ADIPOCYTOKINES AND OBESITY-LINKED DISORDERS

ABSTRACT Obesity is closely associated with an increased risk for metabolic and cardiovascular diseases. Adipose tissue produces a number of secretory bioactive substances affecting adjacent or distant organs. Most adipocytokines are pro-inflammatory, thereby promoting the obesity-linked disorders. In contrast, there are a small number of adipocytokines that exhibit antiinflammatory properties. It is now recognized that dysregulated production or secretion of adipocytokines caused by adipocyte dysfunction leads to the development of obesity-linked complications. In this review, we focus on the functional role of several adipocytokines in metabolic and cardiovascular diseases.

INTRODUCTION

The prevalence of obesity has been increasing in the Western countries during the last decades, and obesity has become the major health problems. Obesity is strongly associated with a cluster of type 2 diabetes, hypertension and dyslipidemia, also known as metabolic syndrome, and it is associated with increased cardiovascular mortality and morbidity[1,2]. It is estimated that obesity increases the risk of disease as much as 20 years of aging[3]. Accumulating evidence indicates that obesity contributes to chronic inflammation, thereby leading to the development of insulin resistance and the metabolic syndrome[4-6]. Recent findings indicate that fat tissue is an endocrine organ, which produces and secretes a variety of bioactive substances, referred to as adipocytokines or adipokines[7-9]. Furthermore, it is recognized that dysregulation of adipocytokines caused by dysfunctional adipocytes could contribute to the pathogenesis of various obese complications. This review article focuses on the functional significance of key adipocytokines that display favorable effects on obesity-linked disorders.

ADIPOSE TISSUE AS AN ENDOCRINE ORGAN

Adipose tissue has been considered as an energy storage organ with less attention, but it is now recognized that it functions as an endocrine organ by producing numerous adipocytokines[7,8,10]. Adipsin/compliment factor D was initially identified as an adipocytokine that is primarily produced by adipocytes in 1987[11]. In 1993, tumor necrosis factor-α (TNF-α) was identified as a pro-inflammatory adipocytokine that is potentially involved in the pathogenesis of insulin resistance[12]. In 1994, leptin was identified as a fat-specific adipocytokine that plays an important role in regulation of food intake and energy expenditure[13]. Furthermore, plasminogen activator inhibitor-1 (PAI-1) was identified as an adipocytokine that is highly expressed in adipose tissue[10]. PAI-1 is robustly upregulated in visceral adipose depots during the development of fat accumulation in a rat model of obesity. Plasma levels of PAI-1 positively correlate with visceral adiposity in humans, indicating that PAI-1 can serve as a thrombogenic adipocytokine that potentially contributes to the pathogenesis of obesity-linked thrombotic disorders. About the same time, adiponectin/ACRP30 was found as an adipose-specific adipocytokine[14-16], and its expression is unexpectedly decreased in obesity . Adiponectin is now recognized as a key factor that modulates various diseases usually found in obesity, and this adipocytokine is protective against a number of metabolic and cardiovascular disorders. It has also been shown that several inflammatory mediators including IL-6, IL-18 and MCP-1 induce the metabolic dysfunction[9]. A recent report showed that angiopoietin-like protein 2 (Angptl2) acts as an adipocytokine that promotes inflammation and insulin resistance[17] . Most of adipocytokines such as TNF-α, IL-6 and PAI-1 are upregulated in obese states and promote obesity-inducible metabolic and cardiovascular

