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Mini Review

Parenchymal-stromal cell interaction in metabolic diseases

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Evidence has suggested that parenchymal-stromal cell interaction is implicated in the development of a variety of metabolic diseases. In obese adipose tissue, saturated fatty acids, which are released as a danger signal from hypertrophied adipocytes, stimulates a pathogen sensor TLR4 in the infiltrating macrophages, thus establishing a vicious cycle that augments adipose tissue inflammation. Histologically, macrophages aggregate to constitute crown-like structures (CLS), where they are thought to scavenge the residual lipid droplets of dead adipocytes. In obese adipose tissue, macrophage-inducible C-type lectin (Mincle) is induced in macrophages constituting CLS, the number of which is correlated with the extent of interstitial fibrosis. Mincle, when activated by an as-yet-unidentified danger signal released from dead or dying adipocytes, may play a key role in adipose tissue inflammation and fibrosis. Free fatty acids, when released from obese visceral fat depots, are transported in large quantities to the liver via the portal vein, where they are accumulated as ectopic fat, thus developing nonalcoholic steatohepatitis (NASH). There is a unique histological feature termed "hepatic CLS (hCLS)" in the NASH liver, where macrophages aggregate to surround dead hepatocytes with large lipid droplets. Notably, the number of hCLS is positively correlated with the extent of liver fibrosis, which suggests that hCLS serves as an origin of hepatic inflammation and fibrosis during the progression from simple steatosis to NASH. We postulate that CLS/hCLS represent the unique microenvironment for parenchymal-stromal cell interaction in metabolic diseases.

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Introduction

Inflammation, particularly, one which persists for a long time or chronic inflammation, plays a critical role in the pathogenesis of multiple chronic diseases such as cardiovascular and metabolic diseases, autoimmune diseases, some of neurodegenerative disorders, and even every stage of cancers; carcinogenesis, invasion, and metastasis[1]. Evidence has accumulated suggesting that chronic inflammation represents a major common molecular basis underlying a variety of non-communicable diseases[1].

Inflammation is an adaptive response to either exogenous or endogenous stimuli. It is classified into acute and chronic inflammation based upon the duration of inflammatory responses[2]. The "acute inflammation" is a short-term and self-limited process characterized by classic signs and symptoms of inflammation; rubor, calor, tumor, and dolor. This may be a physiological adaptive response to tissue injury and is mostly resolved by an active termination program; damaged and/or stressed parenchymal cells are being dead and removed with the aid of stromal cells such as lymphocytes and macrophages, which are thereafter replaced by healthy parenchymal cells through the process of regeneration. However, under pathological conditions, inflammation may persist inappropriately in response to tissue stress or malfunction in a given parenchymal organ, thereby leading to tissue remodeling and irreversible loss of organ function. The molecular mechanism underlying the progression from acute to chronic inflammation is ill-defined, and whether they are totally differentiated is note well established.

Metabolic diseases may be viewed as a state of chronic low-grade inflammation. For instance, obese adipose tissue is characterized by adipocyte hypertrophy, increased angiogenesis and infiltration of immune cells such as macrophages, tissue fibrosis, and increased production of proinflammatory adipokines, which may be referred to as "adipose tissue remodeling"[3]. This review article is to describe the molecular mechanisms underlying the interaction between parenchymal and stromal cells in metabolic tissue; the adipose tissue and liver, and to discuss their pathophysiological and therapeutic implications.

Parenchymal-stromal Cell Interaction in the Adipose Tissue

During the progression of obesity, hypertrophied adipocytes secrete a number of chemokines such as monocyte chemoattractant protein-1 (MCP-1) to stimulate the recruitment of CCR2-positive monocytes into obese adipose tissue as proinflammatory M1 macrophages[4]. Once infiltrated, macrophages may be activated in response to saturated fatty acids released from hypertrophied adipocytes and produce a large amount of proinflammatory cytokines such as tumor necrosis factor α (TNFα), which, in turn, augments inflammatory responses and adipocyte lipolysis to increase the release of fatty acids[5]. It is conceivable that crosstalk between parenchymal cells (adipocytes) and stromal cells (macrophages) establishes a viscous cycle that augments obesity-induced adipose tissue inflammation[5]. Interestingly, saturated fatty acids may serve as a naturally occurring ligand for TLR4 complex, a well-known pathogen sensor expressed on macrophages[6].

As the site of crosstalk between parenchymal and stromal cells in obese adipose tissue, there is a unique histological feature termed "crown-like structures (CLS)", which are composed of dead or dying adipocytes surrounded by macrophages[7]. In CLS, macrophages may scavenge the residual lipid droplets of dying hypertrophied adipocytes. These observations led us to speculate that the macrophage-induced adipocyte lipolysis within CLS accelerates the release of free fatty acids from obese adipose tissue, which may be accumulated in non-adipose tissue as ectopic fat, thus inducing a variety of metabolic effects called lipotoxicity[8].

