Special Issue: Cutting-edge research exploring mechanisms of tissue homeostasis in health and disease

Review Article

The role of ANGPTL2-induced chronic inflammation in lifestyle diseases and cancer

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Repeated cellular stress due to aging and lifestyle-related activities causes tissue damage. That damage is repaired by a homeostatic process consisting first of acute inflammation and then of adaptive physiologic tissue remodeling meditated by communication between parenchymal and stromal cells. That signaling can occur via cell-to-cell contact or through secreted factors. However, excessive or prolonged stress leads to chronic inflammation and pathologic tissue remodeling, perturbing homeostasis and promoting development of lifestyle-related diseases or cancer. Expression of Angiopoietin-like protein 2 (ANGPTL2) is induced both normally and by disease-associated stresses. In the former, ANGPTL2 promotes proper adaptive inflammation and tissue reconstruction and thus maintains homeostasis; however, in the latter, excess ANGPTL2 activation impairs homeostasis due to chronic inflammation and irreversible tissue remodeling, promoting metabolic and atherosclerotic diseases and some cancers. Thus, it is important to define how ANGPTL2 signaling is regulated in order to understand mechanisms underlying tissue homeostasis and disease development. Here, we focus on ANGPTL2 function in these activities and discuss whether excess ANGPTL2 function is a common molecular mechanism underlying lifestyle diseases and cancer.

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Introduction

Various external and internal stresses due to aging and lifestyle cause tissue damage in organs. Such damage is normally repaired first by acute inflammation and then by physiologic tissue remodeling[1]. These activities are regulated primarily by communication between parenchymal and stromal cells via either direct cell-to-cell contact and/or by paracrine mechanisms mediated by secreted factors[1]. However, excess stress leads to continuous unresolved inflammation and subsequent irreversible tissue remodeling associated with metabolic diseases, such as obesity, glucose or lipid metabolism diseases, atherosclerosis, and even some forms of cancer[2]. Thus prevention of these diseases requires clarification of molecular mechanisms underlying breakdown of normal tissue homeostasis.

The angiopoietin-like protein (ANGPTL) family

The proteins angiopoietin-1, -2, -3 and -4 (ANGPT-1, -2, -3 and -4) exhibit an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain and function as Tie2 ligands (Fig. 1). Tie2 signaling plays an essential role in regulating angiogenesis/lymphangiogenesis and maintaining hematopoietic stem cells (HSCs)[3, 4]. Around 2000, a family of proteins structurally similar to ANGPTs was identified and designated “angiopoietin-like proteins” (ANGPTLs) (Fig. 1B). Like ANGPTs, ANGPTLs 1-7 exhibit an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain (ANGPTL 8/betatrophin lacks the C-terminal fibrinogen-like domain). However, ANGPTLs do not bind to the ANGPT receptor Tie2, indicating that they function differently from ANGPTs[5]. To date, several studies show that most ANGPTLs are potent regulators of angiogenesis, although a subset of ANGPTLs also functions in glucose, lipid, and energy metabolism. For example, ANGPTL3 and ANGPTL4 regulate lipid metabolism by inhibiting lipoprotein lipase activity[6]. The activity of ANGPTL6, also known as “angiopoietin-like growth factor” (AGF), reportedly counteracts obesity by increasing systemic energy expenditure and thus antagonizing related metabolic diseases[7-9]. More recently, ANGPTL8/betatrophin has been shown to function in triglyceride (TG)[10] and glucose metabolism[11].

In several studies, we have reported that normal ANGPTL2 signaling functions in angiogenesis and tissue repair[5, 12-14], while excess ANGPTL2 signaling causes chronic inflammation and irreversible tissue remodeling,

