The Role of Angiogenesis in Obesity

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Angiogenesis, the formation of new capillary blood vessels, is a tightly regulated process. Under normal physiological conditions, angiogenesis only takes place during embryonic development, wound healing, and female menstruation. Dysregulation of angiogenesis is associated with many diseases, such as cancer, rheumatoid arthritis, psoriasis, and proliferative retinopathy. The growth and expansion of adipose tissue require the formation of new blood vessels. Adipose tissue is probably the most highly vascularized tissue in the body, as each adipocyte is surrounded by capillaries, and the angiogenic vessels supply nutrients and oxygen to adipocytes. Accumulating evidence shows that capillary endothelial cells communicate with adipocytes via paracrine signaling pathways, extracellular components, and direct cell-cell interactions. Activated adipocytes produce multiple angiogenic factors, including VEGF, FGF-2, leptin, and HGF, which either alone or cooperatively stimulate the expansion and metabolism of adipose tissue by increasing adipose tissue vasculature. Recently, it was demonstrated that antiangiogenic herbal Ob-X extracts and Korean red ginseng extracts reduce adipose tissue mass and suppress obesity by inhibiting angiogenesis in obese mice. Thus, angiogenesis inhibitors provide a promising therapeutic approach for controlling human obesity and related disorders.

Key words : Adipose tissue growth, angiogenesis, angiogenesis inhibitors, obesity, MMP

Introduction

Obesity is the result of an energy imbalance caused by an increased ratio of caloric intake to energy expenditure. In conjunction with obesity, related metabolic disorders such as dyslipidemia, atherosclerosis, and type 2 diabetes have become global health problems. Obesity is characterized by increased adipose tissue mass that results from both increased fat cell number (hyperplasia) and increased fat cell size (hypertrophy) [23]. Development of obesity is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis, and remodeling of extracellular matrix (ECM) [24].

Angiogenesis means the formation of new blood vessels from preexisting vessels (Fig. 1). Angiogenesis is a fundamental requirement for the survival of new tissue in embryonic development as well as for wound healing, placental development, and cyclical changes within the endometrium in the mature adult [30]. However, angiogenesis is also the

underlying pathological process of all major diseases of the developed world. It is a prominent feature of cancer, atherosclerosis, diabetes, rheumatoid arthritis, and proliferative retinopathy [14, 18]. Similarly, the growth and development of adipose tissue require the formation of new blood vessels to provide oxygen and nutrients to adipocytes [13, 63].

Similar to neoplastic tissues, angiogenesis occurs in the growing adipose tissue of adults [24]. Adipose tissue can expand and shrink throughout life, whereas most tissues normally do not grow throughout adulthood, and the supporting vasculature is quiescent [44]. Adipose tissue is highly vascularized, and each adipocyte is nourished by an extensive capillary network [8, 24, 58]. It is suggested, therefore, that growth and expansion of adipose tissue is angiogenesis-dependent and may be inhibited by angiogenesis inhibitors. This is supported by reports that treatment with angiogenesis inhibitors resulted in weight reduction and adipose tissue loss, showing that adipose tissue mass can be regulated by its vasculature [10, 74, 81].

Prominent changes in ECM remodeling have also been observed during adipose tissue growth. Two types of proteolytic systems, the plasminogen/plasmin (fibrinolytic) and matrix metalloproteinase (MMP) systems, have been implicated in tissue remodeling, via degradation of ECM and basement membrane components, or activation of adipocyte growth factors [66, 94]. The MMP system plays important

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Fig. 1. Schematic representation of angiogenesis. Angiogenesis is the formation of new blood vessels from pre-existing vessels by the proliferation and migration of differentiated endothelial cells.

roles in the development of adipose tissue and microvessel maturation by modulating ECM [9, 35, 66]. Increasing evidence suggests that endogenous and exogenous MMPs regulate adipogenesis [9, 21, 52]. During obesity, MMP expression is modulated in adipose tissue, and MMPs (e.g., MMP-2 and MMP-9) potentially affect adipocyte differentiation [9, 19, 71]. More clearly, MMP inhibition impairs development of adipose tissue in mice [66].

Growing adipocytes produce angiogenic factors, such as vascular endothelial growth factor (VEGF)-A and fibroblast growth factor (FGF)-2, contributing to the formation of new blood vessels inside the adipose tissue [13, 22, 95]. VEGF-A and FGF-2 stimulate proliferation and migration of endothelial cells and enhance adipocyte differentiation [7, 16, 52]. Adipose tissue also secretes several MMPs, including MMP-2 and MMP-9 [9]. Indeed, it is well established that degradation of ECM represents the first step in the angiogenic process, and that MMP-2 and MMP-9 have been shown to be necessary for this event [85], indicating the synergistic actions of angiogenesis and the MMP system on the regulation of adipose tissue growth.

Based on my published results showing the actions of anti-angiogenic herbs on obesity, this review will discuss the role of angiogenesis in modulating adipose tissue growth and the regulation of adipose tissue growth by anti-angiogenic agents.

Adipose tissue vasculature

Adipose tissue is primarily a site of fat storage, but also serves as an endocrine gland secreting hormones, angiogenic factors, growth factors, cytokines, and free fatty acids. Development of fat cells is characterized by the appearance of a number of fat cell clusters, or 'primitive fat organs,' which are vascular structures in the adipose tissue with few or no fat cells. Adipose tissue consists of diverse cell populations including preadipocytes, mature adipocytes, adipose stromal cells, endothelial cells, fibroblasts, and in-

flammatory cells.