diseases . In contrast, there are a smaller number of adipocytokines that exerts beneficial actions on obese complications with anti-inflammatory properties. Thus, it is conceivable that the imbalance in the production of pro-inflammatory and anti-inflammatory adipocytokines under conditions of obesity contributes to the development of obesity-linked disorders. ADIPONECTIN Adiponectin overview Adiponectin is abundantly present in human blood stream, and its plasma levels range 3 to 30 μg/ml, which accounts for approximately 0.01% of total plasma protein[7,18]. Adiponectin is a 244-amino acid protein that contains a putative signal sequence and a collagen-like domain followed by a globular domain similar to collagens VIII and X and compliment factor C1q. It forms trimers through a collagen domain and further combines to make multimeric oligomers. Adiponectin exists in blood stream as three major oligomeric complexes: trimers, hexamers and high-molecular weight form[7,18]. Of importance, plasma adiponectin levels are paradoxically decreased in obese subjects[18]. Large adipocytes, found in obese subjects, produce lower levels of adiponectin but higher levels of pro-inflammatory adipocytokines such as TNF-α, and IL-6, which, in turn, inhibit the production of adiponectin in adipocytes 7, 8. PPAR-ɣ agonists, which promote adipocyte differentiation, increase adiponectin expression in vitro and in vivo[19]. Other factors that negatively regulate adiponectin expression include hypoxia and oxidative stress[20,21]. In addition, low plasma adiponectin levels are closely associated with obesity-linked complications including type 2 diabetes, coronary heart disease and hypertension. Insulin-sensitizing actions of adiponectin Large evidence from experimental models indicates that adiponectin acts as a protective adipocytokine against obesity-linked metabolic dysfunction. Systemic delivery of adiponectin has been shown to reduce hyperglycemia in diabetic mice via enhancement of insulin action[22]. Administration of adiponectin increases fatty acid oxidation in muscle and reduces plasma levels of glucose, free fatty acids and triglycerides[23]. Consistent with these observations, adiponectin-deficient (APN-KO) mice develop severe diet-induced insulin resistance on a high caloric diet[24-26]. Adiponectin promotes insulin sensitivity by its ability to activate AMP-activated protein kinase (AMPK) in skeletal muscle[27,28] and liver[28]. Adenovirus-mediated delivery of adiponectin is reported to enhance AMPK activation in skeletal muscle and increase insulin sensitivity in rats[29]. Adiponectin transgenic mice also show improved insulin sensitivity and increased AMPK activation in liver[30]. Adiponectin is supposed to stimulate AMPK activation through interactions with its cell surface receptors AdipoR1 most abundantly on skeletal muscle and AdipoR2 mainly expressed in liver [31]. AdipoR1-deficiency causes reduced adiponectin-induced . In contrast, AdipoR2-deficiency causes decreased activity of PPAR-α signaling pathways and enhanced insulin resistance. Disruption of both AdipoR1 and AdipoR2 abolishes adiponectin binding and actions, thereby leading to exacerbation of insulin resistance and glucose intolerance. These data suggest that adiponectin acts as an insulin-sensitizing adipocytokine. Regulation of macrophage function by adiponectin Increasing evidence documents that adiponectin modulates macrophage function and phenotype, leading to resolution of inflammation. The accumulation of lipid-laden foam cells and macrophage-related inflammation are key features of atherosclerotic lesion progression. Adiponectin inhibits macrophage-to-foam cell transformation and reduces intracellular cholesteryl ester content in human macrophages by suppressing expression of class A scavenger receptor (SR-A)[33] Adiponectin suppresses lipopolysaccharide (LPS)-stimulated TNF-α production in

macrophages34,35) . Furthermore, globular adiponectin reduces TNF−α production from leptin−stimulated macrophages36). Adiponectin treatment also inhibits Toll−like receptor−mediated NF−ｶB activation in mouse macrophages37). Adiponectin stimulates the production of IL−10, an anti−inflammatory cytokine in porcine macrophages35), and it increases the production of tissue inhibitor of metalloproteinase−1 in human macrophages through its ability to stimulate IL−10 expression38). Adiponectin is structurally similar to the members of collectin family including C1q, surfactant protein A and surfactant protein D34), which are abundantly expressed in serum and form stable multimers like adiponectin39). This collectin proteins promote the rapid removal of apoptotic debris from the body which is critical in preventing inflammation and immune system dysfunction40). We have shown that adiponectin is functionally similar to collectin family proteins. Adiponectin preferentially binds to apoptotic cells and facilitates the phagocytosis of early apoptotic cells by macrophages41) (Figure 2). Of importance, adiponectin promotes the uptake of dead cells by macrophages through interactions with calreticulin and its adaptor protein CD91 on the cell surface. APN−KO mice display the reduced ability of macrophages to clear early apoptotic cells in the peritoneal cavity. Conversely, adiponectin administration promotes the efficient clearance of apoptotic cells by macrophages in both APN−KO and wild−type mice. Adiponectin overexpression also promotes macrophage ingestion of apoptotic cells and reduces features of autoimmunity in lpr mice, which possess a mutation in the Fas gene and exhibit impaired clearance of dying cells, systemic inflammation and lymphadenopathy. Furthermore, adiponectin−deficiency in lpr mice exacerbates chronic inflammatory phenotypes, which are associated with further impairment of apoptotic cell clearance. Taken together, these data suggest that adiponectin protects the organism from systemic inflammation, at least in part, through its ability to promote the phagocytosis of early apoptotic cells by macrophages via a receptor−dependent pathway involving calreticulin/ CD91 co−receptor system . Macrophages that infiltrate into adipose tissue of obese mice predominantly express the genes related to the M1 or "classically activated" macrophage, whereas macrophages from adipose tissue of lean mice express markers of the M2 or "alternatively activated" macrophages42). A recent study has shown that adiponectin can switch the macrophage polarization towards anti−inflammatory phenotype43) (Figure 2). APN−KO mice show increased expression of proinflammatory M1 markers and decreased expression of anti−inflammatory M2 markers in peritoneal macrophages and the stromal vascular fraction (SVF) cells of adipose tissue. Systemic delivery of adiponectin stimulates the expression of M2 markers in peritoneal macrophages and SVF cells in both wild−type and APN−KO mice. Stimulation with recombinant adiponectin protein results in an increase in the levels of M2 markers and a reduction of reactive oxygen species (ROS) generation in cultured macrophages. These data suggest that adiponectin functions as a regulator of macrophage polarization. Phagocytosis of early apoptotic debris by macrophages can favor the M2 phenotype40). Collectively, high levels of adiponectin can confer an anti−inflammatory phenotype in macrophages, thereby protecting against obesity−linked diseases. Vascular protection by adiponectin Increasing evidence from experimental studies indicates that adiponectin is protective against various obesity−linked cardiovascular diseases. Initial observations demonstrate that adiponectin reduces TNF−α−stimulated expression of endothelial adhesion molecules such as vascular cell adhesion molecule−1 (VCAM−1) and