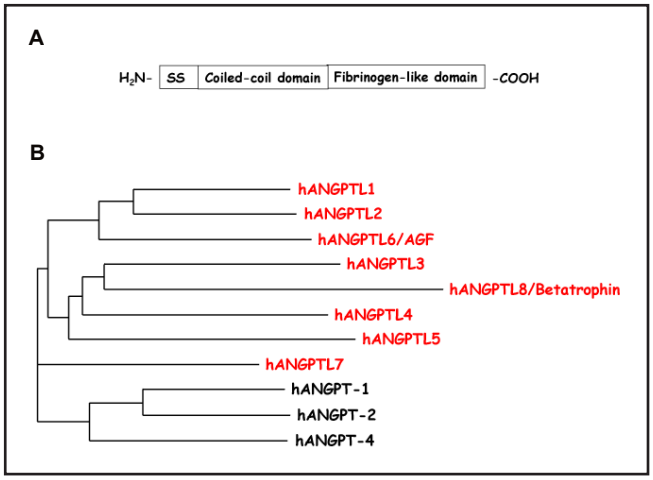

Fig. 1 Characterization of angiopoietin (ANGPT) and angiopoietin-like (ANGPTL) protein families

(A)Classical angiopoietin (ANGPT) and angiopoietin-like (ANGPTL) proteins exhibit an N-terminal signal sequence (SS) plus a coiled-coil domain and (with the exception of ANGTPL-8) a C-terminal fibrinogen-like domain.

(B)Evolutionary relationships of human ANGPT-1, -2 and -4, and human ANGPTL-1, 2, 3, 4, 5, 6, 7, and -8. The length of each horizontal line is proportional to the degree of amino acid sequence identity.

leading to development of obesity, metabolic disease, type 2 diabetes, atherosclerotic disease, and some cancers[12, 14-18]. Thus, excessive ANGPTL2 signaling is a potential common molecular mechanism underlying all of these conditions.

ANGPTL2 function in tissue remodeling

The presence of a C-terminal fibrinogen-like domain suggests that ANGPTL2 binds integrin receptors[19]. We have shown that ANGPTL2 binds to and signals through integrin α5β1[12]. In brief, we reported that via integrin α5β1 ANGPTL2 enhances cell motility by activating the Rho family GTPase Rac1 and increasing degradation of IκB, a factor that inhibits nuclear localization of nuclear factor κB (NF-κB). Thus ANGPTL2 signaling induces expression of inflammation-related NF-κB target genes[12]. Moreover, ANGPTL2 activates extracellular matrix (ECM) remodeling by upregulating and activating p38 mitogen-activated protein kinase (MAPK)-dependent matrix metalloproteinases (MMPs)[18]. Thus, ANGPTL2 signaling through integrin α5β1 increases cell motility, tissue inflammation, and ECM remodeling, resulting in subsequent tissue remodeling (Fig. 2)[14].

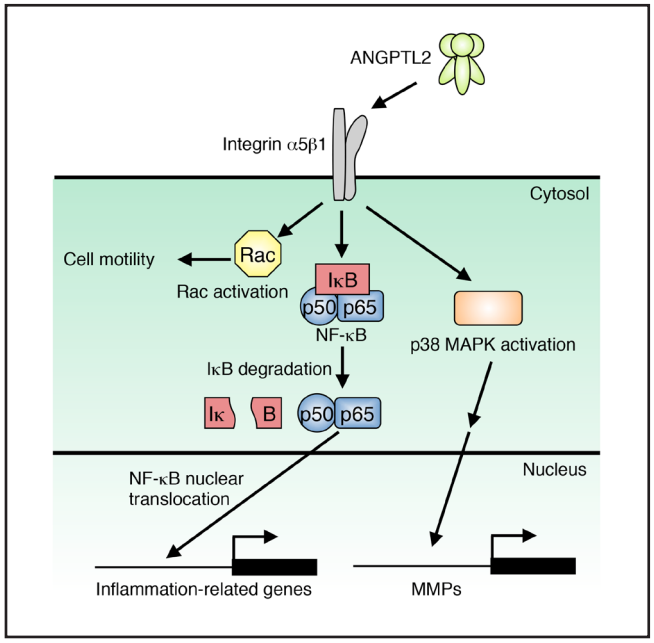

Fig. 2 Signaling downstream of ANGPTL2

Schematic diagram of ANGPTL2/integrin α5β1 signaling[14]. In integrin α5β1-expressing cells, such as endothelial and tumor cells, ANGPTL2 promotes cell motility by activating Rac. ANGPTL2 also induces expression and activity of matrix metalloproteinases (MMPs) via the integrin α5β1/p38 mitogen-activated protein kinase (MAPK) pathway, promoting extracellular matrix (ECM) remodeling and tumor invasivity. ANGPTL2 also induces inflammation-1 related gene expression by promoting IκB degradation and nuclear localization of nuclear factor κB (NF-κB). Figure 2 is a modified reprint of a figure published in our previous paper, Kadomatsu T, et al. Diverse roles of ANGPTL2 in physiology and pathophysiology. Trends Endocrinol Metab. 25: 245-254, 2014.