Adipose tissues exhibit extensive vascularity and each adipocyte is surrounded by an extensive capillary network. The adipose vasculature supplies nutrients and oxygen to growing adipocytes by increasing the size and number of new blood vessels. The vessels also support infiltration of a number of inflammatory cells [80] and remove waste products. In addition to adipocytes, activated endothelial cells produce various growth factors and cytokines, and fenestrated vessels play an essential part in local or systemic effects of adipokines [12]. Accumulating evidence shows that capillary endothelial cells communicate with adipocytes via paracrine signaling pathways, extracellular components, and direct cell-cell interactions [8, 43, 45]. Interestingly, adipocytes and their accompanying endothelial cells might share a common progenitor that could differentiate into adipocytes or endothelial lineages depending upon exposure to different environments [79]. Human adipose tissue - derived stem cells can differentiate into endothelial cells and improve postnatal neovascularization [15]. These findings raise an interesting and exciting possibility that targeting a common adipose progenitor is probably an effective approach for therapeutic intervention of obesity.

Growth and differentiation of adipocytes are spatially and temporally associated with angiogenesis [24]. The growth and development of white adipose tissue requires extensive remodelling of the vascular network, primarily of primitive capillary networks. Expansion of adipose tissue can be supported by both neovascularization (for adipocyte hyperplasia) and dilation and remodeling of existing capillaries (for adipocyte hypertrophy). Brown adipose tissue is largely responsible for energy metabolism, and its function requires efficient blood perfusion to supply nutrients and oxygen and to produce heat. Hyperplasia of brown adipose tissue is critically dependent on angiogenesis, as it requires rapid activation of mitosis in fat precursor cells and endothelial cells to develop capillaries [11].

To adapt to changes in the size and metabolic rate of adipose depots, adipose vasculature requires constant regulation by several angiogenic modulators. Adipocytes seem to regulate angiogenesis both by cell to cell contact and by adipokine secretion [13]. Conditioned media obtained from preadipocytes and tissue homogenates from omentum or subcutaneous fat induce angiogenesis in the chick chorioallantoic membrane and in the mouse cornea [17, 38, 60, 88]. Both white and brown adipose tissue produce several proangiogenic growth factors, such as VEGF-A, FGF-2, and leptin [12] as well as antiangiogenic factors, such as thrombospondin-1 (TSP-1), or other angiogenic modulators including plasminogen activator inhibitor or adiponectin, whose expression ratio will determine the angiogenic phenotype in the adipose tissue [90]. During differentiation of 3T3-F442A pre-adipocytes into mature adipocytes, proangiogenic factors are upregulated, whereas TSP-1 and TSP-2 are transiently downregulated [95]. In addition to adipocytes, other cell types contribute to angiogenesis modulation, including resident macrophages, other inflammatory cells and stromal cells [20].

Maturation of capillary networks and the size of fetal adipose clusters are inversely correlated with the degree of ECM deposition, and the presence of gelatinous protein mixture reduces microvessel and preadipocyte maturation [24, 53]. Adipose tissue produces several MMPs including MMP-2 and -9, which could potentially affect preadipocyte differentiation and microvessel maturation by modulating ECM [9]. Moreover, MMP-9 is able to release the matrix-bound VEGF and indirectly induces angiogenesis [6]. It is suggested that endogenous and exogenous MMPs regulate adipogenesis [9, 21, 53]. In expanding adipose tissue, upregulation of MMP-3, -11, -12, -13, and -14 and downregulation of MMP-7, -9, -16, and -24 have also been found although most of these modulations are specific to gonadal fat depots [21]. Deletion of tissue inhibitor-1 of MMP (TIMP-1), a known angiogenesis inhibitor, leads to reduced obesity in mice fed a high-fat diet [65]. In contrast, significantly higher vessel density and sizes are present in the adipose tissue of TIMP-1 knockout mice compared with control mice.

Blood vessel density in adipose tissue may not truly reflect angiogenic activity. Indeed, a tumor study suggested that the number of cells that can be supported by a blood vessel varies, influencing in turn the vascular density [42]. Similarly, the number and/or size of adipocytes in adipose tissue may affect blood vessel density. This is supported by

a study on capillary fenestrations in adipose tissue, showing that microvessel density was lower in genetically obese ob/ob mice than in wild-type controls, possibly as a result of increased adipocyte size in ob/ob mice [12]. To take this into account, blood vessel density in adipose tissues can be normalized to the adipocyte density. These paradoxical findings might be explained by normalizing blood vessel density with the number of adipocytes [21, 65]. Collectively, these findings demonstrate that MMPs and TIMPs play a pivotal role in controlling adipogenesis via regulation of angiogenesis.

Adipose tissue as an endocrine gland

Adipose tissue is very well developed and is substantial amount of the total body mass (up to 40% of body weight). Adipose tissue is considered as the largest endocrine gland because it produces free fatty acids, hormones, growth factors, and cytokines that either individually or jointly regulate vessel growth.

Adipose tissue-derived angiogenic factors

Growing adipocytes produce a dozen angiogenic factors including leptin, VEGF, FGF-2, hepatocyte growth factor (HGF), insulin-like growth factor (IGF), tumor necrosis factor α (TNF- α), tumor necrosis factor β (TNF- β), placental growth factor (PlGF), VEGF-C, resistin, tissue factor (TF), neuropeptide Y (NPY), heparin-binding epidermal growth factor, angiopoietin (Ang)-1 and Ang-2 [13, 14]. Preadipocytes and adipocytes also produce non-protein small lipid molecules such as monobutyrin that stimulate angiogenesis in the adipose tissue [29, 97]. Adipose-derived stem cells secrete high levels of a number of angiogenic factors including VEGF, HGF, granulocyte macrophage colony-stimulating factor (GM-CSF), FGF-2, and TGF- β [9]. Recruitment of inflammatory cells also significantly contributes to adipose neovascularization. For example, activated macrophages produce potent angiogenic factors such as TNF- α , VEGF, FGF-2, interleukin-1 beta (IL-1 β), IL-6, and IL-8 [95] (Fig. 2).

