Diverse roles of ANGPTL2 in physiology and pathophysiology

Tsuyoshi Kadomatsu¹, Motoyoshi Endo¹, Keishi Miyata¹, and Yuichi Oike^{1,2}

¹Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan

²Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Chiyoda-ku, Tokyo 102-0075, Japan

Corresponding author: Oike, Y. (oike@gpo.kumamoto-u.ac.jp).

Keywords: angiopoietin-like protein 2; tissue repair; chronic inflammation; tissue remodeling; circadian rhythm; lifestyle disease.

Stresses based on aging and lifestyle can cause tissue damage. Repair of damage by tissue remodeling is often mediated by communications between parenchymal and

stromal cells via cell–cell contact or humoral factors. However, loss of tissue homeostasis leads to chronic inflammation and pathological tissue remodeling. Angiopoietin-like protein 2 (ANGPTL2) maintains tissue homeostasis by promoting adaptive inflammation and subsequent tissue reconstruction, whereas excess ANGPTL2 activation induced by prolonged stress promotes breakdown of tissue homeostasis due to chronic inflammation and irreversible tissue remodeling, promoting development of various metabolic diseases. Thus, it is important to define how ANGPTL2 signaling is regulated in order to understand mechanisms underlying disease development. Here, we focus on ANGPTL2 function in physiology and pathophysiology.

Introduction

External and internal stresses due to aging and lifestyle cause tissue damage in various organs. Such damage is repaired by inflammation and subsequent physiological tissue remodeling, a phenomenon called tissue homeostasis [1]. Intercellular communication between parenchymal and stromal cells via either cell–cell contact or humoral factors is important to maintain tissue homeostasis [1]. However, breakdown in tissue homeostasis due to excess

stress leads to continuous unresolved inflammation and subsequent pathological irreversible tissue remodeling. These conditions promote development and progression of metabolic diseases, such as obesity, diseases of glucose or lipid metabolism, atherosclerotic diseases, and even some forms of cancer [2].

Angiopoietins, such as angiopoietin-1, are secreted proteins that play important roles in angiogenesis and maintenance of hematopoietic stem cells (HSCs) [3]. Recently, a family of proteins structurally similar to angiopoietin and marked by an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain was identified and designated ‘angiopoietin-like proteins’ (ANGPTLs; see Glossary) [4]. Interestingly, ANGPTLs are also secreted but do not bind to either Tie2, which is an angiopoietin receptor, or the related receptor Tie1 [5], suggesting that these orphan ligands function differently from angiopoietins. Several studies show that a subset of ANGPTLs function in glucose, lipid, and energy metabolism, although most ANGPTLs potentially regulate angiogenesis [4]. For example, ANGPTL3 and ANGPTL4 regulate triglyceride (TG) metabolism by inhibiting lipoprotein lipase activity [4,6]. ANGPTL6/angiopoietin-like growth factor (AGF) counteracts obesity by increasing systemic energy expenditure, thereby antagonizing obesity-induced metabolic disease [7,8]. ANGPTL8/betatrophin reportedly functions in TG

[9] and glucose metabolism [10]. Interestingly, normal ANGPTL2 signaling functions in angiogenesis and tissue repair [5,11,12], whereas excess ANGPTL2 signaling causes chronic inflammation and subsequent pathological irreversible tissue remodeling, leading to the development of obesity, metabolic disease, type 2 diabetes, atherosclerotic diseases, and possibly some cancers [11,13–17]. Here, we review ANGPTL2 function in physiology and diseases and discuss whether therapies designed to inhibit excess ANGPTL2 signaling could be devised to prevent or treat lifestyle diseases.

Cellular ANGPTL2 signaling through integrin $\alpha 5\beta 1$

ANGPTL2 enhances cell motility by activating Rac1, a Rho family GTPase, via integrin $\alpha 5\beta 1$ [11]. In addition, ANGPTL2 signaling through integrin $\alpha 5\beta 1$ promotes degradation of I κ B, a factor that inhibits nuclear localization of nuclear factor κ B (NF κ B), thereby inducing expression of inflammation-related NF κ B target genes [11]. It was recently shown that ANGPTL2 increases expression and activity of matrix metalloproteinases (MMPs) through integrin $\alpha 5\beta 1$ -mediated activation of p38 mitogen-activated protein kinase (MAPK) [16], leading to extracellular matrix (ECM) remodeling. Thus, ANGPTL2 signaling through integrin $\alpha 5\beta 1$ increases cell motility, tissue inflammation, and ECM remodeling, resulting in

activation of tissue remodeling (Figure 1).

Roles of ANGPTL2 in angiogenesis in development and disease

In a mouse cornea assay of neovascularization, implanted pellets containing ANGPTL2 markedly induce neovascularization [11]. Furthermore, ANGPTL2 increases migration and chemotactic activity of endothelial cells, such as human umbilical vein endothelial cells (HUVECs) and human coronary artery endothelial cells (HCAECs), by activating Rac1 [11]. These findings suggest that ANGPTL2 is a proangiogenic factor in mammals [5,11]. However, *Angptl2*-deficient mice show grossly normal vascular development [11]. Among mammalian ANGPTLs, ANGPTL1 and ANGPTL2 are highly homologous [12]. Both also cooperate to exert antiapoptotic effects on HUVECs via phosphatidylinositol 3-kinase (PI3-K)/Akt signaling [18], suggesting that they have a complementary function in vascular development during mouse embryogenesis. ANGPTL1 and ANGPTL2 are also highly homologous in zebrafish [12]. Single knockdown of *Angptl2* in zebrafish using morpholino antisense constructs results in fish showing normal vascular phenotypes [18]. However, knockdown of *Angptl1* and *Angptl2* induces severe defects in vascular development partially due to increased apoptosis of endothelial cells [18]. Thus, ANGPTL1 and ANGPTL2

function cooperatively in vascular development in zebrafish and mice.