IL-8 in human aortic endothelial cells as well as monocyte attachment to TNF-α-stimulated endothelial cells via suppression of NF-ҡB activation44-46) (Figure 3). The inhibitory effect of adiponectin on NF-ҡB pathway is mediated, at least in part, by its ability to promote signaling through cyclicAMP (cAMP)/protein kinase A (PKA). Similarly, adiponectin attenuates high glucose-induced production of ROS in endothelial cells through a cAMP-PKA-dependent mechanism47). In line with these in vitro findings, adenovirus-mediated overexpression of adiponectin attenuates the atherosclerotic lesion formation and decreases the expression of SR-A, TNF-α and VCAM-1 in the vascular wall in a model of atherosclerosis48,49). These data indicate the ability of adiponectin to attenuate atherogenesis through anti-inflammatory actions on macrophages and vascular endothelial cells. Several studies have shown that adiponectin also exerts beneficial actions on endothelial function. Adiponectin acts as an important regulator of endothelial nitric oxide synthase (eNOS), a key determinant of endothelial cell function. Adiponectin promotes eNOS phosphorylation in endothelial cells through its ability to activate AMPK50,51) (Figure 3). Furthermore, adiponectin stimulates endothelial cell migration and differentiation into capillary-like structures, and prevents apoptosis of endothelial cells through activation of AMPK signaling50,52-54). These in vitro observations were supported by the in vivo findings with mouse genetic models. APN-KO mice show impaired endothelium-dependent vasodilation on an atherogenic diet55) and salt-induced hypertension with reduced eNOS expression in aorta56). Furthermore, adiponectin protects against cerebral ischemia-reperfusion injury through eNOS-dependent mechanisms57). Adiponectin also stimulates ischemia-induced revascularization partly via activation of AMPK signaling58). Of note, caloric restriction promotes revascularization in response to tissue ischemia through an adiponectin-mediated activation of eNOS59). These studies suggest that adiponectin plays a crucial role in retaining vascular tone and function, at least in part, by its ability to promote AMPK/ eNOS signaling pathways A recent report demonstrates that adiponectin promotes ischemia-induced revascularization in muscle through cyclooxygenese-2 (COX-2)-dependent mechanism60). Ablation of COX-2 in an endothelial specific manner in mice results in attenuation of adiponectin-mediated revascularization response in ischemic muscle. Adiponectin stimulates COX-2 expression and its metabolites prostaglandin I2 (PGI2) production, and promotes endothelial cell function via activation of COX-2 signaling pathway within endothelial cells (Figure 3). Importantly, adiponectin promotes the COX-2 regulatory pathways and endothelial cell function via CRT/CD91 co-receptor systems on the surface of endothelial cells60). Therefore, adiponectin can regulate endothelial cell function, at least in part, through COX-2-PGI2-dependent pathway. Taken together, adiponectin regulates vascular homeostasis via at least two regulatory pathways involving AMPK-eNOS and COX-2PGI2 within endothelial cells. Cardioprotection by adiponectin A number of experimental findings have shown that adiponectin exerts beneficial actions on the heart under pathological conditions. Lack of adiponectin results in enhancement of myocardial ischemia-reperfusion injury, which is associated with increased myocardial cell apoptosis and TNF-α production61). Adiponectin stimulates COX-2 expression and prostaglandin E2 (PGE2) synthesis in cardiac cells, thereby preventing LPS-induced secretion of TNF-α from cardiac cells. Adiponectin also inhibits apoptosis of cardiac cells in response to hypoxia-reoxygenation via AMPK-dependent pathways61). Collectively, adiponectin protects against myocardial