It is generally accepted that the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) system accounts for most of the angiogenic activity in adipose tissue, making it an attractive target to reduce obesity [33, 39, 96]. Among all adipose tissues examined in the body, omentum expresses the highest level of VEGF. Localization studies have shown that adipocytes are the primary source of VEGF, which may act as an angiogenic

Fig. 2. Regulation of adipose tissue angiogenesis by multiple factors. A variety of cells in the adipose tissue, including preadipocytes, adipocytes, and adipose stromal cells contributes to production of multiple angiogenic factors and inhibitors that regulate adipose tissue angiogenesis.

and vascular survival factor for the omental vasculature. Additionally, adipose-infiltrated inflammatory cells and adipose stromal cells also significantly contribute to VEGF production. VEGF-A (17 - 23 kDa) is a major angiogenic factor that stimulates proliferation and migration of endothelial cells [63]. Three forms of VEGF-A are produced in the mouse as a result of alternative splicing (VEGF-A121, VEGF-A165, and VEGF-A189). Several studies indicate that VEGF-A stimulates both physiological and pathological angiogenesis by signaling through VEGFR-2 in a strict dose-dependent manner. VEGF-B (21 kDa) is 43% identical to VEGF-A165; it also promotes angiogenesis and is implicated in ECM degradation via regulation of plasminogen activation. VEGF-C (23 kDa) displays 30% homology with VEGF-A165 and plays an important role both in angiogenesis and lymph angiogenesis. VEGF-D (22 kDa) is 48% identical to VEGF-C and also promotes the growth of lymphatic vessels.

Another member of the VEGF family, placental growth factor (PlGF, 25 kDa) is 53% identical to VEGF-A165 and enhances angiogenesis, but only in pathological conditions [63]. Loss of PlGF impairs angiogenesis in the ischemic retina, limb, and heart, in wounded skin and in tumors, without affecting physiological angiogenesis. Inactivation of PlGF function in mice leads to impaired adipose tissue development due to defective angiogenesis, suggesting that other VEGF members also modulate adipogenesis via the vascular system [64].

FGF-2 (25 kDa) is a potent stimulator of differentiation,

migration, and proliferation of endothelial cells, and enhances adipocyte differentiation *in vivo*. During angiogenesis, FGF-2 stimulates the synthesis of proteinases such as collagenase and urokinase-type plasminogen activator (u-PA), and of integrins to form new capillary cord structures.

Leptin is an adipocyte-derived hormone that regulates appetite and energy homeostasis. Leptin (16 kDa) deficiency leads to severe obesity, diabetes, and infertility [31]. Interestingly, leptin is also defined as a potent angiogenic factor to promote migration of endothelial cells. Interaction of leptin with its receptor on endothelial cells leads to activation of the Stat3 pathway and enhancement of its DNA-binding activity. Besides a direct pro-angiogenic activity, leptin upregulates VEGF expression via activation of the Jak/Stat3 signaling pathway. Similar to VEGF-A, leptin induces the formation of fenestrated capillaries, as confirmed by the absence of fenestrations in leptin deficient *ob/ob* mice [12]. Leptin has a synergistic effect on stimulation of angiogenesis by VEGF or FGF-2 [12]. Leptin has been shown to induce MMP-2 and MMP-9 activity, which indirectly facilitates angiogenesis [13]. Interestingly, leptin modulates FGF-2- and VEGF-induced vascular activity by synergistically promoting neovascularization *in vivo*. These studies show that leptin also acts as an indirect angiogenic factor or a modulator for other known angiogenic factors.

NPY is a small protein peptides acting as both endocrine and paracrine factors to control adipogenesis and obesity. NPY stimulates angiogenesis *in vitro* and *in vivo* via activa-

tion of its Y2 receptor distributed in vascular endothelial cells, and deletion of the Y2 receptor in mice leads to delayed wound healing [13]. Resistin is an angiogenic factor that is produced in adipose tissue and directly promotes endothelial cell proliferation, migration, and tube formation [73]. IGF-1 and TNF- α are two other upregulated angiogenic factors in expanding adipose tissues. IGF-1 is a survival factor for many cell types and may play an important role in maintenance of vascular integrity in adipose tissue. In addition to its direct angiogenic activity, TNF- α is a potent inflammatory cytokine that links between inflammation, angiogenesis, and adipogenesis. In fact, adipose-infiltrated inflammatory cells produce high levels of proangiogenic cytokines such as TNF- α , IL-1b, IL-6, and IL-8 [96] (Fig. 2). Intriguingly, IL-8 could be a survival factor for adipocytes *in vivo*, probably via stimulation of angiogenesis.

TGF- β and TF are expressed in both adipocytes and stromal cells and increased in adipose tissue of obese mice [84]. TGF- β could positively and negatively regulate angiogenesis depending on the concentration and receptor types expressed in endothelial cells. Preadipocytes and adipocytes produce high levels of HGF, which is an important angiogenic factor for vessel growth and remodeling [83]. Remarkably, Ang-2 as a vascular remodeling factor is consistently upregulated during adipose tissue growth [95]. Other angiogenic factors including VEGF-B, VEGF-C, and angiogenin have also been positively correlated with BMI.

