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Mini Review

The pathological role of adipose tissue aging in the progression of systemic insulin resistance

Yohko Yoshida^{1,2,*}, Ippei Shimizu^{1,2,*} and Tohru Minamino^{1,3,**}

¹)Department of Cardiovascular Biology and Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

²)Division of Molecular Aging and Cell Biology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

³)PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

The prevalence of obesity, diabetes and heart failure increases with aging, and there is evidence that these age-related disorders share common pathologic features such as chronic low-grade inflammation and systemic metabolic dysfunction. Cellular senescence was initially discovered by *in vitro* studies, but it is now widely accepted that this process also occurs *in vivo* with aging. The p53 signaling pathway has a central role in the regulation of cellular senescence, and several lines of evidence indicate that cellular senescence has a causative role in the progression of age-related disorders. In obesity, chronic low-grade inflammation of visceral fat is characterized by production of pro-inflammatory cytokines and infiltration of immune cells, which induces systemic insulin resistance (hyperinsulinemia) that leads to the development of diabetes. Recently, p53-induced cellular senescence was reported to have a critical role in this process. In murine heart failure models, cellular senescence due to activation of p53 not only contributes to adipose tissue inflammation and systemic insulin resistance, but also to the progression of heart failure. This review outlines the pathological role of cellular senescence occurring in white adipose tissue in the development of obesity, diabetes, and heart failure.

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*These authors contributed equally to this work.

**Correspondence should be addressed to:

Tohru Minamino, M.D., Ph.D., Department of Cardiovascular Biology and Medicine, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachidori, Chuo-ku, Niigata 951-8510, Japan. Phone: +81-25-227-2182, Fax: +81-25-228-0774, E-mail: t_minamino@yahoo.co.jp

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Introduction

Obesity has become one of the chief health problems around the world. In 2014, the World Health Organization estimated that more than 1.9 billion adults (>18 years old) were overweight (body mass index; BMI >25 kg/m²) and more than 600 million were obese (BMI >30 kg/m²). Obese individuals have a high risk of developing diabetes, cardiovascular disease, and heart failure, which are conditions with high mortality rates^{1, 2}. Chronic low-grade sterile inflammation of adipose tissue, characterized by production of pro-inflammatory cytokines and infiltration of inflammatory cells, is widely accepted to have a central role in the development of systemic metabolic dysfunction^{3, 4}.

Aging is characterized by a decline of physiological function through the accumulation of diverse deleterious changes to cells and tissues, leading to an increase in vulnerability to illness and death. Chronological aging is associated with an increase of DNA damage, shortening of telomeres, oncogenic stress, and damage due to reactive oxygen species, and p53-mediated transcriptional activity is reported to increase under these conditions^{5, 6}. p53 protein is a transcriptional factor involved in the coordination of DNA repair, cell cycle regulation, apoptosis, and senescence to preserve genomic stability and inhibit tumorigenesis, so it has been described as the “guardian of the genome”⁷. Recently, p53 was also shown to regulate cell metabolism through modulation of mitochondrial function, glycolysis, and autophagy^{7, 8}. A p53 signaling pathway is known to be the critical regulator of cellular senescence⁶. The p53 level is increased in aged vessels, failing hearts, and visceral fat in the presence of metabolic stress or impaired cardiac function, and there are several lines of evidence that p53-induced cellular senescence promotes the progression of atherosclerotic disease, heart failure, obesity, and diabetes. In murine models of obesity or heart failure, p53-induced visceral fat inflammation causes the progression of pathological changes, indicating that modulation of aging-related signaling in adipose tissue could potentially become a next-generation therapy for obesity and/or heart failure⁹⁻¹². Recently, the secreted protein “semaphorin3E” was reported to have chemo-attractant activity for inflammatory macrophages and to promote infiltration of these cells into visceral fat, leading to the development of adipose tissue inflammation and systemic metabolic dysfunction. Importantly, the gene coding for semaphorin 3E (*Sema3e*) undergoes transcriptional regulation by p53, suggesting that inhibition of semaphorin3E is a possible new therapeutic

target for diabetes¹⁰. This review outlines the effects of cellular senescence in adipose tissue, focusing on the pathological role of p53 in cardio-metabolic disorders.