Physiological roles of ANGPTL2 in adipose tissue and obesity

Adipose tissue consists of adipocytes and stromal cells such as macrophages and endothelial cells. Adipocyte hypertrophy, adipogenesis, angiogenesis, and infiltration by hematopoietic cells occur as adipose tissue undergoes remodeling in early phases of obesity[20]. As obesity develops, MMPs secreted from adipose tissue also play crucial roles in adipose tissue remodeling by promoting ECM remodeling[20]. ANGPTL2 is abundantly expressed in visceral adipose tissues and those levels increase in diet-induced obese mice[12]. As obesity develops, increased adipose tissue-secreted ANGPTL2 contributes to adipose tissue remodeling by promoting angiogenesis, macrophage recruitment, and ECM remodeling in order to store excess lipids into adipocytes (Fig. 3A)[14].

Pathological roles of ANGPTL2 in adipose tissue in obesity and metabolic disease

Mice fed a high-fat diet develop obesity accompanied by chronic adipose tissue inflammation due to vascular inflammation and abundant infiltration of inflammatory macrophages, causing pathologic and irreversible adipose tissue remodeling and leading to systemic insulin resistance[20]. *ANGPTL2* expression levels in visceral adipose tissues increase of these mice. *Angptl2*-deficient mice fed a high-fat diet show decreased chronic adipose tissue inflammation than do wild-type mice, likely because *Angptl2*-deficiency attenuates macrophage infiltration and vascular inflammation[12]. Wild-type mice made obese through a high-fat diet show impaired glucose tolerance and insulin sensitivity, whereas *Angptl2*-deficient mice fed the same diet exhibit better glucose tolerance and insulin sensitivity[12]. Transgenic (Tg) mice expressing *Angptl2* in adipose tissue do not show an obese phenotype when fed a normal diet but do exhibit adipose tissue inflammation with vascular inflammation and increased inflammatory macrophage infiltration, leading to decreased glucose tolerance and increased insulin resistance[12]. These studies suggest that increased adipose tissue-secreted ANGPTL2 in response to excessive food intake is a physiological response to store excess lipid into adipocytes and contributes to adipose tissue remodeling. However, in severe obesity, excess ANGPTL2 signaling leads to irreversible adipose tissue remodeling with chronic inflammation, resulting in metabolic diseases, such as obesity-related insulin resistance or type 2 diabetes (Fig. 3A)[14].

In obese mice, circulating levels of ANGPTL2 increase in parallel with ANGPTL2 expression in visceral adipose tissues and adipose tissue inflammatory status[12]. In a human study, circulating ANGPTL2 concentrations have been positively correlated with systemic insulin resistance in diabetes patients[12]. A 7-year follow-up of an epidemiological study of a general population with no history of diabetes showed that elevated serum ANGPTL2 levels are positively associated with future *de novo* development of type 2 diabetes, independent of other risk factors, including high-sensitivity C-reactive protein (hs-CRP) levels[21]. Moreover, in overweight subjects, decreased serum ANGPTL2 levels reflect positive effects of lifestyle intervention in terms of weight loss and improved metabolic parameters, such as TG and/or insulin activity and the homeostasis model assessment-insulin resistance (HOMA-IR) index[22]. These findings suggest that high circulating ANGPTL2 levels