ECM proteolysis is required for cell migration during the development of blood vessels and also for adipose tissue expansion. Several MMPs including MMP-2 and MMP-9 affects microvessel maturation and adipocyte differentiation by modulating ECM [9]. Endogenous and exogenous MMPs regulate adipogenesis [9, 21, 53]. High expression of MMPs was reported in adipose tissue of diet-induced and genetically obese mice, and in obese human adipose tissue [5, 31], whereas TIMP levels are modulated during adipocyte differentiation, and in adipose tissue of obese mice [31]. Cathepsins belong to a family of cysteine proteases that play important roles in human pathobiology, through their proteolytic activity toward extracellular elastins and collagens. Some members, including cathepsins -S, -L, and -K, have been implicated in atherogenesis [41]. Cathepsin B, regulates both pro and anti-angiogenic factors [57] and is secreted by human adipose tissue [64]. Finally, the proteins of the ADAM (a disintegrin and metalloproteinase) and ADAMTS (ADAM with TSP motif) may also contribute to the angio-

genesis and adipogenesis regulation [73].

Adipose tissue-derived angiogenesis inhibitors

Adipose vasculature may be modulated by a net balance between angiogenic factors and their inhibitors, which cooperatively determine growth or regress of the vasculature. Adipose tissue also produces several angiogenesis inhibitors including TSP-1, TIMPs, and adiponectin [13]. In contrast to proangiogenic factors, regulation of adipose vessel growth and remodeling by endogenous angiogenesis inhibitors is relatively poorly understood. Adiponectin accumulates to high levels in the circulation of lean individuals and may protect against diabetes and atherosclerosis. Blood levels of adiponectin have inversely been correlated with BMI and are significantly decreased in obese animals and humans, suggesting its negative role in regulation of adipogenesis. Adiponectin inhibits endothelial cell proliferation, migration, and survival via activation of caspase-triggered endothelial cell apoptosis. *In vivo* it inhibits mouse corneal, CAM, and tumor angiogenesis. However, deletion or overexpression of adiponectin in mice does not seem to affect body weight, suggesting that the adiponectin system might be redundant.

TSP-1 (145 kDa) and TSP-2 (145 kDa) are components of the ECM in remodeling tissues and binds to matrix proteins and cell-surface receptors, including proteoglycans, non-integrin, and integrin receptors [63]. MMP activity is modulated through interactions with TIMPs. TIMP-1, which is synthesized by most types of connective tissue cells as well as macrophages, acts against all members of the collagenase, stromelysin, and gelatinase classes. Analysis of mRNA expression in adipose tissue of lean and obese mice revealed significant upregulation of TIMP-1 with obesity. In contrast, TIMP-4 was downregulated with obesity, whereas TIMP-2 and TIMP-3 expression levels were not significantly modulated, at least in gonadal adipose tissue.

Several reports describe the inexplicable finding that a number of endogenous angiogenesis inhibitors including angiostatin, endostatin, TSP-1, and soluble VEGFR-2 are produced at high levels in overweight and obese subjects [95]. These contradictory findings can be explained by that when the growth rate of an adipose tissue becomes stabilized, high expression levels of angiogenesis inhibitors are required to restrict further vessel growth. In agreement with this hypothesis, TSP-1 expression is downregulated in pre-adipocytes, followed by upregulation in differentiated

adipocytes. High expression of endostatin could be due to the fact that expanding adipose tissue produces an excessive amount of proteases such as MMPs that cleave collagen XVIII into endostatin. Although TSP-1 is a relatively well characterized angiogenesis inhibitor, deletion of the TSP-1 gene in mice did not result in severe vasculature-related abnormalities. Similarly, exposure of TSP-1 knockout mice to a high-calorie diet does not significantly alter body weight or adipose tissue development as compared with control animals. Thus the role of TSP-1 in regulation of adipose angiogenesis needs further to be investigated. Thus, it is possible that a number of endogenous angiogenesis inhibitors are upregulated in order to maintain a homeostatic state of the adipose tissue by counteracting the excessive proangiogenic activity.

Modulation of obesity by angiogenesis inhibitors

Substantial evidence suggests that different angiogenesis inhibitors significantly reduced body weight and adipose tissue mass [81] and newly formed adipose tissue depends on continued angiogenesis for further growth [68], strongly indicating a role of angiogenesis in adipose tissue growth.

Anti-obesity effects of angiogenesis modulators

Several types of angiogenesis inhibitors, such as angiostatin, endostatin, TNP-470 and CKD-732 (TNP-470 analog) inhibited fat mass expansion in mice (Table 1) [10, 54, 63, 81].

It is known that TNP-470 inhibits endothelial cell proliferation in vitro and angiogenesis *in vivo* [72]. TNP-470 also suppresses non-endothelial cell proliferation [46, 59]. TNP-470 suppresses 3T3-L1 preadipocyte proliferation [58]

and significantly reduced body weight in obese *ob/ob* mice [81]. Mice from other obesity models (*A^y*, *Cpe^{fat}*, C57BL/6J mice on a high-fat diet) treated with TNP-470 also weighed less than controls. Aged, relatively weight-stable *ob/ob* mice with negligible adipose endothelial cell proliferation lost weight when treated with TNP-470, whereas controls slightly gained. This suggests adipose vasculature is susceptible to inhibitors, even when not proliferating. CDK-732 also significantly decreased body weight, mesenteric fat pads and the size of adipocytes in arcuate nucleus lesion mice and *ob/ob* mice.

