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Review

Molecular mechanism of obesity-induced adipose tissue inflammation; the role of Mincle in adipose tissue fibrosis and ectopic lipid accumulation

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Abstract. Metabolic syndrome is a common metabolic disorder that involves multiple organs and is predominantly influenced by obesity, especially the accumulation of visceral fat. It is also known that macrophages that infiltrate obese adipose tissue play an important role in inflammation of the adipose tissue. Macrophage-inducible C-type lectin (Mincle), a new inflammatory regulator found in obese adipose tissue, is expressed in pro-inflammatory M1 macrophages in adipose tissue. In addition, Mincle is expressed in macrophages that form a crown-like structure, where dead or dying adipocytes are surrounded by pro-inflammatory M1 macrophages; within this crown-like structure, adipocyte–macrophage crosstalk may occur in close proximity. Although there is no significant difference in body weight between Mincle-deficient and wild-type mice under high-fat diet, the epididymal fat weight is significantly higher and the liver weight is significantly lower in Mincle-deficient mice than those in wild-type mice. It has been shown that adipose tissue inflammation and fibrosis are attenuated in Mincle-deficient mice when compared with wild-type mice. In addition, Mincle-deficient mice have reduced hepatic lipid accumulation and better glucose metabolism. These results suggest that Mincle signaling in adipose tissue macrophages activates adipose tissue fibroblasts, which leads to adipose tissue fibrosis.

Key words: Obesity, Adipose tissue inflammation, Adipose tissue fibrosis, Macrophage, Ectopic lipid accumulation

Introduction

Adipose tissue is a metabolic organ that accumulates triglycerides as energy reserve; however, recent studies have revealed that adipose tissue is also an endocrine organ that produces and secretes various hormones called adipocytokines. In obesity, adipose tissue dysfunction is caused by adipose tissue inflammation, which leads to metabolic syndrome [1, 2]. In addition to adipocytes, adipose tissue contains various stromal cells, such as preadipocytes, vascular cells, immune cells, and fibroblasts, and the number and type of cells change dramatically during the progression of obesity. Since two groups independently had reported macrophage infiltration in obese adipose tissue [3, 4], many other researchers have revealed the effect of macrophages on the tissue. It is generally accepted that the interaction between adipocytes and macrophages leads to chronic inflammation of adipose tissue resulting in disruption of endocrine function such as adipocytokine production [5]. However, little is known about the effects of adipose tissue inflammation on the metabolic function of adipose tissue. In this article, we provide an overview of the significance of macrophage-inducible C-type lectin (Mincle), a new inflammatory regulator of adipose tissue inflammation, with an emphasis on its role in the adipose tissue inflammation, fibrosis, and ectopic lipid accumulation.

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Adipose Tissue Macrophages

Metabolic syndrome is a common metabolic disorder that involves multiple organs and is associated with obesity and adipose tissue inflammation. It is considered that adipose tissue inflammation is mainly exacerbated by the interaction between enlarged adipocytes and macrophages that infiltrate into the obese adipose tissue. It has also been reported that the interaction between saturated

fatty acids derived from adipocytes and the pathogen sensor Toll-like receptor 4 (TLR4) expressed in infiltrated macrophages can result in chronic inflammation [6, 7]. Evidence has also suggested that there are at least two types of adipose tissue macrophages, such as pro-inflammatory M1 and anti-inflammatory M2 macrophages [8, 9]. Our study and previous studies have shown that monocyte chemoattractant protein-1 (MCP-1) plays a major role in the recruitment of M1 macrophages from the bone marrow [10-12]. These M1 macrophages form a crown-like structure (CLS) in obese adipose tissue where dead or dying adipocytes are surrounded by pro-inflammatory M1 macrophages; within this CLS, adipocyte–macrophage crosstalk may occur in close proximity [13-15]. In addition, M1 macrophages produce pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα); therefore, the CLS is a hallmark of adipose tissue inflammation, wherein the number of CLS is positively correlated with systemic insulin resistance [16, 17]. On the other hand, M2 macrophages are interspersed in the interstitial spaces between the adipocytes [8, 9]. During the development of obesity, not only did the number of macrophages increase but the M1 to M2 ratio also increased markedly in the adipose tissue [8, 9, 18-20].