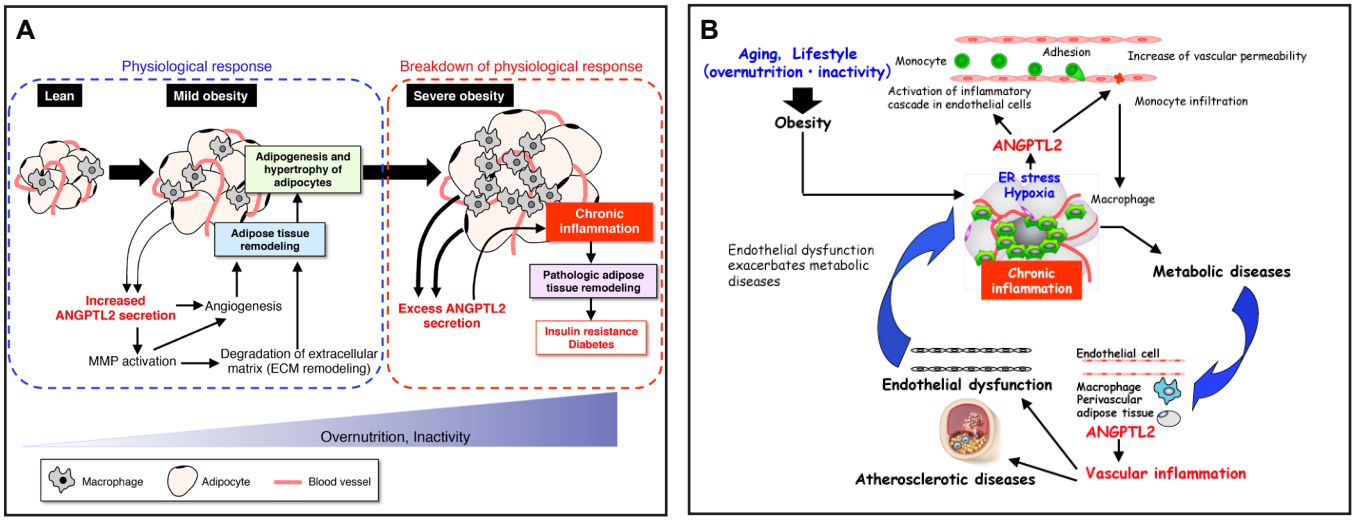

Fig. 3 ANGPTL2 activity in mild and severe obesity and in CVD

(A)*ANGPTL2* expression in adipose tissues increases under obese conditions[14]. In the early phases of obesity (mild obesity), increased ANGPTL2 secretion from adipose tissues promotes MMP activation and induces adipose tissue remodeling. These activities promote angiogenesis and ECM remodeling, leading to adipogenesis and adipocyte hypertrophy. Lifestyle changes, such as overnutrition or inactivity, induce higher adiposity (severe obesity) and result in excess ANGPTL2 secretion and enhanced vascular inflammation and macrophage infiltration into adipose tissue. These conditions lead to chronic inflammation and subsequent pathologic adipose tissue remodeling, resulting in obesity-related insulin resistance and type 2 diabetes.

(B)ANGPTL2 links metabolic disorders seen in obesity to CVD. Perivascular adipose tissue-secreted ANGPTL2 accelerates vascular inflammation, pathologic vascular tissue remodeling and subsequent CVD development. *ANGPTL2* expression in endothelial cells is also abundantly induced by obesity and associated metabolic dysregulation and is a predisposing condition for atherosclerotic disease. Increased endothelial cell-derived ANGPTL2 due to obesity and/or metabolic disturbance promotes vascular inflammation, leading to endothelial dysfunction and atherosclerosis. Figure 3(A) is reprinted by courtesy of Elsevier. Kadomatsu T, et al. Diverse roles of ANGPTL2 in physiology and pathophysiology. Trends Endocrinol Metab. 25:245-254, 2014.

could serve as an indicator of irreversible adipose tissue remodeling with chronic inflammation and predict a risk of *de novo* development of type 2 diabetes.

ANGPTL2-induced chronic inflammation links obesity and associated metabolic disease to atherosclerotic disease

Coronary heart disease (CHD) is the major common form of cardiovascular disease (CVD), and its underlying pathology is atherosclerosis[23]. Recently, investigators have recognized that atherosclerosis progression, including plaque instability, is associated with chronic inflammation in the vessel wall and is a risk factor for major CHD events[24]. Therefore, therapies designed to inhibit chronic inflammation in vessel walls could slow atherosclerosis progression. In addition, chronic adipose tissue inflammation, previously recognized as a leading cause of metabolic disturbance in obesity, is now known to be a predisposing factor for CHD; however, mechanisms linking these conditions have not been identified. Perivascular adipose tissue-secreted pro-inflammatory adipokines, such as TNF-α, contribute to CVD development and enhance vascular remodeling[25, 26]. By contrast, anti-inflammatory adipokines, such as adiponectin, suppress neointimal hyperplasia after endovascular injury.