Angiostatin (crinkle 1-4 domains of plasminogen) [77] and endostatin (a C-terminal fragment of collagen XVIII) are endogenous angiogenesis inhibitors that act exclusively on endothelium. Obese mice treated with angiostatin at 20 mg/kg per day gained one-third that of controls, whereas those receiving 50 mg/kg per 12 hr lost weight. Endostatin-treated *ob/ob* mice gained less or lost weight relative to controls. VEGFR2 inhibitors can limit diet-induced fat tissue expansion and adipocyte differentiation during *in vivo* adipogenesis [91, 33].

D'Amato et al. have demonstrated that orally administered thalidomide is an inhibitor of angiogenesis induced by bFGF in a rabbit cornea micropocket assay [26], although thalidomide is a potent teratogen causing dysmelia (stunted limb growth) in humans. *Ob/ob* mice treated with thalidomide gained less or lost weight relative to controls.

Galardin and Bay-129566 are matrix metalloproteinase inhibitors [36]. Galardin significantly reduced the weight of subcutaneous and gonadal fat deposits, but not body weight in high fat diet-fed wild-type mice, suggesting a role of

Table 1. Anti-obesity effects of angiogenesis modulators in animal models

Angiogenesis inhibitor	Mouse model	Body weight	Angiogenesis
TNP-470	High fat diet-fed mice (HFD)	Reduced	Inhibition
CKD-732 (TNP-470 analogue)	<i>Ob/ob</i> mice	Reduced	Inhibition
MMP inhibitors (Galardin and Bay-129566)	HFD	Reduced	Inhibition
Endostatin	<i>Ob/ob</i> mice	Reduced	Inhibition
Thalidomide	<i>Ob/ob</i> mice	Reduced	Inhibition
Angiostatin	<i>Ob/ob</i> mice	Reduced	Inhibition
VEGFR blockade	HFD	Reduced	Inhibition
PLGF blockade	HFD	Reduced	Inhibition
EGCG (catechin in tea)	HFD and <i>ob/ob</i> mice	Reduced	Inhibition
Curcumin (polyphenol)	HFD	Reduced	Inhibition
Adiponectin	<i>Ob/ob</i> mice	Reduced	Stimulation and inhibition
Leptin	<i>Ob/ob</i> mice	Reduced	Stimulation

Adapted from [14].

MMP inhibitors in the development of adipose tissue. Bay-129566 treated *ob/ob* mice gained less or lost weight relative to controls.

The antiangiogenic herbal composition Ob-X reduces adipose tissue mass

The anti-angiogenic and MMP inhibitory activities were examined in water extracts from medicinal herbs and plants which have been used for a long period of time, and three herbal extracts *Melissa officinalis* L. (Labiatae; lemon balm), *Morus alba* L. (Moraceae; white mulberry), and *Artemisia capillaris* Thunb. (Compositae; injin) were selected to make Ob-X [62]. The antiangiogenic herbal extracts Ob-X reduced body weight gain and adipose tissue mass, and markedly modulated the expression of genes involved in angiogenesis and the MMP system in adipose tissue.

Close examination of developing adipose tissue microvasculature revealed that angiogenesis often precedes adipogenesis [24]. The interaction between adipocytes and endothelium is therefore presumed to be involved in the development and maintenance of adipose tissue. Newly formed adipose tissue depends on continued angiogenesis for further growth [68]. It was shown that different angiogenesis inhibitors significantly reduced body weight and adipose tissue mass [81], strongly indicating a role of angiogenesis in adipose tissue growth. Ob-X reduced the formation of new blood vessels induced by the angiogenic factors VEGF and bFGF in a mouse Matrigel plug assay (Fig. 3A). Plugs from Ob-X-treated mice exhibited decreased blood vessel density and hemoglobin contents in a dose-dependent manner. Ob-X inhibited HUVEC tube formation *in vitro* in a dose-dependent manner (Fig. 3B). Ob-X also produced dose-dependent inhibition of VEGF-induced microvessel outgrowth from aortic tissue in the *ex vivo* rat aortic ring assay (Fig. 3C). These results show that Ob-X has the ability to inhibit angiogenesis.

Ob-X showed the inhibition of two major MMP activities (MMP-2 and MMP-9) *in vitro* markedly [55]. MMPs play major roles in the ECM remodeling occurring in a variety of physiological and pathological conditions, such as embryonic growth and development, wound healing, atherosclerosis, and tumor invasion and metastasis. Moreover, adipocytes are surrounded by a basement membrane that has to be extensively remodeled in order to allow the hypertrophic development of adipocytes observed in obesity [78]. MMP-2 and MMP-9 can remodel the ECM of murine and

Fig. 3. Inhibitory effects of Ob-X on angiogenesis. (C) Matrigel plugs containing VEGF plus bFGF (control group) or VEGF plus bFGF plus Ob-X (Ob-X group) were photographed. (B) Tube formation of human endothelial cells. HUVECs were plated in Matrigel-coated wells with varying amounts of Ob-X (25, 50, and 100 µg/ml). After incubation capillary-like tube formation was photographed (original magnification 100×). (C) Microvessel outgrowth arising from rat aortic ring. Aortic rings were embedded in Matrigel, then fed with medium containing various concentrations of Ob-X for 10 days, and photographed (original magnification 12.5×). Adapted from [55].

human adipogenic cells to facilitate the adipogenic process [9, 67], and regulate the bio-availability of adipocyte growth factors sequestered as inactive molecules in the matrix, or blocked by interaction with their binding proteins [82]. These results strongly suggest that Ob-X, which has the ability to inhibit MMP activity as well as angiogenesis, can regulate adipose tissue growth.