Mincle Expression in Obese Adipose Tissue

To screen for a new regulator involved in adipose tissue inflammation, we performed microarray analysis and identified Mincle, the expression of which is upregulated in macrophages *via* saturated fatty acid-TLR4 signaling [21]. Mincle is a type II transmembrane Ca^{2+}-dependent lectin that is induced in macrophages by lipopolysaccharide [22]. Although the function of Mincle was previously unknown, it is now understood that Mincle recognizes trehalose-6,6'-dimycolate (TDM), a mycobacterial cell wall glycolipid, and pathogenic fungi (*Malacethia*, *Candida*) to induce the production of pro-inflammatory cytokines and chemokines [23, 24]. Therefore, pathogen sensors such as Mincle and TLR4 play a central role in the defense against infection. In addition, recent studies have demonstrated that these pathogen sensors also recognize endogenous ligands released from damaged and dead cells. Interestingly, it has been reported that Mincle can sense cell death [25], suggesting the role of Mincle in sterile inflammation. We examined Mincle expression during the progression of obesity and revealed that it is expressed in obese adipose tissue in humans and mice, especially in visceral adipose tissue. In addition, Mincle is highly expressed in pro-inflammatory M1 macrophages in various immune cells in obese adipose tissue [26]. Histological analysis revealed that Mincle expression is localized to macrophages in the CLS. Because the CLS is associated with adipose tissue inflammation, these results suggest that Mincle is involved in adipose tissue inflammation.

Role of Mincle in Adipose Tissue Inflammation and Fibrosis

Adipose tissue is composed of mature adipocytes and various stromal cells, whose cellular components change greatly with body weight. In chronic inflammation, continuous interaction between parenchymal and stromal cells results in dynamic morphological changes termed "adipose tissue remodeling." Hypertrophic adipocytes produce and secrete large amounts of inflammatory cytokines, such as TNFα, interleukin-6 (IL-6), and saturated fatty acids, which induce insulin resistance. On the other hand, adiponectin, an anti-inflammatory cytokine, is in lesser amount and inversely correlates with obesity. Furthermore, obese adipose tissue reportedly becomes fibrotic and accumulates less triglycerides [27]. In order to examine the role of Mincle in adipose tissue inflammation, we analyzed diet-induced obese Mincle-deficient and wild-type mice. Although there was no significant difference in body weight, the epididymal fat weight was significantly higher and the liver weight was significantly lower in Mincle-deficient mice compared with those of wild-type mice on a high-fat diet [26]. Histological analysis revealed that wild-type mice showed extensive interstitial fibrosis in adipose tissue, whereas Mincle-deficient mice showed a marked reduction. Furthermore, the diameter of adipocytes in obese adipose tissue extracted from Mincle-deficient mice was larger than that from wild-type mice, and the number of adipose tissue macrophages was not significantly different between the genotypes. However, the number of CLS was significantly reduced in Mincle-deficient mice. Moreover, Mincle-deficient mice exhibit lower serum free fatty acid levels than those of wild-type mice on a high-fat diet. These results suggest that Mincle activation induces adipose tissue inflammation and fibrosis, which limits lipid accumulation in adipose tissue; excess lipids are released as serum free fatty acids.

Molecular Mechanism of Adipose Tissue Fibrosis through Mincle Signaling

Several molecules are reportedly involved in adipose tissue fibrosis. Mice lacking type VI collagen, which is highly expressed in adipose tissue, showed adipocyte hypertrophy and increased adipose tissue weight during the progression of obesity [28]. Hypoxia-inducible factor-1α (HIF-1α) in adipocytes reportedly promotes

adipose tissue fibrosis [29], whereas peroxisome proliferator-activated receptor γ (PPARγ)-fibroblast growth factor 1 (FGF1) axis suppresses adipose tissue fibrosis [30]. In addition, a recent study reported that obese TLR4-deficient mice showed attenuation of adipose tissue fibrosis and improvement in glucose metabolism [31]. However, little is known about the molecular mechanism of adipose tissue fibrosis. In order to investigate the molecular mechanism of adipose tissue fibrosis through Mincle signaling, peritoneal macrophages were stimulated by TDM, a previously identified exogenous Mincle ligand. This stimulation resulted in the upregulation of not only inflammatory cytokines and chemokines such as TNFα and macrophage inflammatory protein-2 (MIP-2) but also fibrosis-related genes such as transforming growth factor-β (TGF-β) and tissue inhibitor of metalloproteinase 1 (TIMP-1) in a spleen tyrosine kinase (Syk)-dependent manner. In addition, co-culture of fibroblasts from obese adipose tissue and peritoneal macrophages with TDM stimulation resulted in the upregulation of α-smooth muscle actin (αSMA), a marker of activated fibroblasts, and collagen genes, in addition to TGF-β and TIMP-1. These results suggest that Mincle is involved not only in inflammation but also in fibrosis. Notably, αSMA-positive fibroblasts were found to accumulate around CLS in obese adipose tissue in wild-type mice, whereas they were decreased in Mincle-deficient mice. Furthermore, adipose tissue fibrosis with CLS formation and accumulation of activated fibroblasts was induced when TDM was administered directly to the adipose tissue in lean wild-type mice. These results suggest that the activation of Mincle plays a central role in the induction of adipose tissue fibrosis.