By contrast, in both chemically induced carcinogenesis models and xenograft tumor models in mice, tumor angiogenesis decreases in *Angptl2*-deficient or *ANGPTL2*-knockdown tumor cells, whereas tumor angiogenesis is accelerated in mice in which *ANGPTL2* is overexpressed in tumor cells [14]. Thus, *ANGPTL2* plays a crucial role in pathological angiogenesis, such as tumor angiogenesis. By contrast, *ANGPTL1*, also known as Angioarrestin, is reportedly downregulated in tumor tissues and suppresses tumor angiogenesis [19,20]. In addition, angiogenesis is not increased in transgenic mice overexpressing *ANGPTL1* [21]. Therefore, *ANGPTL1* does not have an effect on angiogenesis under normal conditions, and in fact *ANGPTL1* and *ANGPTL2* may function competitively in tumor angiogenesis in mammals.

Role of *ANGPTL2* in tissue repair

Zebrafish exhibit a high capacity for tissue regeneration. Fin regeneration, which has been characterized in this model, proceeds through the following steps [22]: epidermal wound healing (~12 h post-amputation), blastema formation (12–24 h post-amputation), regenerative outgrowth (24–48 h post-amputation), and patterning (~48 h post-amputation). Zebrafish

Angptl2 expression is induced abundantly at the second stage in blastema tissue, a mass of undifferentiated progenitor cells functioning as the origin of tissue regeneration [12]. MMPs induce degradation of ECM during not only tissue repair [23] but also physiological processes, such as embryonic development and reproduction [24]. Similar to *Angptl2* induction in blastema tissue, zebrafish *Mmps* are also expressed in the blastema during fin regeneration [25]. Because ANGPTL2 signaling induces MMP expression and activity in mammals [16,26], ANGPTL2 may contribute to fin regeneration through promoting MMP-dependent ECM remodeling. Further studies are necessary to investigate whether ANGPTL2 signaling plays roles in tissue repair in mammals.

ANGPTL2 function in obesity and metabolic diseases

Adipose tissue remodeling, consisting of adipocyte hypertrophy, adipogenesis, angiogenesis, and ECM remodeling, in the early phase of obesity is a physiological response to the need to store excess lipids into adipocytes [27]. As obesity develops, MMPs secreted from adipocytes and stromal cells, such as macrophages, play crucial roles in adipose tissue remodeling by promoting ECM remodeling [27]. ANGPTL2, which increases angiogenesis and macrophage recruitment, is abundantly expressed in visceral adipose tissues and both ANGPTL2 mRNA

levels and circulating protein levels increase in obese mice [11]. Over the course of obesity, excess energy is stored in adipocytes. Thus, ANGPTL2 may contribute to adipose tissue remodeling by promoting angiogenesis, macrophage recruitment, and ECM remodeling (Figure 2).

In high-fat diet-induced obese mice, pathological irreversible adipose tissue remodeling, including vascular inflammation and abundant inflammatory macrophage infiltration, is induced, causing chronic adipose tissue inflammation and leading to systemic insulin resistance [27]. *Angptl2*-deficient mice fed a high-fat diet show fewer infiltrated macrophages and decreased chronic adipose tissue inflammation than do wild type mice [11]. In addition, wild type mice fed 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 [11]. Conversely, transgenic (Tg) mice expressing *Angptl2* in adipose tissue show vascular inflammation and increased inflammatory macrophage infiltration in adipose tissue but are not obese [11]. Furthermore, *Angptl2* Tg mice exhibit decreased glucose tolerance and increased insulin resistance [11]. These findings suggest that ANGPTL2 signaling in response to obesity functions in adipose tissue remodeling to store excess energy into adipocytes. In cases of severe obesity, ANGPTL2 expression is abundant

in adipose tissues and excess ANGPTL2 signaling leads to chronic inflammation, resulting in metabolic diseases, such as obesity-related insulin resistance and type 2 diabetes (Figure 2).

In obese humans, circulating ANGPTL2 concentrations increase and correlate with systemic insulin resistance [11]. A 7-year follow-up of an epidemiological study of a general population with no history of diabetes shows 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 [28]. Moreover, changes in serum ANGPTL2 levels reflect positive effects of lifestyle intervention in terms of weight loss and improved metabolic parameters, such as TG, insulin, and homeostasis model assessment-insulin resistance (HOMA-IR) index in overweight individuals [29]. These findings suggest that alterations in circulating ANGPTL2 levels could serve as a biomarker to assess these disease conditions.

Role of ANGPTL2 in atherosclerotic disease

Coronary heart disease (CHD) is the major common form of cardiovascular disease (CVD), and its underlying pathology is atherosclerosis [30]. It has recently been 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 [31]. Therefore, therapies designed to inhibit chronic inflammation in the vessel wall could slow atherosclerosis progression. Abundant ANGPTL2 expression is observed in atheromatous plaques of CHD patients, particularly in endothelial cells and infiltrated macrophages [13], suggesting that ANGPTL2 secreted from these cells contributes to atherosclerosis progression. Furthermore, ANGPTL2 protein levels in the aorta of *apolipoprotein E* (*ApoE*)-deficient mice are positively correlated with atherosclerosis severity [13]. In addition, *Angptl2* deficiency in *ApoE*-deficient (*ApoE*^{-/-}/*Angptl2*^{-/-}) mice attenuates atherosclerosis severity by decreasing vascular inflammation through decreasing the number of macrophages infiltrating atheromatous plaques [13]. Conversely, *ApoE*-deficient mice crossed with Tg mice expressing *Angptl2* driven by the Tie2 promoter (*ApoE*^{-/-}/Tie2-*Angptl2* Tg), which show increased *Angptl2* expression in endothelial cells but not in differentiated hematopoietic lineage cells including monocytes/macrophages, exhibit increased vascular inflammation and more severe atherosclerosis than do *ApoE*-deficient mice [13]. Tie2-*Angptl2* Tg mice alone do not develop atheromatous plaques but show endothelium-dependent vasodilatory dysfunction (hereafter designated ‘endothelial dysfunction’), probably due to decreased production of endothelial cell derived nitric oxide (NO) [13]. Conversely, *Angptl2*-deficient

mice exhibit less severe endothelial dysfunction than do wild type mice when fed a high-fat diet [13]. Interestingly, in humans aged 85–99 years old with no history of CVD, serum ANGPTL2 levels were found to be positively correlated with vascular inflammatory status, based on estimation of circulating levels of vascular inflammatory markers, such as E-selectin and intercellular adhesion molecule-1 (ICAM-1) [13]. Moreover, serum ANGPTL2 levels were found to be positively correlated with both intimal-medial thickness and the presence of arterial plaques, and that association was maintained even after adjustment for traditional CHD risk factors and other inflammatory mediators including CRP, tumor necrosis factor (TNF)- α , and interleukin (IL)-6 [13]. These findings have potential clinical relevance because they suggest that circulating ANGPTL2 concentrations reflect vascular inflammatory status and progression of arteriosclerosis in humans.