Body weight gain and adipose tissue mass of Ob-X-treat-

Table 2. Effects of Ob-X on body weight gain and adipose tissue weight in nutritionally obese mice

	Normal	Control	Ob-X
Body weight (g)	26.9±0.70	30.7±0.71*	28.4±0.62**
Body weight gain (g)	5.61±0.43	9.60±0.54*	7.29±1.14**
VSC fat (g)	1.58±0.11	2.64±0.41*	1.65±0.27**
Epididymal (g)	0.88±0.19	1.62±0.23*	0.95±0.20**
Mesenteric (g)	0.38±0.07	0.61±0.16*	0.35±0.08**
Retroperitoneal (g)	0.32±0.06	0.41±0.11	0.35±0.07
SC fat (g)	0.89±0.09	1.77±0.25*	0.97±0.13**
Brown fat (g)	1.13±0.11	1.38±0.20*	1.20±0.17**

Adult male mice received a low fat (normal group), high fat (control group), or Ob-X-supplemented (0.2% w/w) high fat diet (Ob-X group) for 12 weeks. All values are expressed as the mean ± SD. * $p < 0.05$ compared with normal group. ** $p < 0.05$ compared with control group. VSC, visceral; SC, subcutaneous. Adapted from [55].

ed mice were significantly less than those of untreated mice (Table 2). Ob-X treatment for 12 weeks in high fat diet-fed mice decreased adipose tissue mass by 43%, and this effect was higher than the results showing a 38% reduction after a 12-week administration of galardin, a broad spectrum inhibitor of angiogenesis and MMP [66]. These data are also supported by other results indicating that body weight gain and adipose tissue mass of obese animals were reduced significantly by several kinds of angiogenesis inhibitors [63, 81]. Human studies also showed that 1.5 g of Ob-X per day for a 12-week treatment reduced VSC fat area by 9.5% (from $81.5 \pm 4.40 \text{ cm}^2$ to $73.8 \pm 4.72 \text{ cm}^2$; $p < 0.01$) (unpublished data). In addition to weight reduction, Ob-X inhibited adipocyte hypertrophy in high fat diet-fed obese mice. The size of adipocytes was considerably smaller in Ob-X-treated mice than in controls, eventually resulting in the decreased body weight gain and adipose tissue mass.

Consistent with the inhibitory effects of Ob-X on angiogenesis *in vitro*, blood vessel density of visceral adipose tissue sections from Ob-X-treated mice was much lower than in untreated mice (Fig. 4A). In contrast, the *in vivo* administration of galardin results in a higher blood vessel density in adipose tissue of mice than in untreated control mice [66]. These inconsistent findings can be explained by normalizing blood vessel density with the number of adipocytes [21, 65]. Zymographic analysis revealed that administration of Ob-X suppressed gelatinolytic activity, especially MMP-2 activity, since proMMP-2 activity was markedly reduced in adipose tissues, even though MMP-9 activity was not detectable in our tissue samples. It has also been reported that *in situ* zy-

Fig. 4. Effects of Ob-X on blood vessel density and mRNA expression of genes involved in angiogenesis in adipose tissues of diet-induced obese mice. (A) Histological analysis of the blood vessels in visceral adipose tissue stained with an antibody against von Willebrand Factor. The blood vessels of epididymal white adipose tissue derived from mice fed with a high fat diet (control group) or a high fat diet supplemented with Ob-X (Ob-X group) for 12 weeks were stained and analyzed (original magnification 100×). (B) mRNA expression of angiogenic factors, MMPs, and their inhibitors in adipose tissues of diet-induced obese mice. Adapted from [55].

mography with gelatin-containing gels on cryosections of adipose tissues confirmed a lower MMP activity in tissues of galardin-treated animals. These results indicate that the reduction of adipose tissue mass by Ob-X may be due to its anti-angiogenic and MMP-inhibiting actions.

Several kinds of cells in adipose tissue, including pre-adipocytes, adipocytes, adipose stromal cells, endothelial cells, and inflammatory cells contribute to production of multiple angiogenic factors and inhibitors that regulate adipose angiogenesis. Angiogenic factors, such as VEGF-A and FGF-2, promote the proliferation and differentiation of endothelial cells within fat pad [7, 17, 52], whereas TSP-1 inhibits angiogenesis *in vivo* and impairs migration and proliferation of cultured microvascular endothelial cells [2]. Adipocytes also produce MMPs and MMP inhibitors that are differentially expressed in adipose tissue during obesity in murine obesity models [19, 71, 95]. Interplay between different factors is presumed to be involved in the development and maintenance of adipose tissue. Ob-X administration to high

fat diet-induced obese mice decreased the mRNA expression of VEGF-A and FGF-2 responsible for angiogenesis, whereas it increased mRNA level of the anti-angiogenic TSP-1 in adipose tissues (Fig. 4B). Similarly, Ob-X decreased MMP-2 and MMP-9 mRNA levels, but increased TIMP1 and TIMP-2. These data indicate that Ob-X exerts a specific regulatory effect on genes involved in angiogenesis and the MMP system in adipose tissues. Moreover, inhibition of adipose tissue growth by Ob-X may alter the expression of genes responsible for angiogenesis and the MMP system.

MMPs play important roles in angiogenesis. MMP inhibitors, both synthetic and endogenous, inhibit angiogenic responses both *in vivo* and *in vitro* [3, 40, 50, 90]. Moreover, MMP-deficient mice exhibit delayed or diminished angiogenic responses during development or in response to tumor xenograft [47]. But on the other hand, it was reported that MMP-based proteolysis of the ECM proteins releases cryptic fragments, which are very potent anti-angiogenic substances such as angiostatin and endostatin [76, 77]. High expression of endostatin could be due to the fact that expanding adipose tissue produces a MMPs that cleave collagen XVIII into endostatin, showing that inhibiting MMP activity may decrease endogenous angiogenic inhibitors.