Adipose tissue inflammation induces systemic insulin resistance

Adipose tissue was once thought to be largely involved in the storage and release of fats to provide energy, but evidence has been obtained indicating its role as an active endocrine organ that secretes various cytokines and chemokines described as “adipokines”¹³. Metabolic homeostasis is maintained through the physiological balance between production of anti-inflammatory and pro-inflammatory adipokines. In obesity, adipose tissue is characterized by overflow of free fatty acids (FFA), elevated levels of oxidative stress, infiltration of inflammatory cells, and increased production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin 6 (IL-6), and chemokine (C-C motif) ligand 2 (CCL2, also known as monocyte chemoattractant protein-1; MCP-1)^{3, 13}. Circulating pro-inflammatory cytokines and FFA released from inflamed adipose tissue inhibit insulin signaling in key organs, such as the skeletal muscles and the liver, resulting in the development and progression of systemic metabolic dysfunction and diabetes³. Adiponectin is an anti-diabetic adipokine that enhances insulin sensitivity and promotes oxidation of FFA in key metabolic organs, thus contributing to the clearance of FFA, glucose, and triacylglycerol from the blood¹⁴⁻¹⁶. The plasma adiponectin level is low in obesity and type 2 diabetes¹⁷, suggesting that modulation of inflammatory responses and adiponectin signaling are critically important in relation to establishment of next-generation therapies for diabetes.

Adipose tissue aging and insulin resistance

1) Chronological aging and p53-induced cellular senescence

Chronological aging is associated with accumulation of DNA damage, shortening of telomeres, oncogenic stress, and increased production of reactive oxygen species^{6, 18}. These multiple sources of endogenous damage eventually cause cells to enter a state of irreversible growth arrest that is called “cellular senescence”. Somatic cells have a finite lifespan and eventually reach a state of irreversible growth arrest known as “replicative senescence”¹⁹. Telomeres are non-coding TTAGGG repeats located at the ends of mammalian chromosomes, and shortening of telomeres

occurs with cell division due to incomplete replication. Because telomeres play an essential role in maintaining the stability of chromatin and in DNA replication, critical telomere shortening is a form of DNA damage that triggers cellular senescence. Another type of cellular senescence has been described as “stress-induced premature senescence”, and this is triggered by signals such as DNA damage induced by oxidative stress or irradiation, constitutive activation of mitogenic stimuli, oncogenic activation, and metabolic stress^{20, 21}. Dermal fibroblasts from patients with Hutchinson-Gilford progeria syndrome (HGPS) show genomic instability and have a shorter lifespan than normal²². The telomere length of cells in human vessels is known to show a strong inverse correlation with aging and elderly people with shorter telomeres in leukocyte DNA show worse survival²³. These reports suggest a potential link between cellular senescence and aging. The p53/p21 and/or p16/Rb signaling pathways are critically involved in regulating cellular senescence, among which p53 has been studied extensively. p53 is a tumor suppressor protein involved in maintenance of genomic stability. It regulates a broad range of biological processes that include DNA repair, cell metabolism, autophagy, cell cycle progression, and apoptosis^{8, 24, 25}. Chronological aging is associated with an increase of p53-mediated transcriptional activity, suggesting a potential role of p53 signaling in the aging process^{12, 26}. Recently, p53-induced cellular senescence was reported to have a pathological role in age-related disorders such as heart failure, atherosclerotic diseases, and diabetes^{9-11, 20}.