Perivascular adipose tissue-secreted adipokines contribute to CVD development [32]. Anti-inflammatory adipokines, such as adiponectin, reportedly suppressed neointimal hyperplasia after endovascular injury, whereas such vascular remodeling was enhanced by proinflammatory adipokines, such as TNF- α , secreted by perivascular adipose tissue [33]. ANGPTL2 is also a proinflammatory adipokine, which is expressed in mouse perivascular adipose tissues surrounding the femoral artery at levels equivalent to those seen in visceral

adipose tissues [34]. In adipose tissue transplantation experiments, wild type mice with perivascular adipose tissue derived from adipose-specific Tg mice expressing *Angptl2* exhibited accelerated neointimal hyperplasia after endovascular injury relative to wild type mice transplanted with wild type tissue [35]. Conversely, vascular inflammation and neointimal hyperplasia after endovascular injury were significantly attenuated in wild type mice transplanted with *Angptl2*-deficient mouse-derived perivascular adipose tissue, compared with wild type mice transplanted with wild type tissue [35]. Interestingly, *Angptl2* expression in mouse perivascular adipose tissue was significantly increased by aging, hypercholesterolemia, and endovascular injury, all of which are CHD risk factors [35]. Taken together, perivascular adipose tissue-secreted ANGPTL2 accelerates vascular inflammation, pathological vascular tissue remodeling, and subsequent CVD development.

Abdominal aortic aneurysm (AAA) is also characterized pathologically by atherosclerotic changes accompanying chronic inflammation and characterized by infiltrating inflammatory cells, such as macrophages [36]. Infiltrating cells persistently secrete inflammatory cytokines and chemokines, resulting in chronic inflammation of the aortic wall [37]. Furthermore, infiltrating macrophages in aortic aneurysmal vessel walls release MMPs, particularly MMP-9 [38], resulting in ECM degradation and AAA progression attributable to

destructive remodeling of elastic media and of the outer aortic wall. ANGPTL2 is abundantly expressed in infiltrating macrophages within the vessel wall in a CaCl₂-induced AAA mouse model and in patients with AAA [26]. In the mouse model, *Angptl2*-deficient animals show decreased AAA development compared with wild type mice, as evidenced by reduced aneurysmal size, less severe destruction of vessel structure, and lower expression of proinflammatory cytokines and *Mmp-9* [26]. Wild type mice that undergo *Angptl2*-deficient bone marrow transplantation show significantly suppressed AAA development compared with mice transplanted with wild type marrow [26]. Furthermore, expression levels of proinflammatory cytokines and *Mmp-9* in *Angptl2*-deficient macrophages were significantly decreased compared with macrophages derived from wild type mice [26]. These results suggest that macrophage-derived ANGPTL2 contributes to AAA development by promoting pathological tissue remodeling in the vessel wall via continuous inflammation and MMP activation.

Vascular inflammation, a common pathology for various atherosclerotic diseases, emerges from the interplay of different cell types in vascular tissue structures, including endothelial cells, smooth muscle cells, and perivascular adipocytes as resident cells, and macrophages as infiltrating cells. In these conditions, vessel wall cells cooperate to produce

ANGPTL2, which accelerates vascular tissue inflammation and pathological remodeling, leading to atherosclerotic disease progression [13,26,35,39]. Further clinical investigation is needed to determine whether reduction of circulating or tissue ANGPTL2 levels would constitute an effective treatment for CVD patients.

Roles of ANGPTL2 in carcinogenesis and metastasis

Cancer is an increasingly prevalent medical and social problem and remains a major cause of mortality. Therefore, the identification of molecular and cellular mechanisms underlying its pathogenesis is essential to develop new therapeutic and preventive approaches. Recently, the concept that chronic inflammation and tissue remodeling play an important role at different stages of cancer development, including carcinogenesis, invasion, and metastasis, has been the focus of much attention [40].

Sunlight exposure and aging promote severe skin tissue damage and increase susceptibility to skin carcinogenesis [41]. ANGPTL2 expression is induced in skin tissues under these conditions [14]. Prolonged or excessive induction of ANGPTL2 expression in skin tissue of Tg mice expressing *Angptl2* in that tissue induces chronic skin tissue inflammation [14]. Furthermore Tg mice exhibit increased preneoplastic changes and

malignant conversion rates, which increase cancer susceptibility, in a chemically induced skin squamous cell carcinoma (SCC) mouse model [14]. Conversely, in this model, *Angptl2*-deficient mice show suppressed preneoplastic changes and decreased malignant conversion rates compared with wild type mice [14]. Thus, abundant ANGPTL2 expression is highly correlated with frequency of carcinogenesis. Interestingly, compared with wild type mice, Tg mice in this model show significantly decreased expression of *mutator small subunit homologue 2* (*Msh2*), which encodes a DNA mismatch repair enzyme, due to epigenetic silencing of the gene, and increased genomic microsatellite instability and rates of DNA replication errors, leading to accumulation of oncogenic DNA mutations and increased cancer susceptibility [42]. In skin tissue damage by sun exposure or aging, ANGPTL2 is induced to repair damage by tissue inflammation and subsequent tissue remodeling. However, when skin tissue damage is severe due to repetitive or continuous stresses, excess ANGPTL2 induction occurs, increasing carcinogenesis susceptibility due to accumulation of oncogenic DNA mutations (Figure 3A).