Several studies demonstrated that MMPs have novel function in adipogenesis which modulate adipocyte differentiation independent of angiogenesis and therefore MMP inhibitors can block the adipocyte differentiation process [9, 19, 25, 71]. Ob-X is capable of suppressing adipogenesis and adipocyte-specific gene expression. Ob-X treatment prevented lipid accumulation in 3T3-L1 adipocytes. Consistent with the effects of Ob-X on lipid accumulation, Ob-X decreased the expression of PPAR γ and the PPAR γ target gene aP2, which are directly implicated in lipogenic pathways in 3T3-L1 cells, indicating that Ob-X has an inhibitory effect on adipogenesis. Treatment with MMP inhibitors impairs adipose tissue development in mice fed a high fat diet [44, 66]. Furthermore, the secretion of MMP-2 and MMP-9 increases during adipocyte differentiation in both human adipocytes and mouse preadipocyte cell lines [9, 19, 71]. This suggests that Ob-X can regulate growth and development of adipose tissue by inhibiting MMP activities.

Metabolic changes were accompanied during Ob-X-induced weight loss. Ob-X decreased serum triglyceride and free fatty acid levels. Serum glucose and insulin levels were also decreased by Ob-X in obese mice, which exhibited hyperinsulinemia and hyperglycemia. These results are con-

sistent with our previous study showing that Ob-X treatment to obese mice increases mRNA expression of enzymes for fatty acid β -oxidation [61]. Thus, it is likely that Ob-X may be used to prevent and treat obesity and obesity-related disorders. Since there is a strong association between visceral adiposity and insulin resistance, Ob-X may have an important role in alleviating hyperlipidemia, insulin resistance and diabetes [28, 56].

In conclusion, these studies demonstrate that Ob-X, which inhibits angiogenesis and MMP activity, regulates adipose tissue growth of nutritionally induced obesity and related disorders in mice. These events may influence changes in the expression of genes involved in angiogenesis and the MMP system.

Korean red ginseng prevents obesity by inhibiting angiogenesis

Ginseng is widely used in Asian societies as a valuable medicine. Extensive research indicates that ginseng has many pharmacological effects on the central nervous, endocrine, immune, and cardiovascular systems [1, 37, 69]. Ginseng has also been reported to inhibit tumor growth by modulating MMP-2 and MMP-9 [98, 102], which are regarded as markers of tumor invasion and metastasis, and suppression of their expression may inhibit malignant tumor invasion and metastasis. Ginseng and ginsenosides, its major active components, exhibit potential as potent cancer chemopreventive agents due in part to their downregulation of MMP expression [32, 43, 98, 100, 102]. Based on reports suggesting that the growth and development of adipose tissue are thought to be associated with angiogenesis and ECM remodeling [13, 66, 81], and that ginseng both inhibits angiogenesis and reduces the activity of MMPs [43, 86], its effects on obesity were examined in high fat diet-fed obese mice.

Similar to previous results showing that anti-angiogenic herbal composition Ob-X reduces adipose tissue mass and body weight gain in obese mice [55, 101], body weights and adipose tissue mass were much less in ginseng-treated mice compared with untreated mice (Fig. 5A and B) [61]. Treatment of high fat diet-fed mice with 0.5 and 5% ginseng for 8 weeks decreased adipose tissue mass by 49 and 60%, respectively. These results also provide evidence that adipose tissue growth and development may be prevented by inhibiting angiogenesis. Ginseng also significantly inhibited adipocyte hypertrophy in high fat diet-fed obese mice. The size of adipocytes was considerably smaller in ginseng-

Fig. 5. Regulation of body weight, adipose tissue mass, adipocyte size, and food intake by ginseng in high-fat diet-fed obese mice. Adult male mice ($n=8$ /group) were fed a low fat diet (LFD), a high fat diet (HFD), or HFD supplemented with 0.5 or 5% ginseng for 8 weeks. (A) Body weights at the end of the treatment period are significantly different between the LFD and HFD groups ($p<0.05$) and between the HFD group and those fed HFD supplemented with 0.5 or 5% ginseng ($p<0.05$). (B) Adipose tissue weights. (C) Light microscopic analysis of the size of adipocytes in visceral and subcutaneous adipose tissues. We measured the size of adipocytes in a fixed area ($1,000,000 \mu\text{m}^2$). (D) Food intake. All values are expressed as mean $\pm$ SD. # $p<0.05$ versus LFD group. * $p<0.05$ versus HFD group. Adapted from [61].

treated mice than in untreated obese mice (Fig. 5C), eventually resulting in decreased adipose tissue mass and body weight gain. Visceral obesity due to adipocyte hypertrophy is known to be closely associated with various metabolic syndromes, including insulin resistance, and large adipocytes are associated with insulin resistance, and smaller adipocytes are associated with insulin sensitivity [49, 75]. Therefore, ginseng may alleviate insulin resistance due to its ability to inhibit adipocyte hypertrophy in obese animals.

Weight loss and appetite suppression are common non-specific responses, maybe due to drug toxicity. However, appetite changes were not observed during ginseng-induced weight loss (Fig. 5D), showing a nontoxic mechanism. It was reported that treatment with angiogenesis inhibitors endostatin and low doses of either TNP-470 or angiostatin as well as Ob-X did not change caloric intake [55, 81]. Thus, anti-angiogenic ginseng may selectively target adipose tissue and cause weight reduction because angiogenesis inhibitors target only growing or newly formed, immature vessels.