Evidence has suggested that macrophage-inducible C-type lectin (Mincle) is implicated in adipose tissue remodeling[9]. Mincle, a type II transmembrane Ca^{+}-dependent carbohydrate binding lectin, is expressed abundantly in macrophages, where it is induced in response to lipopolysaccharide (LPS)[10]. It is a *bona fide* pathogen sensor that recognizes the mycobacterial glycolipid trehalose dimycolate (TDM)[11]. More interestingly, Mincle can also recognize an endogenous danger signal that is released from dead cells[12]. During the interaction between adipocytes and macrophages, Mincle is induced in macrophages through the activation of TLR4 by saturated fatty acids released from hypertrophied adipocytes as a danger signal[13]. In obese adipose tissue, Mincle is mostly induced in the stromal-vascular cells. Indeed, Mincle occurs in infiltrating M1 macrophages in obese adipose tissue[13]. Interestingly, Mincle is localized to the macrophages comprising CLS in obese adipose tissue, suggesting its pathophysiologic role in adipose tissue remodeling[9].

Mice with targeted disruption of Mincle (Mincle-KO mice) show increased adipose tissue weight with reciprocal

reduction of liver weight during the high-fat diet (HFD) feeding, although there is no significant difference in body weight between the genotypes throughout the experimental period[9]. The adipocytes are enlarged in Mincle-KO mice relative to wildtype mice[9]. Although there is no significant difference in F4/80 positive macrophage between the genotypes, the number of CLS is significantly reduced in Mincle-KO mice relative to wildtype mice[9]. There is extensive interstitial fibrosis in the epididymal fat depot from wildtype mice, which is markedly attenuated in Mincle-KO mice[9]. Interestingly, hepatic steatosis is markedly reduced in Mincle-KO mice relative to wildtype mice[9]. Accordingly, hepatic triglyceride content and serum ALT concentrations are reduced in Mincle-KO mice[9]. They also show improved glucose metabolism and insulin sensitivity relative to wildtype mice[9].

During the paracrine interaction between adipocytes and macrophages in obese adipose tissue, saturated fatty acids, which are released via the macrophage-induced adipocyte lipolysis, is able to induce Mincle in the infiltrating CLS macrophages through the TLR4/NF-κB pathway[13]. Mincle may recognize as-yet-unidentified endogenous ligands released from dead adipocytes as a danger signal, thereby stimulating adipose tissue fibrosis. In Mincle-KO mice, where adipose tissue fibrosis is attenuated, adipocytes can be enlarged enough to store lipid, which may reduce ectopic fat accumulation in the liver[9]. Accordingly, Mincle plays a key role in the interaction between adipocytes and macrophages within CLS, an origin of adipose tissue inflammation and fibrosis during the course of obesity, thereby reducing lipid-storage capacity in adipose tissue and enhancing ectopic lipid accumulation.

Parenchymal-stromal Cell Interaction in the Liver

Nonalcoholic fatty liver disease (NAFLD) is defined as increased accumulation of lipids in the liver without a history of excess alcohol consumption. It is considered the hepatic manifestation of the metabolic syndrome[14]. Nonalcoholic steatohepatitis (NASH), a progressive form of NAFLD, are closely associated with the development to liver cirrhosis (LC) and hepatocellular carcinoma (HCC). As the pathogenesis of NASH, the well-known "two-hit hypothesis" has been proposed for years, although the detailed mechanism from simple steatosis to NASH is currently unclear[15]. It is partly because of no appropriate animal models that reflect the liver condition of human NASH.

Melanocortin-4 receptor (MC4R), a seven-transmembrane G protein-coupled receptor that is expressed in the hypothalamic nuclei implicated in the regulation of food intake and body weight[16]. We recently reported that MC4R-deficient mice (MC4R-KO mice), when fed HFD, is a novel rodent model of NASH; they develop diet-induced obesity and hepatic steatosis, liver fibrosis, and HCC[17]. Histological analysis revealed hepatocyte ballooning degeneration, infiltration of inflammatory cells, and pericellular fibrosis in MC4R-KO mice fed HFD, all of which are a hallmark of human NASH[17]. Because MC4R expression is relatively restricted to the brain, especially the hypothalamic nuclei, it is conceivable that the brain participates in the development of NASH. Further studies are required to investigate the role of central nervous system in the development of NASH.