Tumor metastasis is the most common cause of death in cancer patients. The tumor microenvironment consists of stromal cells, including immune cells, fibroblasts, and endothelial cells, all of which contribute to tumor cell proliferation, survival, migration, and

invasion [43]. Loss of oxygen or nutrients such as glucose or amino acids occurs as a primary tumor mass expands, creating a microenvironment unfavorable to tumor cell growth and survival at the primary site [44] and encouraging acquisition of aggressive phenotypes to enhance invasion and metastasis [45]. It was recently reported that *ANGPTL2* expression is significantly increased in human osteosarcoma (OS) cells implanted into the tibia of immunodeficient mice relative to that seen prior to implantation [16]. It was concluded that tumor microenvironmental conditions, such as hypoxia and undernutrition, induce expression of genes encoding demethylase-related enzymes, resulting in demethylation of the *ANGPTL2* promoter [16]. *ANGPTL2* is expressed in human cancer cells, including lung and breast cancers, and that expression is induced by activating nuclear factor of activated T cells, cytoplasmic (NFATc), activating transcription factor 2 (ATF2), and c-Jun [15], transcription factors activated by hypoxia, oxidative stress, and endoplasmic reticulum (ER) stress conditions [46]. All of these conditions are commonly observed in the tumor microenvironment [43]. Thus, *ANGPTL2* expression in tumor cells can be increased by both epigenetic modification [16,47] and transcriptional activation [15] induced by the tumor microenvironment (Figure 3B).

Using tumor cell implanted mouse models, it was demonstrated that tumor cell

derived ANGPTL2 accelerates metastasis and shortens survival periods but that decreasing ANGPTL2 expression in tumor cells significantly attenuates metastasis and extends survival [15,16]. Furthermore, these experiments indicated that tumor cell derived ANGPTL2 increases tumor cell motility and angiogenesis through the integrin $\alpha 5 \beta 1$ –Rac pathway [15]. Recently, it was also reported that tumor cell derived ANGPTL2 enhances tumor cell invasion and tumor cell intravasation of blood vessels at primary tumor sites by increasing expression and activity of MMPs, such as MMP-1, MMP-9, and MMP-13, in tumor cells via the integrin $\alpha 5 \beta 1$ /p38 MAPK pathway [16]. Moreover, ANGPTL2 promotes the epithelial-to-mesenchymal transition (EMT) via activating the transforming growth factor- β (TGF- β)–Smad pathway in a chemically induced mouse model of SCC expressing ANGPTL2 [14]. Overall, tumor cell derived ANGPTL2, which is increased by the tumor microenvironment, accelerates tumor metastasis through promoting tumor cell motility, tumor cell invasion, and tumor angiogenesis in an autocrine/paracrine manner (Figure 3B).

More recently, it was reported that serum ANGPTL2 levels in patients with metastatic breast cancer are significantly higher than those in healthy individuals or in patients with ductal carcinoma *in situ* or non-metastatic invasive ductal carcinoma [48]. This finding raises a possibility that serum ANGPTL2 levels in breast cancer patients could be a

potential marker of breast cancer metastasis. Further studies are needed to investigate whether serum ANGPTL2 levels could serve as a biomarker to assess metastasis in other types of cancer.

Blocking excess ANGPTL2 signaling as a potential therapeutic strategy

Recent results have shown that a cleaved form of ANGPTL2 lacks the functional capacity of its full-length counterpart to promote tumor progression [16]. It was found that full-length ANGPTL2 secreted from HEK293 cells was cleaved extracellularly by tolloid-like 1 (TLL1) [16], which is a member of the bone morphogenetic protein-1 (BMP-1)/tolloid (TLD) family of proteinases [49,50] and is essential for heart development [51]. By contrast, cleavage of endogenous ANGPTL2 was not observed in several types of OS cell cultures, although no mutations in the ANGPTL2 cleavage site have been identified in these cell lines [16], suggesting that there are no mutations in the ANGPTL2 cleavage site in many types of OS tumors. *TLL1* expression in HEK293 cells is abundant, whereas it is markedly lower in OS cell lines and OS patient samples [16], suggesting that levels of TLL1 in human OS tumors may not be sufficient to cleave endogenous ANGPTL2. Thus, ANGPTL2 cleavage could be a novel therapeutic strategy to delay metastasis. *TLL1* is reportedly silenced by aberrant

methylation of its 5' upstream region in human pancreatic cancers [52], and recent findings revealed that some human pancreatic cancer cell lines abundantly secrete only full-length ANGPTL2 [16]. These findings suggest that a compound that could increase *TLL1* expression or activity in a primary tumor could serve as a novel therapeutic strategy to delay metastasis in human cancers.

Other groups have reported that a single variant (rs1503298) in human *TLL1* is positively associated with coronary artery disease (CAD), specifically in patients with type 2 diabetes and CAD [53]. This single nucleotide polymorphism (SNP) is located in *TLL1* intron 12 [53], suggesting that it alters *TLL1* transcriptional regulation. Serum ANGPTL2 was significantly increased in patients with type 2 diabetes or CAD [11], and ANGPTL2-associated inflammation and pathological tissue remodeling contributed to the development of these diseases [54]. Taken together, these findings suggest that promoting TLL1-mediated ANGPTL2 cleavage could serve as a novel therapeutic strategy for type 2 diabetes and CAD.

Physiological significance of circadian regulation of *ANGPTL2* expression

Various physiological and behavioral processes exhibit circadian rhythmicity [55,56]. The

molecular machinery underlying the generation of these rhythms requires a transcriptional/translational feedback loop in the suprachiasmatic nucleus (SCN) of the hypothalamus, and in most types of cells in peripheral tissues [57]. The mammalian circadian system is composed of a set 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) [57–59]. In mice, targeted or spontaneous mutations of core genes and resulting disruption of circadian rhythmicity promotes conditions reminiscent of metabolic dysfunction, such as hypertension, lipid or glucose metabolic disease, or of some cancers [60]. *Angptl2* mRNA and protein, which is widely expressed in many mouse tissues [11], shows circadian rhythmicity in some of these tissues [61,62], similar to that of *PER2*, which is regulated by the CLOCK/BMAL1 complex [62]. Activity of the human *ANGPTL2* promoter is significantly induced by CLOCK and BMAL1, an induction markedly attenuated by CRY coexpression [62]. Dysregulation of periodic *ANGPTL2* expression in Tg mice constitutively expressing abundant *Angptl2* in adipose tissue induces adipose tissue inflammation, pathological adipose tissue remodeling, and subsequent systemic insulin resistance [11]. Moreover, as noted, continuous *ANGPTL2* expression in skin tissue of Tg mice constitutively

expressing *Angptl2* in that tissue induces inflammation and increased susceptibility to skin carcinogenesis [11]. Periodic expression or secretion of hormones and cytokines is also important to maintain *in vivo* homeostasis [57]. Taken together, these findings suggest that circadian regulation of *ANGPTL2* expression or secretion contributes to maintenance of tissue homeostasis *in vivo*, potentially due to communication between parenchymal and stromal cells (Figure 4A).