Inhibition of angiogenesis in growing adipose tissue and weight loss in obese mice are both associated with a reduction in vascular density [14]. Blood vessel density of adipose tissue sections from ginseng-treated mice was much less than that from untreated obese mice (Fig. 6A). This finding is consistent with reports that adipose tissue mass is sensitive to angiogenesis inhibitors and can be regulated by the adipose tissue vasculature [10, 55, 81]. It has been suggested that ginseng exerts anti-angiogenic activities as a potential cancer chemopreventive agent [86]. The active ginsenosides including Rb1 and Rb3 inhibit the early step in angiogenesis and the chemoinvasion of endothelial cells, and they suppress tumor metastasis in part due to inhibition of angiogenesis; the ginsenosides metabolite compound K exerts anti-angiogenic activity by inhibiting the migration and tube formation of endothelial cells [48, 87, 98, 102]. These results suggest that ginseng can reduce adipose tissue mass and body weight through its angiosuppressive actions.

MMPs are essential regulators of various phases of the

angiogenic process, indicating synergistic actions of angiogenesis and MMPs on the regulation of adipose tissue growth. MMPs can influence endothelial cell survival and proliferation by modifying the balance between angiogenic and anti-angiogenic molecules [34]. Both synthetic and endogenous MMP inhibitors inhibit angiogenic responses [90, 92]. Zymographic analyses revealed that administration of ginseng suppressed MMP-2 activity, because proMMP-2 ac-

tivity was markedly reduced in adipose tissues, although MMP-9 activity was not detectable (Fig. 6B). Moreover, MMP-2 activity was even lower in ginseng-treated group than in the low fat diet group, suggesting that other potential mechanisms may also be involved in the ginseng-mediated regulation of obesity. Similarly, ginseng decreases MMP activities; Rg3 inhibits MMP-2 and MMP-9 protein expression, and compound K suppresses MMP-9 protein expression in endothelial cells and human astrogloma cells [48, 51, 98, 102]. Recent studies suggest that MMPs play roles in the tissue remodeling events associated with adipogenesis. These results indicate that the reduction in adipose tissue mass by ginseng may be due to reductions in MMP activities.

Angiogenic factors, such as VEGF-A and FGF-2, promote the proliferation and differentiation of endothelial cells within fat tissue [16, 52], whereas TSP-1 inhibits angiogenesis in vivo and impairs the migration and proliferation of cultured microvascular endothelial cells [2]. Adipocytes also produce MMPs and MMP inhibitors that are differentially expressed in adipose tissue in murine obesity models [9, 19, 71]. Ginseng treatment of high fat diet-induced obese mice decreased VEGF-A and FGF-2 mRNA levels, whereas ginseng increased the mRNA levels of the anti-angiogenic agent TSP-1 in adipose tissues (Fig. 6C). Similarly, ginseng decreased MMP-2 and MMP-9 mRNA levels but increased the levels of TIMP-1 and TIMP-2. These data indicate that ginseng exerts a specific regulatory effect on genes involved in both angiogenesis and MMPs in adipose tissues.

In conclusion, these studies suggest that ginseng may inhibit adipose tissue growth and obesity in nutritionally induced obese mice and that this process may be mediated in part through the inhibition of angiogenesis.

Conclusions

Obesity is a complex metabolic disorder that is deeply associated with type 2 diabetes, dyslipidemia, hypertension, atherosclerosis, stroke, hepatic steatosis, sleep apnea, gallbladder disease, and cancer. Emerging evidence suggests that modulation of angiogenesis seems to have the potential to reduce fat mass and impair the development of obesity by regulating adipose tissue vasculature. Actually, anti-angiogenic agents including herbal extracts Ob-X and ginseng could inhibit obesity as well as hepatic steatosis, dyslipidemia, and hyperglycemia without any toxic effects. Thus angiogenesis inhibitors may be an attractive pharmacological

Fig. 6. Effects of ginseng on blood vessel density, MMP activity, and mRNA expression of genes involved in angiogenesis in adipose tissues of obese mice. (A) Histological analysis of the blood vessels in adipose tissue stained with an antibody against vWF. Adult male mice were fed a low fat diet (LFD), a high fat diet (HFD), or HFD supplemented with 5% ginseng for 8 weeks. (B) Zymographic analysis of adipose tissue. Protein extracts from adipose tissues were applied to a gelatin-containing gel. Gelatinolytic activity was measured by zymography. (C) mRNA expression of angiogenic factors, MMPs, and their inhibitors in adipose tissues in HFD-induced obese mice. All values are expressed as mean $\pm$ SD. $\#p < 0.05$ versus LFD group. $*p < 0.05$ versus HFD group. Adapted from [61].

Fig. 7. Regulation of obesity by angiogenesis inhibitors.

target for the treatment of obesity and related metabolic disorders (Fig. 7).

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초록 : 비만에서의 혈관신생의 역할

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혈관신생은 모든 조직의 성장과 발달, 그리고 상처회복 등에 매우 중요하다. 지방조직은 우리 몸에서 가장 혈관이 발달된 조직으로서 각 지방세포들은 모세혈관에 둘러싸여 있으며 신생혈관들은 지방세포에 영양분과 산소를 공급한다. 혈관의 내피세포들은 파라크린 신호경로, 세포외 성분, 세포들 간의 직접적인 작용을 통해 지방세포와 교류한다. 활성화된 지방세포들은 VEGF, FGF-2, leptin, HGF와 같은 혈관신생인자들을 생성하며, 이들은 단독으로 혹은 협력하여 혈관신생을 증가시키고 지방조직의 성장과 대사를 촉진한다. 따라서 혈관신생 억제제들은 비만과 비만관련 질환을 치료하는데 유용할 것으로 생각된다.