ANGPTL2 also is expressed in mouse perivascular adipose tissues surrounding the femoral artery at levels equivalent to those seen in visceral adipose tissues[27]. In this context, ANGPTL2 accelerates vascular inflammation, pathologic vascular tissue remodeling and subsequent CVD development[28]. Abundant ANGPTL2 expression in endothelial cells also occurs with obesity and associated metabolic disturbances, and is viewed as a predisposing condition for atherosclerotic disease[15]. Increases in endothelial cell-derived ANGPTL2 expression in these conditions thus promotes vascular inflammation and associated pathologies (Fig. 3B). Moreover, several lines of evidence demonstrate that endothelial cell dysfunction in fact worsens diabetes and obesity by exacerbating insulin resistance and metabolic disturbance[29, 30]. Overall, these findings suggest that ANGPTL2 links metabolic disorders seen in obesity to

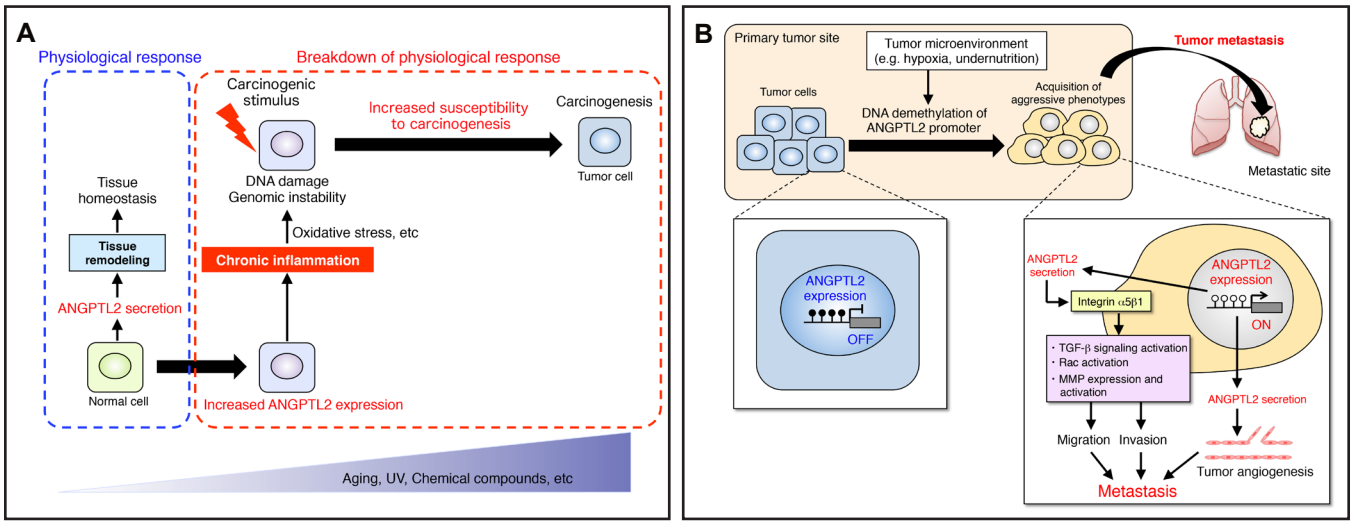

Fig. 4 ANGPTL2 function in cancer

(A)Model proposing linkage of *ANGPTL2* expression to carcinogenesis[14]. Normally, ANGPTL2 secretion promotes tissue remodeling and maintains tissue homeostasis. However, prolonged stresses due to aging or exposure to UV light or chemical compounds increase ANGPTL2 expression and secretion, resulting in chronic inflammation. Chronic inflammation increases cancer susceptibility by inducing oxidative stress, which promotes DNA damage and genomic instability.