The function of ANGPTL2 signaling independent of integrin $\alpha 5 \beta 1$

Zheng *et al.* reported that the immune-inhibitory human leukocyte immunoglobulin-like receptor B2 (LILRB2) is a receptor for ANGPTL2 and ANGPTL5, and that signaling through that receptor contributes to maintenance of HSC stemness and to leukemia development [63]. ANGPTL5 enhances calmodulin-dependent protein kinase (CAMK) phosphorylation in human cord blood mononuclear cells and increases association of the Src homology region 2-containing protein tyrosine phosphatase (SHP) with the paired immunoglobulin-like receptor B (PirB) [64], an LILRB2 mouse ortholog [65], in mouse HSCs [66]. These findings suggest that ANGPTL2 also contributes to maintenance of HSC stemness or possibly plays some role in leukemia development via these signaling pathways

(Figure 1). Interestingly, LILRB2 is reportedly expressed on immune cells, such as monocytes, macrophages, and dendritic cells, and functions in the immune response [67]. Because these cells function not only in host defense but also in both physiological and pathological tissue remodeling [1], ANGPTL2 signaling through LILRB2 may contribute to regulation of both the immune response and tissue remodeling. Recent studies showed that LILRB2 is not expressed on epithelial or mesenchymal cells such as endothelial and tumor cells, whereas these cells express integrin $\alpha 5\beta 1$ [13,16] through which ANGPTL2 signaling is transduced. Thus, ANGPTL2 may exert different activities depending on which receptors are expressed by target cells. Further studies are necessary to examine whether ANGPTL2 plays roles in the immune response and tissue remodeling via LILRB2 and characterizes its downstream signaling.

The role of ANGPTL2 as a cytoplasmic protein

ANGPTL2, which has also been called ARAP1, has been characterized as a protein that interacts with the angiotensin II type 1 (AT1) receptor in the cytoplasm [68]. *In vitro* and *in vivo* studies demonstrate that ARAP1/ANGPTL2 increases AT1 receptor recycling, thereby facilitating its surface expression and acting as a positive modulator of vascular AT1

receptors [68]. During endotoxemia, *Arap1/Angptl2* expression is markedly downregulated and sepsis-induced circulatory failure is aggravated in *Arap1/Angptl2*-deficient mice [69]. Loss of ARAP1/ANGPTL2 function in endotoxemia may therefore contribute to vascular hyporeactivity to angiotensin II in septic patients. Thus, ANGPTL2 functions not only as a secreted protein but also as a cytoplasmic protein.

Concluding remarks

In this review, we have focused on diverse functions of ANGPTL2 in physiological conditions and diseases. ANGPTL2 signaling plays roles in tissue homeostasis in physiological conditions, and excess ANGPTL2 signaling leads to chronic inflammation and pathological tissue remodeling, the so-called breakdown in tissue homeostasis. We propose that circadian regulation of *ANGPTL2* expression might contribute to maintenance of tissue homeostasis and that dysregulation of its expression is a contributing factor to the development and progression of metabolic diseases (Figure 4B). Moreover, circulating ANGPTL2 levels may serve as a biomarker that indicates whether tissue homeostasis is a physiological or pathological state.

Suppression of excess ANGPTL2 signaling could represent novel and effective

therapeutic strategies against metabolic diseases and cancer. Recent findings suggest that full-length ANGPTL2 cleavage by TLL1 could be a potential strategy to suppress excess ANGPTL2 signaling [16]. Similar to ANGPTL2, ANGPTL3 and ANGPTL4 are also cleaved into an N-terminal fragment and a C-terminal fragment [70,71]. Interestingly, N-terminal fragments of ANGPTL3 and ANGPTL4 alone alter lipid metabolism [70,71]. However, because it remains to be determined whether the truncated form of ANGPTL2 shows biological activity, further investigation is needed. In addition, recent findings suggest that intercellular communication via integrin $\alpha 5 \beta 1$ - or LILRB2-mediated ANGPTL2 signaling is important to maintain tissue homeostasis. To clarify the significance of intercellular communication via ANGPTL2 signaling in tissue homeostasis, it is necessary to investigate what type of cells express ANGPTL2 and/or its receptors and how integrin $\alpha 5 \beta 1$ - and LILRB2-mediated ANGPTL2 signaling are regulated in ANGPTL2 target cells. In advance of clinical applications, further studies are necessary to investigate the physiological functions of ANGPTL2 in maintenance of tissue homeostasis and molecular mechanisms underlying *ANGPTL2* dysregulation under pathological conditions. Outstanding questions for further research in the field are presented in Box 1.

Acknowledgments

This work was supported by the Japan Society for the Promotion of Science (JSPS) through the ‘Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)’ (LS098) initiated by the Council for Science and Technology Policy (CSTP). This work was also supported by the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Agency (JST) and by JSPS KAKENHI grant numbers 25870543, 25461192, and 25461114.