2) Adipose tissue aging in obesity and insulin resistance

Obesity is associated with chronic low-grade inflammation of adipose tissue that is characterized by the infiltration of immune cells, such as T cells and macrophages, and by increased production of pro-inflammatory cytokines leading to the development of systemic insulin resistance²⁷⁻³⁰. It is also generally accepted that metabolic syndrome promotes premature aging. Since the prevalence of metabolic disorders such as diabetes increases with aging, cellular senescence may have a pathological role in the development of systemic insulin resistance and progression of diabetes. Consistent with this concept, our previous study showed that p53-induced cellular senescence was central to inducing adipose tissue inflammation and systemic insulin resistance in a mouse model of dietary obesity^{10, 11}. Excessive calorie intake led to elevation of oxidative stress in adipose tissue and promoted senescence-like changes,

characterized by elevation of senescence-associated β -galactosidase, increased p53 expression, and production of pro-inflammatory cytokines. In diabetic mice, inhibition of p53 in adipose tissue markedly ameliorated these senescence-like changes, suppressed the production of pro-inflammatory cytokines, and improved systemic insulin resistance. Conversely, forced expression of p53 in the adipose tissue of normal chow-fed mice induced inflammation and led to the development of systemic insulin resistance. Importantly, adipose tissue from diabetic patients also displays these senescence-like features. Analysis of mice with the combination of p53 knockout in adipose tissue (aP2-Cre; floxed p53 mice) and bone marrow transplantation indicated that p53 expression in the stromal vascular fraction (SVF) may also contribute to abnormal glucose metabolism in dietary obesity, although glucose tolerance test data suggested that p53 expression in the adipocyte-rich fraction (ARF) has a stronger pathological impact¹¹.

Adipocyte DNA excision repair protein ERCC-1 is involved in nucleotide excision and repair (NER). Deletion of *Ercc-1* results in persistent DNA damage and increased production of pro-inflammatory cytokines such as Tnf and IL6 in adipose tissue³¹. This clearly indicates that DNA damage *per se* is sufficient to trigger the inflammatory response that leads to systemic metabolic dysfunction. There is emerging evidence of negative feedback between diabetes and activation of the DNA damage response, but further studies are needed to delineate the link between DNA damage, DNA damage response (DDR) molecules, and inflammation in adipose tissue¹².

3) Semaphorin is a key regulator of adipose tissue inflammation

Recently, the secreted protein “semaphorin3E” was identified as a key molecule in adipose tissue inflammation associated with obesity. Semaphorins and their receptors (plexins) are axon-guiding molecules that coordinate development of the nervous system during embryogenesis³²⁻³⁵. Semaphorins act both as repellent or attractant molecules and also modulate the responses of immune cells, but a role in metabolic inflammation was unknown. Semaphorin3E is a secreted protein classified as a class 3 semaphorin that binds to its specific receptor (plexinD1). Disruption of *sema3e* or *plxnd1* inhibits the formation of vascular networks. Recently, expression of semaphorin3E and plexinD1 in adipose tissue was found to be increased in a mouse model of dietary obesity. Inhibition

of the semaphorin3E-plexinD1 axis ameliorated adipose tissue inflammation and improved systemic insulin resistance. Conversely, forced expression of semaphorin3E in adipose tissue provoked inflammation and insulin resistance in normal chow-fed mice. Semaphorin3E promoted the infiltration of macrophages, with this effect being inhibited by the suppression of plexinD1 expression on macrophages. Genetic disruption of p53 in adipose tissue led to down-regulation of semaphorin3E expression, inhibited the infiltration of inflammatory cells, and suppressed pro-inflammatory cytokine production. Adipose tissue inflammation induced by a high calorie diet was also ameliorated in *sema3e* null mice. *In vivo* and *in vitro* studies have demonstrated that semaphorin3E has chemoattractant activity for plexinD1-positive macrophages. The chromatin immunoprecipitation assay showed that p53 binds to the presumed promoter region of *sema3e*, indicating that p53 positively regulates *sema3e* at the transcriptional level. These findings suggest that the p53/semaphorin3E/plexinD1 axis has a crucial role in the development of adipose tissue inflammation and systemic metabolic dysfunction associated with obesity. Thus, inhibition of this signaling pathway has the potential to become a new therapeutic option for diabetes¹⁰.

