3 on Obesity-Related Inflammation and Metabolic Dysregulation. *Mediators Inflamm* **2017**, (2017).
153. Zlibut, A. *et al.* Role of pentraxin-3 in risk assessment of patients with metabolic syndrome. *Physiol Int* **106**, 283–293 (2019).
154. Varani, S. *et al.* Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. *Mol Endocrinol* **16**, 1154–1167 (2002).
155. Granneman, J. G., Burnazi, M., Zhu, Z. & Schwamb, L. A. White adipose tissue contributes to UCP1-independent thermogenesis. *Am J Physiol Endocrinol Metab* **285**, (2003).
156. Guo, H. *et al.* Evidence for the regulatory role of lipocalin 2 in high-fat diet-induced adipose tissue remodeling in male mice. *Endocrinology* **154**, 3525–3538 (2013).
157. Mancuso, P. & Bouchard, B. The impact of aging on adipose function and adipokine synthesis. *Frontiers in Endocrinology* vol. 10 (2019).
158. Shin, S. *et al.* Dynamic control of adipose tissue development and adult tissue homeostasis by platelet-derived growth factor receptor alpha. *Elife* **9**, 1–21 (2020).
159. Johnson, A. A. & Stolzing, A. The role of lipid metabolism in aging, lifespan regulation, and age-related disease. *Aging Cell* **18**, (2019).
160. Koh, Y. J. *et al.* Activation of PPAR gamma induces profound multilocularization of adipocytes in adult mouse white adipose tissues. *Exp Mol Med* **41**, 880–895 (2009).

161. Merlin, J. *et al.* Rosiglitazone and a β3-adrenoceptor agonist are both required for functional browning of white adipocytes in culture. *Frontiers in Endocrinology* **9**, 249 (2018).

162. Millner, A. & Ekin Atilla-Gokcumen, G. Lipid Players of Cellular Senescence. *Metabolites* **10**, 1–17 (2020).

163. Klaus, S., Seivert, A. & Boeuf, S. Effect of the beta(3)-adrenergic agonist Cl316,243 on functional differentiation of white and brown adipocytes in primary cell culture. *Biochim Biophys Acta* **1539**, 85–92 (2001).

164. Ho, J. H. *et al.* Cell contact accelerates replicative senescence of human mesenchymal stem cells independent of telomere shortening and p53 activation: roles of Ras and oxidative stress. *Cell Transplant* **20**, 1209–1220 (2011).

165. Dutta, S. & Sengupta, P. Men and mice: Relating their ages. *Life Sci* **152**, 244–248 (2016).

166. Mitchell, A. *et al.* Intermittent cold exposure ameliorates fatty liver disease. *Endocrine Abstracts* **65**, (2019).

167. Heine, M. *et al.* Lipolysis Triggers a Systemic Insulin Response Essential for Efficient Energy Replenishment of Activated Brown Adipose Tissue in Mice. *Cell Metab* **28**, 644-655.e4 (2018).