References

- 1 Medzhitov, R. (2008) Origin and physiological roles of inflammation. *Nature* 454, 428–435
- 2 Handschin, C. and Spiegelman, B.M. (2008) The role of exercise and PGC1 α in inflammation and chronic disease. *Nature* 454, 463–469
- 3 Yancopoulos, G.D. *et al.* (2000) Vascular-specific growth factors and blood vessel formation. *Nature* 407, 242–248
- 4 Hato, T. *et al.* (2008) The role of angiopoietin-like proteins in angiogenesis and metabolism. *Trends Cardiovasc. Med.* 18, 6–14

- 5 Kim, I. *et al.* (1999) Molecular cloning, expression, and characterization of angiopoietin-related protein induces endothelial cell sprouting. *J. Biol. Chem.* 274, 26523–26528
- 6 Mattijssen, F. and Kersten, S. (2012) Regulation of triglyceride metabolism by angiopoietin-like proteins. *Biochim. Biophys. Acta* 1821, 782–789
- 7 Oike, Y. *et al.* (2005) Angiopoietin-like proteins: potential new targets for metabolic syndrome therapy. *Trends Mol. Med.* 11, 473–479
- 8 Oike, Y. *et al.* (2005) Angiopoietin-related growth factor antagonizes obesity and insulin resistance. *Nat. Med.* 11, 400–408
- 9 Wang, Y. *et al.* (2013) Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* 110, 16109–16114
- 10 Yi, P. *et al.* (2013) Betatrophin: a hormone that controls pancreatic b cell proliferation. *Cell* 153, 747–758
- 11 Tabata, M. *et al.* (2009) Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. *Cell Metab.* 10, 178–188
- 12 Kubota, Y. *et al.* (2005) Isolation and expression patterns of genes for three

angiopoietin-like proteins, Angptl1, 2 and 6 in zebrafish. *Gene Expr. Patterns* 5, 679–685

13 Horio, E. *et al.* (2014) The role of endothelial cell-derived ANGPTL2 in vascular inflammation leading to endothelial dysfunction and atherosclerosis progression. *Arterioscler. Thromb. Vasc. Biol.* 34, 790–800

14 Aoi, J. *et al.* (2011) Angiopoietin-like protein 2 is an important facilitator of inflammatory carcinogenesis and metastasis. *Cancer Res.* 71, 7502–7512

15 Endo, M. *et al.* (2012) Tumor cell-derived angiopoietin-like protein ANGPTL2 is a critical driver of metastasis. *Cancer Res.* 72, 1784–1794

16 Odagiri, H. *et al.* (2014) The secreted protein ANGPTL2 promotes metastasis of osteosarcoma cells through integrin $\alpha 5 \beta 1$, p38MAPK, and matrix metalloproteinases. *Sci. Signal.* 7, ra7

17 Oike, Y. and Tabata, M. (2009) Angiopoietin-like proteins – potential therapeutic targets for metabolic syndrome and cardiovascular disease. *Circ. J.* 73, 2192–2197

18 Kubota, Y. *et al.* (2005) Cooperative interaction of angiopoietin-like proteins 1 and 2 in zebrafish vascular development. *Proc. Natl. Acad. Sci. U.S.A.* 102, 13502–13507

19 Dhanabal, M. *et al.* (2005) Angioarrestin: a unique angiopoietin-related protein with anti-angiogenic properties. *Biochem. Biophys. Res. Commun.* 333, 308–315

- 20 Dhanabal, M. *et al.* (2002) Angioarrestin: an antiangiogenic protein with tumor-inhibiting properties. *Cancer Res.* 62, 3834–3841
- 21 Oike, Y. *et al.* (2004) Angiopoietin-related/angiopoietin-like proteins regulate angiogenesis. *Int. J. Hematol.* 80, 21–28
- 22 Akimenko, M.A. *et al.* (2003) Old questions, new tools, and some answers to the mystery of fin regeneration. *Dev. Dyn.* 226, 190–201
- 23 Gill, S.E. and Parks, W.C. (2008) Metalloproteinases and their inhibitors: regulators of wound healing. *Int. J. Biochem. Cell Biol.* 40, 1334–1347
- 24 Vu, T.H. and Werb, Z. (2000) Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev.* 14, 2123–2133
- 25 Bai, S. *et al.* (2005) Matrix metalloproteinase expression and function during fin regeneration in zebrafish: analysis of MT1-MMP, MMP2 and TIMP2. *Matrix Biol.* 24, 247–260
- 26 Tazume, H. *et al.* (2012) Macrophage-derived angiopoietin-like protein 2 accelerates development of abdominal aortic aneurysm. *Arterioscler. Thromb. Vasc. Biol.* 32, 1400–1409
- 27 Sun, K. *et al.* (2011) Adipose tissue remodeling and obesity. *J. Clin. Invest.* 121,

2094–2101

28 Doi, Y. *et al.* (2013) Angiopoietin-like protein 2 and risk of type 2 diabetes in a general Japanese population: the Hisayama study. *Diabetes Care* 36, 98–100

29 Muramoto, A. *et al.* (2011) Angiopoietin-like protein 2 sensitively responds to weight reduction induced by lifestyle intervention on overweight Japanese men. *Nutr. Diabetes* 1, e20

30 Weber, C. and Noels, H. (2011) Atherosclerosis: current pathogenesis and therapeutic options. *Nat. Med.* 17, 1410–1422

31 Ross, R. (1999) Atherosclerosis – an inflammatory disease. *N. Engl. J. Med.* 340, 115–126

32 Aghamohammadzadeh, R. *et al.* (2012) Perivascular adipose tissue from human systemic and coronary vessels: the emergence of a new pharmacotherapeutic target. *Br. J. Pharmacol.* 165, 670–682

33 Takaoka, M. *et al.* (2009) Periadventitial adipose tissue plays a critical role in vascular remodeling. *Circ. Res.* 105, 906–911

34 Ouchi, N. *et al.* (2011) Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* 11, 85–97

- 35 Tian, Z. *et al.* (2013) Perivascular adipose tissue-secreted angiopoietin-like protein 2 (Angptl2) accelerates neointimal hyperplasia after endovascular injury. *J. Mol. Cell. Cardiol.* 57, 1–12
- 36 Rizas, K.D. *et al.* (2009) Immune cells and molecular mediators in the pathogenesis of the abdominal aortic aneurysm. *Cardiol. Rev.* 17, 201–210
- 37 Shimizu, K. *et al.* (2006) Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler. Thromb. Vasc. Biol.* 26, 987–994
- 38 Longo, G.M. *et al.* (2002) Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. *J. Clin. Invest.* 110, 625–632
- 39 Farhat, N. *et al.* (2013) Angiopoietin-like 2 promotes atherogenesis in mice. *J. Am. Heart. Assoc.* 2, e000201
- 40 Grivennikov, S.I. *et al.* (2010) Immunity, inflammation, and cancer. *Cell* 140, 883–899
- 41 Yuspa, S.H. (1998) The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. *J. Dermatol. Sci.* 17, 1–7
- 42 Aoi, J. *et al.* (2013) Angiopoietin-like protein 2 accelerates carcinogenesis by activating chronic inflammation and oxidative stress. *Mol. Cancer Res.* 12, 239–249