Heart failure and adipose tissue inflammation

A close link between heart failure and diabetes has long been recognized in the clinical setting³⁶⁻³⁸. In patients without prior cardiac dysfunction, it was demonstrated that insulin resistance predicts the subsequent onset of heart failure independently of other established risk factors³⁹. In addition, the plasma level of pro-insulin (a marker of insulin resistance) was found to be elevated 20 years before the onset of heart failure⁴⁰, indicating that insulin resistance precedes heart failure rather than being a consequence of it. Patients with insulin resistance have been reported to develop left ventricular hypertrophy and diastolic dysfunction because of their reduced ability to adapt to hypertensive stress^{41, 42}. Another study showed that systemic insulin resistance has a high prevalence among patients with idiopathic dilated cardiomyopathy⁴³. These human studies have suggested a causal role of systemic insulin resistance in the development and progression of heart failure, but the molecular mechanisms of systemic insulin resistance (hyperinsulinemia) and its pathological influence on heart failure have been

largely unknown. Recently, p53-signaling was reported to have causal role in the development of adipose tissue inflammation and systemic insulin resistance in a murine heart failure model⁹. Left ventricular (LV) pressure overload led to marked up-regulation of p53 expression in visceral fat, in association with the infiltration of mononuclear-like cells and production of pro-inflammatory cytokines. An increase of adrenergic signaling, possibly through elevated sympathetic activity, induced excessive lipolysis and increased p53 expression in adipose tissue during LV pressure overload. In the presence of cardiac stress due to pressure overload, inhibition of lipolysis by sympathetic denervation of visceral fat significantly reduced p53 in adipose tissue, ameliorated the inflammatory response, and suppressed the development of systemic insulin resistance. Genetic disruption of p53 in adipose tissue also suppressed the development of adipose tissue inflammation and systemic insulin resistance, and importantly, ameliorated the progression of systolic dysfunction due to LV pressure overload. A number of molecules have been reported to induce physiological and pathological hypertrophy. Among them, insulin-like growth factor 1 (IGF-1) and insulin are critical regulators of physiological hypertrophy^{44, 45}. Akt is the common downstream molecule involved in the growth of cardiomyocytes. Although insulin-Akt signaling is crucial for normal growth of cardiomyocytes, induction of this signaling pathway is known to be detrimental for the maintenance of cardiac homeostasis by inducing excessive cardiac hypertrophy and hypoxia^{46, 47}. While systemic insulin resistance (hyperinsulinemia) develops in the chronic phase of LV pressure overload, insulin signaling is maintained at a high level in cardiac tissue through myocardial stretch-induced activation of this signaling pathway⁴⁷. Although further studies are needed to elucidate how myocardial stretch contributes to the continuous activation of cardiac insulin signaling in the setting of heart failure associated with systemic insulin resistance, these reports indicate that therapies targeting insulin signaling in cardiac tissue and inhibition of adipose tissue inflammation could be crucially important for the treatment of heart failure⁹.

Concluding remarks

Both as an energy store and an active endocrine organ, adipose tissue has a central role in the maintenance of metabolic homeostasis. It is now generally accepted that adipose tissue inflammation is pathological, contributing to the development of systemic insulin resistance and

Fig. 1 Vicious circle of metabolic dysfunction in heart failure and obesity

Heart failure and obesity induce adipose tissue aging and systemic insulin resistance, while systemic insulin resistance has a causal role in the progression of age-related disorders like diabetes and heart failure. (Black lines indicate promotion and red lines indicate aggravation.)

the progression of diabetes. Recently, adipose tissue inflammation and systemic metabolic dysfunction were reported to have a detrimental effect on cardiac function during LV pressure overload. Activation of adrenergic signaling promotes lipolysis and is associated with high ROS levels. Because p53-induced cellular senescence has a causal role in the development of adipose tissue inflammation and systemic insulin resistance (hyper-insulinemia) in both diabetes and heart failure, therapies targeting the modulation of cellular senescence and inhibiting metabolic dysfunction could be vital for suppressing the progression of these disorders (Fig. 1). Recently, the secreted protein semaphorin3E was reported to have chemo-attractant activity for inflammatory macrophages in diabetic mice. *Sema3e*, the gene coding semaphorin3E protein, has been found to be one of the targets of p53 and the blood level of semaphorin3E is increased in patients with diabetes. Thus, inhibition of semaphorin3E signaling may become a new therapeutic option for diabetes.

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Conflict of interests

None

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