(B)Molecular mechanisms underlying ANGPTL2-mediated tumor metastasis[14]. The hypoxic, nutrient-poor tumor microenvironment induces *ANGPTL2* expression in tumor cells by promoting demethylation of the *ANGPTL2* promoter. ANGPTL2 promotes tumor cell migration and angiogenesis by activating Rac in an integrin α5β1-dependent manner. ANGPTL2 also enhances tumor cell invasivity by increasing expression and activity of MMPs via the integrin α5β1/p38 MAPK pathway. Moreover, ANGPTL2 activates transforming growth factor-β (TGF-β) signaling, inducing the epithelial-to-mesenchymal transition associated with metastasis. Open and closed circles indicate unmethylated and methylated CpG dinucleotides, respectively. Figure 4 is reprinted by courtesy of Elsevier. Kadomatsu T, et al. Diverse roles of ANGPTL2 in physiology and pathophysiology. Trends Endocrinol Metab. 25: 245-254, 2014.

CHD (Fig. 3B), suggesting that the ANGPTL2-dependent chronic inflammation axis represents a potential target for developing CHD prevention and treatment strategies.

A 10-year follow-up of an epidemiological study of a general population with no history of CVD showed that elevated serum ANGPTL2 levels were positively associated with future *de novo* development of CVD, independent of other risk factors including hs-CRP levels[31], suggesting that circulating ANGPTL2 concentrations reflect vascular inflammatory status and arteriosclerosis progression in humans. Vascular inflammation, a common pathology underlying atherosclerotic disease, emerges from the interplay of different cell types found in vascular tissue, including endothelial cells, smooth muscle cells, and perivascular adipocytes as resident cells, and macrophages as infiltrating cells[32, 33]. In these conditions, increased ANGPTL2 secretion in vascular tissue accelerates vascular tissue inflammation and pathological remodeling, leading to atherosclerotic disease progression[15, 28, 34, 35]. Further clinical investigation is needed to determine whether reduction of circulating or tissue ANGPTL2 levels would constitute an effective treatment for CVD patients.

ANGPTL2 function in carcinogenesis

Cancer is a major cause of mortality and is increasing world-wide; thus identification of molecular and cellular mechanisms underlying its pathogenesis is critical. Chronic inflammation and pathological tissue remodeling occur at all stages of cancer development, including carcinogenesis, invasion, and metastasis[36]. For example, in skin tissue, sun exposure or aging normally upregulates ANGPTL2 to repair tissue damage by first inducing inflammation and then promoting tissue remodeling[14, 16]. However, repetitive, severe skin damage promotes excessive and prolonged ANGPTL2 induction. Interestingly, unregulated ANGPTL2 signaling epigenetically silences expression of *mutator small subunit homologue 2* (*Msh2*), which encodes a DNA mismatch repair enzyme, thereby increasing genomic microsatellite instability and rates of DNA replication errors[37]. Thus, inappropriate ANGPTL2 signaling causes pathological tissue inflammation and increases carcinogenesis susceptibility through inactivation of DNA repair, an aberration

that likely enables accumulation of oncogenic mutations (Fig. 4A)[14, 16].

ANGPTL2 function in metastasis

Tumor metastasis decreases survival of many cancer patients. The tumor microenvironment, which consists of stromal cells, including immune cells, fibroblasts, and endothelial cells, is a major factor in metastatic activity as it affects tumor cell proliferation, survival, migration, and invasion[37]. A hypoxic microenvironment poor in nutrients such as glucose or amino acids is unfavorable to primary tumor cell growth and survival and thus encourages acquisition of aggressive phenotypes to enhance invasion and metastasis[38, 39]. Interestingly, hypoxia and undernutrition are associated with induction of genes encoding demethylase-related enzymes, resulting in demethylation and subsequent activation of the *ANGPTL2* promoter[18]. Furthermore, *ANGPTL2* expression is increased by activating nuclear factor of activated T-cells, cytoplasmic (NFATc), activating transcription factor 2 (ATF2), and c-Jun[17] – all transcription factors activated by hypoxia, oxidative stress, and endoplasmic reticulum (ER) stress conditions[40] commonly observed in the tumor microenvironment[41]. Thus, *ANGPTL2* expression in tumor cells is increased by both epigenetic modification[18, 42] and microenvironment-dependent transcriptional activation[17] (Fig. 4B)[14]. Increased ANGPTL2 expression in turn promotes tumor cell invasion and angiogenesis in an autocrine/paracrine manner (Fig. 4B)[14]. In mouse xenograft models, tumor cell-derived ANGPTL2 accelerates metastasis and shortens survival periods, while decreasing ANGPTL2 expression in those cells significantly attenuates metastasis and extends survival[17, 18], suggesting that ANGPTL2 suppression could be a potential strategy to decrease tumor metastasis. More recently, it was reported that serum ANGPTL2 levels are associated with pathological progression of some tumor types[43]. Further studies are needed to investigate whether serum ANGPTL2 levels could serve as a biomarker to assess tumor progression and/or metastasis in particular tumor subtypes.