- 43 Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674
- 44 Denko, N.C. (2008) Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nat. Rev. Cancer* 8, 705–713
- 45 Psaila, B. and Lyden, D. (2009) The metastatic niche: adapting the foreign soil. *Nat. Rev. Cancer* 9, 285–293
- 46 Vlahopoulos, S.A. *et al.* (2008) The role of ATF-2 in oncogenesis. *Bioessays* 30, 314–327
- 47 Kikuchi, R. *et al.* (2008) Frequent inactivation of a putative tumor suppressor, angiopoietin-like protein 2, in ovarian cancer. *Cancer Res.* 68, 5067–5075
- 48 Endo, M. *et al.* (2014) Serum ANGPTL2 levels reflect clinical features of breast cancer patients: implications for the pathogenesis of breast cancer metastasis. *Int. J. Biol. Markers* <http://dx.doi.org/10.5301/jbm.5000080>
- 49 Ohnishi, J. *et al.* (2005) Functions for proteinases in the ovulatory process. *Biochim. Biophys. Acta* 1751, 95–109
- 50 Berry, R. *et al.* (2010) Structural and functional evidence for a substrate exclusion mechanism in mammalian tolloid like-1 (TLL-1) proteinase. *FEBS Lett.* 584, 657–661

- 51 Clark, T.G. *et al.* (1999) The mammalian Toll-like 1 gene, Tll1, is necessary for normal septation and positioning of the heart. *Development* 126, 2631–2642
- 52 Hagihara, A. *et al.* (2004) Identification of 27 CpG islands aberrantly methylated and 13 genes silenced in human pancreatic cancers. *Oncogene* 23, 8705–8710
- 53 Cresci, S. *et al.* (2011) Peroxisome proliferator-activated receptor pathway gene polymorphism associated with extent of coronary artery disease in patients with type 2 diabetes in the bypass angioplasty revascularization investigation 2 diabetes trial. *Circulation* 124, 1426–1434
- 54 Kadomatsu, T. *et al.* (2011) Angiopoietin-like proteins: emerging targets for treatment of obesity and related metabolic diseases. *FEBS J.* 278, 559–564
- 55 Bass, J. and Takahashi, J.S. (2010) Circadian integration of metabolism and energetics. *Science* 330, 1349–1354
- 56 Curtis, A.M. *et al.* (2014) Circadian clock proteins and immunity. *Immunity* 40, 178–186
- 57 Green, C.B. *et al.* (2008) The meter of metabolism. *Cell* 134, 728–742
- 58 Ukai, H. and Ueda, H.R. (2010) Systems biology of mammalian circadian clocks. *Annu. Rev. Physiol.* 72, 579–603

- 59 Bass, J. (2012) Circadian topology of metabolism. *Nature* 491, 348–356
- 60 Takahashi, J.S. *et al.* (2008) The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat. Rev. Genet.* 9, 764–775
- 61 Kitazawa, M. *et al.* (2011) Angiopoietin-like 2, a circadian gene, improves type 2 diabetes through potentiation of insulin sensitivity in mice adipocytes. *Endocrinology* 152, 2558–2567
- 62 Kadomatsu, T. *et al.* (2013) A molecular clock regulates angiopoietin-like protein 2 expression. *PLoS ONE* 8, e57921
- 63 Zhang, C.C. *et al.* (2006) Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. *Nat. Med.* 12, 240–245
- 64 Blery, M. *et al.* (1998) The paired Ig-like receptor PIR-B is an inhibitory receptor that recruits the protein-tyrosine phosphatase SHP-1. *Proc. Natl. Acad. Sci. U.S.A.* 95, 2446–2451
- 65 Martin, A.M. *et al.* (2002) Leukocyte Ig-like receptor complex (LRC) in mice and men. *Trends Immunol.* 23, 81–88
- 66 Zheng, J. *et al.* (2012) Inhibitory receptors bind ANGPTLs and support blood stem cells and leukaemia development. *Nature* 485, 656–660

- 67 Takai, T. and Ono, M. (2001) Activating and inhibitory nature of the murine paired immunoglobulin-like receptor family. *Immunol. Rev.* 181, 215–222
- 68 Guo, D.F. *et al.* (2003) Type 1 angiotensin II receptor-associated protein ARAP1 binds and recycles the receptor to the plasma membrane. *Biochem. Biophys. Res. Commun.* 310, 1254–1265
- 69 Mederle, K. *et al.* (2013) The angiotensin II AT1 receptor-associated protein Arap1 is involved in sepsis-induced hypotension. *Crit. Care* 17, R130
- 70 Ge, H. *et al.* (2004) Oligomerization and regulated proteolytic processing of angiopoietin-like protein 4. *J. Biol. Chem.* 279, 2038–2045
- 71 Ono, M. *et al.* (2003) Protein region important for regulation of lipid metabolism in angiopoietin-like 3 (ANGPTL3): ANGPTL3 is cleaved and activated in vivo. *J. Biol. Chem.* 278, 41804–41809

Glossary

Angiopoietin-like proteins (ANGPTLs): proteins structurally similar to angiopoietin. ANGPTLs regulate angiogenesis and some function in glucose and lipid metabolism, energy

metabolism, and inflammation.

DNA demethylation: both active and passive mechanisms of removal of 5-methylcytosine (5mC) have been reported. In active pathways, both DNA demethylation-related enzymes, which function in oxidation or deamination of 5mC, and base excision repair machinery contribute to demethylation.

DNA methylation: an epigenetic transcriptional regulatory mechanism, which in mammalian cells is established by a family of DNA methyltransferases (DNMTs) that catalyze the transfer of methyl groups to the 5' position of cytosine bases in the CpG dinucleotide, generating 5mC. DNA methylation functions in physiological processes, such as embryonic development and cell differentiation. Aberrant DNA methylation is the best-characterized epigenetic hallmark of cancer.