Role of Mincle in Ectopic Lipid Accumulation

It has been reported that there are at least three origins of lipids in the liver; *de novo* lipogenesis, dietary lipids, and lipids from adipose tissue. It has also been demonstrated that more than half of the hepatic lipids originates from the adipose tissue suggesting that the ability of lipids to accumulate in the adipose tissue plays a vital role in hepatic lipid accumulation. Although it has been reported that the balance of lipogenesis and lipolysis in the adipose tissue is tightly regulated by insulin and the sympathetic nervous system, recent studies also suggest a role of chronic inflammation in this balance. For example, inflammatory cytokines can induce insulin resistance as well as directly induce lipolysis, and it has been reported that adipose tissue fibrosis is positively correlated with ectopic lipid accumulation [32-34]. Given that adipose tissue fibrosis was attenuated in Mincle-deficient mice, we examined the hepatic lipid accumulation and found that Mincle-deficient mice showed less hepatic lipid accumulation and lower serum alanine transaminase concentration compared to that of wild-type mice. Specifically, there was no significant difference in body weight between the genotypes; therefore, it is feasible that Mincle may act as a regulator of lipid distribution throughout the body. In addition, Mincle-deficient mice showed better glucose metabolism with increased insulin signaling. These results suggest that Mincle could regulate systemic glucose metabolism by regulating the metabolic function of lipid accumulation in adipose tissue.

Conclusions and Future Perspectives

Many studies have revealed an important role of infiltrating macrophages in obese adipose tissue and have also demonstrated that pathogen sensors, such as TLR4, play an important role not only in innate immunity but also in sterile inflammation. We provided the evidence that Mincle, the pathogen sensor for *Mycobacterium tuberculosis*, is activated in obese adipose tissue, which results in adipose tissue inflammation and fibrosis due to fibroblast activation (Fig. 1). However, the endogenous ligand that activates Mincle is still unknown. Identifying endogenous ligands for pathogen sensors and clarifying their signal pathways will help to develop new understanding and treatment strategies for adipose tissue inflammation and fibrosis.

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Conflict of Interest

The author has no competing interests to declare.

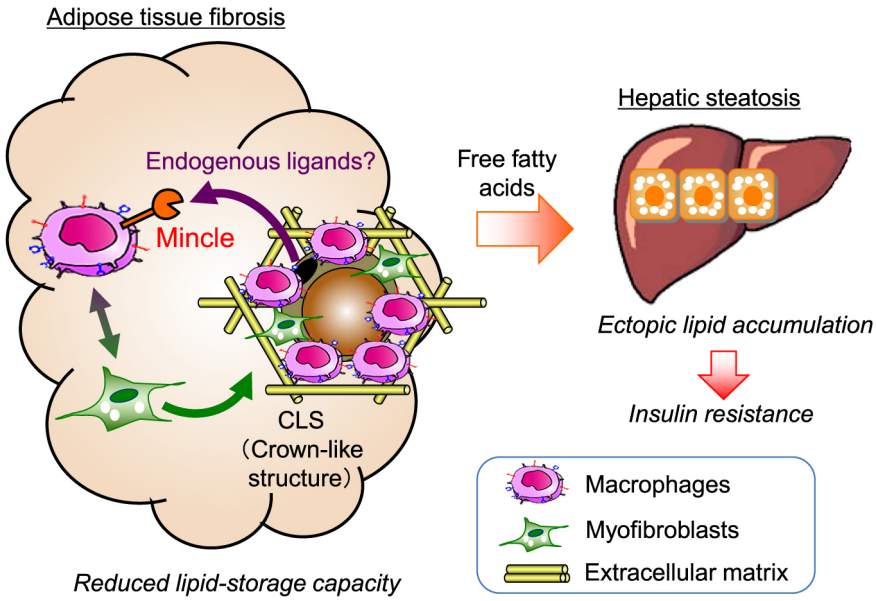

Fig. 1 Potential role of Mincle in obesity-induced adipose tissue inflammation (from Tanaka *et al.* (2014) *Nat Commun* 5: 4982. [26] modified)

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