Effects of inhibiting ANGPTL2 biological activity on tumor progression

Culture supernatants of the human embryonic kidney line HEK293 transfected with an ANGPTL2 expression vector contain not only full-length ANGPTL2 protein but also ANGPTL2 cleavage fragments, suggesting that the protein undergoes proteolytic processing[18]. In fact, cleavage of ANGPTL2 by tolloid-like 1 (TLL1), a member of bone morphogenetic protein-1 (BMP-1)/tolloid (TLD) family of proteinases[44, 45], abrogates the ability of the full-length protein to promote tumor progression[18]. On the other hand, cleavage fragments of endogenous ANGPTL2 have not been observed in culture supernatants of tumor cells, whose metastatic activity is accelerated by tumor cell-secreted ANGPTL2[18]. Interestingly, *TLL1* expression levels in tumor cells are extremely low compared with that seen in HEK293 cells, and no mutations in a potential ANGPTL2 cleavage site have been identified in tumor cells, suggesting that extremely low *TLL1* expression may underlie poor ANGPTL2 cleavage in these cells[18]. Moreover, *TLL1* is reportedly silenced by aberrant methylation of its 5'-upstream region in human pancreatic cancers[46], and recent findings reveal that some human pancreatic cancer lines abundantly secrete full-length ANGPTL2[18]. These studies suggest that TLL1 levels in some human tumors are not sufficient to cleave endogenous ANGPTL2. Thus one novel strategy that could be exploited therapeutically to inactivate ANGPTL2 would be to promote its cleavage. These findings also suggest that compounds capable of increasing *TLL1* expression or activity in a primary tumor could serve as anti-metastatic drugs.

Other groups have reported that a single nucleotide polymorphism (SNP) located in *TLL1* intron 12 (a single human *TLL1* variant rs1503298) is positively associated with coronary artery disease (CAD) in patients with type 2 diabetes and CAD[47], however, the molecular mechanisms of relationship between the SNP and CAD development remains unknown. Serum ANGPTL2 significantly increases in patients with type 2 diabetes or CAD[8, 12], and ANGPTL2-associated inflammation and pathologic tissue remodeling contribute to development of these diseases[9]. Taken together, these findings suggest that promoting TLL1-mediated ANGPTL2 cleavage could serve as a novel therapeutic strategy for type 2 diabetes and CAD as well as a way to block tumor progression and metastasis.

Circadian regulation of *ANGPTL2* expression

Several important physiological and behavioral processes exhibit circadian rhythmicity[48, 49]. Periodic expression or secretion of hormones and cytokines is critical to maintain *in vivo* homeostasis[50]. Mice showing disrupted circadian rhythms exhibit metabolic pathologies, such as