ischemiareperfusion injury through COX–2–mediated anti–inflammatory and AMPK–mediated anti–apoptotic mechanisms. Adiponectin also prevents pathological cardiac remodeling following pressure overload or angiotensin II infusion in vivo, in part, through activation of AMPK signaling62,63). Furthermore, adiponectin protects against detrimental cardiac remodeling (e.g. myocardial fibrosis, systolic dysfunction) after myocardial infarction64). APN–KO mice following aldosterone infusion also show increased left ventricular hypertrophy and pulmonary congestion, which are accompanied by severe diastolic dysfunction65). A recent study has demonstrated that adiponectin attenuates doxorubicin–induced cardiotoxicity through modulation of cardiomyocyte survival66). Overall, adiponectin serves as a protective adipocytokine against the development of various obesity–linked heart diseases. SFRP5 Recently we identified secreted frizzled–related protein 5 (Sfrp5) as a novel adipocytokine that exerts salutary effects on metabolic function with anti–inflammatory properties67). Sfrp proteins act as soluble modulators that sequester Wnt proteins in the extracellular space between cells and prevents their binding to receptors68). Sfrp5 is expressed abundantly in white adipose tissue among various adult mouse tissues. Sfrp5 expression is down–regulated in fat tissue of obese rodents such as ob/ob mice, wild–type obese mice after long–term treatment with high caloric diet, and Zucker diabetic fatty rats (Figure 1). Wnt5a protein, which is antagonized by Sfrp5, is upregulated in fat tissues of obese rodents, and the Wnt5a/Sfrp5 protein expression ratio in adipose tissue is also increased in obese states. Sfrp5–knockout (Sfrp5–KO) mice show normal glucose tolerance on a regular diet, but exhibit impaired insulin sensitivity, severe glucose intolerance and severe hepatic steatosis on a high calorie diet compared with control mice67). Sfrp5–deficiency also causes increased accumulation of macrophages in white adipose tissue, which is associated with enhanced production of pro–inflammatory adipocytokines including TNF–α and IL–6. Of note, the signaling of c–Jun N–terminal kinase (JNK), a downstream target of the non–canonical Wnt signaling, is activated in fat tissue in Sfrp5–KO mice on a high calorie diet. A number of in vitro experiments indicate that Sfrp5 reduces Wnt5a–stumulated phosphorylation of JNK in adipocytes and macrophages, and that Sfrp5 blocks Wnt5a–induced production of pro–inflammatory mediators in macrophages. JNK1 plays a key role in regulation of insulin resistance and inflammation69–72). Therefore, Sfrp–5 deficiency exacerbates fat inflammation and insulin resistance under conditions of obesity through enhancement of JNK1 activation in fat tissue. Sfrp5 may represent a potential target for manipulation of obesity–linked metabolic disease73,74). ADIPOLIN More recently we identified C1q domain containing 2 (C1qdc2)/C1q/TNF–related protein 12 (CTRP12) as a novel adipocytokine and designated this adipocytokine as adipolin (adipose–derived insulin–sensitizing factor) to indicate its potential function75). Adipolin belongs to the CTRP family proteins, which are conserved adiponectin paralogs. Adipolin is predominantly expressed in adipocytes, and its expression in adipose tissue and plasma is decreased in mouse models of obesity (Figure 1). Of importance, systemic administration of adipolin to diet–induced obese mice leads to improvement of glucose intolerance and insulin resistance, which is associated with reduced macrophage infiltration and attenuated expression of pro–inflammatory adipocytokines in fat tissue. In cultured macrophages, conditioned media from adipolin–expressing cells suppresses the expression of pro–inflammatory mediators following inflammatory stimuli. These findings suggest that adipolin functions as an anti–inflammatory

adipocytokine that promotes insulin sensitivity, at least in part, through suppression of macrophage activation. Therefore, adipolin can represent a target molecule for the treatment of insulin resistance. CONCLUSION Adipocytokines include pro-inflammatory and anti-inflammatory molecules, and the balance of production of these adipocytokines can be a crucial determinant for homeostasis related to nutritional status. Adipocyte dysfunction induced by obese states contributes to dysregulation of adipocytokine production, leading to the initiation and progression of obesity-induced metabolic and cardiovascular disorders. In particular, the reduced production of the beneficial adipocytokines with anti-inflammatory properties including adiponectin, Sfrp5 and adipolin, causes the development of obese complications. Thus, further elucidation of the function and regulation of adipocytokines will lead to better understanding for the pathogenesis of obesity-linked disorders.