MC4R-KO mice show a number of CLSs in the adipose tissue. Interestingly, there are bizarre histological features, which we can call "hepatic crown-like structures (hCLS)"; where macrophages aggregate to surround hepatocytes with large lipid droplets[18]. Although there is no significant difference in the number of macrophages between the genotypes, the number of hCLS is increased in MC4R-KO mice during the progression from simple steatosis to NASH[18]. Accordingly, the number of hCLS is positively correlated with the extent of fibrosis[18]. Importantly, hCLS occurs in patients with NASH or even in those with simple steatosis[18]. Immunofluorescent and electron microscopic analysis revealed that hCLS is composed of CD11c-positive macrophages and dead hepatocytes with large lipid droplets, which is closely associated with activated fibroblasts and collagen deposition[18]. Indeed, eicosapentaenoic acid, a clinically available ω3-polyunsaturated fatty acid, reduces the number of hCLS in parallel with the improvement of liver fibrosis in MC4R-KO mice[19]. These observations suggest the pathophysiological role of hCLS in the development of liver fibrosis.

Increased flow of free fatty acids from visceral adipose tissue to the liver as a result of adipose tissue inflammation and fibrosis induces simple steatosis[17]. In fatty liver, parenchymal hepatocytes which are overloaded with lipid and thus being dead are surrounded by macrophages to form hCLS[18]. In the NASH liver, macrophages can interact with and engulf dead hepatocytes within hCLS, thereafter activating fibrogenic cells to stimulate fibrosis as an adaptive repair response to tissue injury. It is, therefore, likely that hCLS serves as an origin of hepatic inflammation and fibrosis during the progression from simple steatosis to NASH.

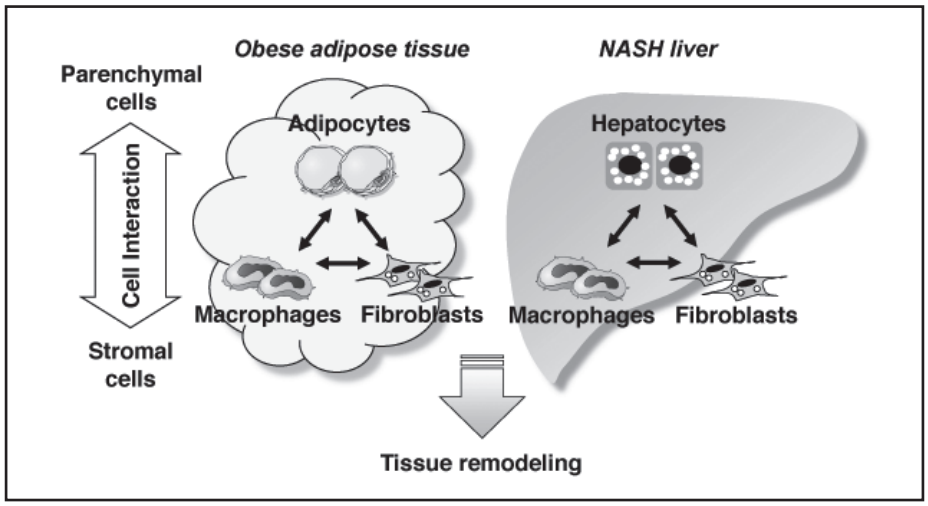

Fig. 1 Role of parenchymal-stromal cell interaction in metabolic diseases

In obese adipose tissue and NASH liver, parenchymal cells; adipocytes and hepatocytes, when overloaded with lipids and thus being dead or dying, may report their dysfunctional state via multiple danger signals or dying messages to the adjacent stromal cells. Such parenchymal-stromal cell interaction constitutes a unique microenvironment, which may be a molecular basis of obesity-induced tissue remodeling in metabolic organs.

Conclusion

In obese adipose tissue and NASH liver, parenchymal cells; adipocytes and hepatocytes, when are overloaded with lipids and thus being dead or dying, may report their dysfunctional state via multiple danger signals or dying messages to the adjacent stromal cells (Fig. 1). It is conceivable that CLS/hCLS serves as an origin of tissue inflammation and fibrosis in response to tissue injury. Mincle is essential for the TDM induced-granuloma formation in the lung[19]. Given the structural and functional similarities between CLS and tuberculous granuloma, it is interesting to speculate that Mincle is involved in the development of tissue fibrosis and, more broadly, tissue remodeling in metabolic diseases. Considering the role Mincle in adipose tissue remodeling, it is also interesting to speculate that Mincle also plays a role in hepatic inflammation and fibrosis in hCLS. Further studies are required to understand the pathophysiologic role of Mincle during the progression from simple steatosis to NASH. By analogy with mycobacterial infection, unhealthy lipids such as saturated fatty acids may act as an infectious agent to disseminate CLS and to propagate the CLS-mediated chronic inflammation and tissue damage from the adipose tissue to multiple metabolic organs. We, therefore, postulate that CLS/hCLS provides the unique microenvironment where dead or dying parenchymal cells and stromal cells crosstalk in close proximity *in vivo*. This discussion highlights the role of parenchymal-stromal cell interaction in metabolic diseases.

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Conflict of interests

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