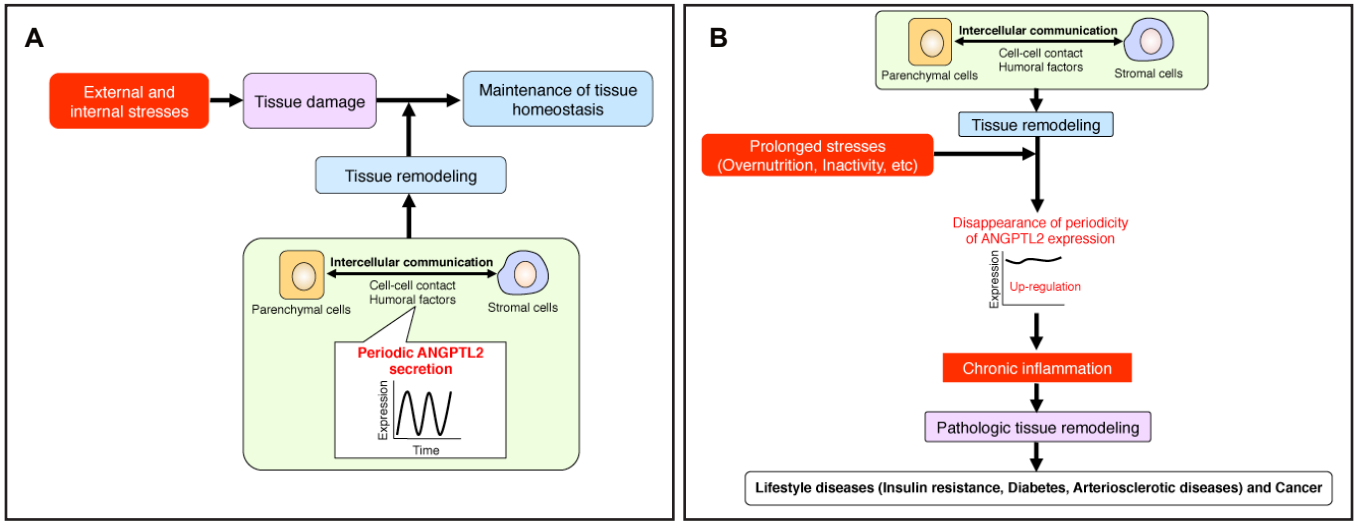

Fig. 5 Models representing potential mechanisms underlying ANGPTL2-mediated tissue homeostasis and development of lifestyle diseases and cancer

(A)Potential roles of ANGPTL2 in maintenance of tissue homeostasis[14]. External and internal stresses due to aging or lifestyle damage tissues, which are normally repaired via tissue remodeling by interactions of parenchymal with stromal cells, either directly or via humoral factors. ANGPTL2 contributes to tissue repair by inducing such remodeling, while its periodic expression or secretion maintains homeostasis.

(B)Loss of *ANGPTL2* periodicity and development of lifestyle diseases and cancer[14]. Periodic *ANGPTL2* expression maintains tissue homeostasis; however, prolonged stresses induce loss of periodicity of *ANGPTL2* transcription. Continuous *ANGPTL2* expression causes chronic inflammation and subsequent pathologic irreversible tissue remodeling, resulting in development and progression of lifestyle diseases and cancer. Figure 5 is a modified reprint of a figure published in our previous paper, Kadomatsu T. et al. Diverse roles of ANGPTL2 in physiology and pathophysiology. Trends Endocrinol Metab. 25: 245-254, 2014.

hypertension, lipid or glucose metabolic disease, or some cancers[51]. The mammalian circadian system is composed of core clock genes that encode proteins such as circadian locomotor output kaput (CLOCK), brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like protein 1 (BMAL1), period (PER), and cryptochrome (CRY)[50, 52, 53]. Both *Angptl2* mRNA and protein, which are widely expressed in many mouse tissues[12], show circadian rhythmicity and have been shown to be regulated by core clock genes in some tissues[54, 55]. Dysregulation of periodic *ANGPTL2* expression in transgenic (Tg) mice constitutively expressing abundant *Angptl2* in adipose, vascular, or skin tissues induces respective tissue chronic inflammation and pathologic tissue remodeling, resulting in development of systemic insulin resistance, vascular dysfunction, or increased susceptibility to carcinogenesis, respectively. These findings suggest that circadian regulation of *ANGPTL2* expression or secretion is required for maintenance of tissue homeostasis *in vivo* (Fig. 5A)[14].

Concluding remarks

In this review, we have focused on diverse ANGPTL2 functions in both normal and pathological conditions. In the former, ANGPTL2 signaling is critical for tissue homeostasis; in the latter, however, excess and prolonged ANGPTL2 signaling leads to chronic inflammation and pathologic tissue remodeling, triggering a breakdown in tissue homeostasis. Circadian regulation of *ANGPTL2* might contribute to maintenance of tissue homeostasis, and dysregulation of *ANGPTL2* expression likely contributes to development and progression of metabolic diseases and even some cancers (Fig. 5B)[14]. Moreover, circulating ANGPTL2 levels may serve as a biomarker of whether tissue homeostasis is proceeding in a physiological or pathological manner. Suppression of excess and prolonged ANGPTL2 signaling might represent a novel and effective therapeutic strategy against metabolic diseases and cancer. In advance of clinical applications, further pre-clinical studies are necessary using patient tissues.

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Conflict of interests

No conflicts of interest to be disclosed.

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