Leukocyte immunoglobulin-like receptor B2 (LILRB2): a member of the leukocyte immunoglobulin-like family of receptors containing cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) domains. LILRB2 is expressed on immune cells, such as monocytes, macrophages, and dendritic cells. Major histocompatibility complex class I (MHCI) is a well-characterized LILRB2 ligand, and LILRB2 downstream signaling inhibits activation of the immune response. In addition, LILRB2 functions as a receptor for

ANGPTLs, such as ANGPTL2 and ANGPTL5.

Matrix metalloproteinases (MMPs): a family of zinc-dependent endopeptidases that induce ECM remodeling. MMPs function in various physiological processes, including embryonic development, reproduction, and tissue remodeling, and in pathological processes, such as tumor metastasis.

Molecular clock: a set of interlocking transcription/translation feedback loops that result in expression of core clock genes with 24-h periodicity. Molecular clocks play essential roles in generating circadian rhythms underlying various physiological and behavioral processes, such as sleep–wake cycles, body temperature, hormone secretion, blood pressure, and metabolism.

Tolloid-like 1 (TLL1): a member of the BMP-1/TLD family of proteinases that functions in processing of ECM precursors and TGF- β superfamily members. TLL1 plays important roles in heart development and is essential for formation of the interventricular septum.

Box 1: Outstanding questions

- How does ANGPTL2 contribute to tissue repair in mammals and what types of cells secrete ANGPTL2 during tissue repair?

- What molecular mechanisms underlie dysregulation of ANGPTL2 expression induced by stresses brought on by aging or lifestyle?
- Does cleaved ANGPTL2 show biological activity and, if so, what are the functional differences between full-length and cleaved ANGPTL2?
- Does ANGPTL2 cleavage by TLL1 suppress ANGPTL2-associated inflammation and pathological tissue remodeling?
- Does ANGPTL2 regulate immune responses via LILRB2?
- How do ANGPTL2 target cells use both LILRB2- and integrin $\alpha 5\beta 1$ -mediated signaling to regulate the immune response or undergo tissue remodeling? Does ANGPTL2 signaling depend on the type of target cells expressing LILRB2 or integrin $\alpha 5\beta 1$?
- Are there additional ANGPTL2 receptors and, if so, what is their function?

Figure 1. Angiopoietin-like protein 2 (ANGPTL2) downstream signaling. In integrin $\alpha 5\beta 1$ -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 $\alpha 5\beta 1$ /p38 mitogen-activated protein kinase

(MAPK) pathway, thereby promoting extracellular matrix (ECM) remodeling and tumor invasivity. ANGPTL2 also induces inflammation-related gene expression by promoting I κ B degradation and nuclear localization of nuclear factor κ B (NF κ B). Leukocyte immunoglobulin-like receptor B2 (LILRB2), a recently identified receptor for ANGPTL proteins including ANGPTL2 and ANGPTL5, is expressed on hematopoietic stem cells, immune cells, and leukemia cells. ANGPTL2 can induce association of Src homology region 2-containing protein tyrosine phosphatase (SHP) with LILRB2 via immunoreceptor tyrosine-based inhibitory motif (ITIM) domains and promote calmodulin-dependent protein kinase (CAMK) activation. Such activity could contribute to stemness maintenance of hematopoietic stem cells and to leukemia development.

Figure 2. Role of angiopoietin-like protein 2 (ANGPTL2) in obesity. *ANGPTL2* expression in adipose tissues increases under obese conditions. In the early phase of obesity (mild obesity), increased ANGPTL2 secretion from adipose tissues promotes MMP activation and induces adipose tissue remodeling, such as angiogenesis and ECM remodeling, leading to adipogenesis and adipocyte hypertrophy, which function to store excess energy in adipose tissue. 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 pathological adipose tissue remodeling, resulting in metabolic disease, such as obesity-related insulin resistance and type 2 diabetes.

Figure 3. Role of angiopoietin-like protein 2 (ANGPTL2) in cancer. **(A)** Model proposing mechanism linking increased *ANGPTL2* expression to carcinogenesis. In physiological conditions, secreted ANGPTL2 promotes tissue remodeling and contributes to maintenance of tissue homeostasis. However, prolonged stresses derived from aging, UV light exposure, and chemical compounds increased ANGPTL2 expression and secretion, resulting in chronic inflammation. Chronic inflammation increases susceptibility to carcinogenesis through inducing oxidative stress that promotes DNA damage and genomic instability. **(B)** Molecular mechanisms of ANGPTL2-mediated tumor metastasis. The tumor microenvironment, marked by hypoxia and undernutrition, induces *ANGPTL2* in tumor cells by inducing demethylation of its promoter. Tumor cells expressing and secreting increased ANGPTL2 acquire aggressive phenotypes associated with metastasis. ANGPTL2 promotes tumor cell migration and angiogenesis by activating Rac in an integrin $\alpha 5\beta 1$ -dependent manner. Furthermore,

ANGPTL2 enhances tumor cell invasivity by increasing expression and activity of MMPs via the integrin $\alpha 5\beta 1$ /p38 MAPK pathway. ANGPTL2 also activates transforming growth factor- β (TGF- β) signaling, which induces an epithelial-to-mesenchymal transition. Open and closed circles indicate unmethylated and methylated CpG dinucleotides, respectively.

Figure 4. Models proposing potential mechanisms underlying angiopoietin-like protein 2 (ANGPTL2)-mediated tissue homeostasis and development of lifestyle diseases and cancer.

(A) Potential roles of ANGPTL2 in maintenance of tissue homeostasis. External and internal stresses based on aging or lifestyle cause tissue damage, which is normally repaired via tissue remodeling due to interactions between parenchymal cells and stromal cells resulting from cell–cell contact or humoral factors. ANGPTL2 contributes to tissue repair by inducing tissue remodeling, whereas its periodic expression or secretion contributes to maintenance of tissue homeostasis. **(B)** Loss of *ANGPTL2* periodicity and development of lifestyle diseases and cancer. Periodic *ANGPTL2* expression maintains tissue homeostasis. However, prolonged stresses, such as overnutrition and inactivity, induce dysfunction of *ANGPTL2* transcriptional regulation, leading to loss of periodicity. Prolonged decreased *ANGPTL2* expression results in development and progression of lifestyle diseases resulting from insufficient tissue repair.

By contrast, continuous *ANGPTL2* expression causes chronic inflammation and subsequent pathological irreversible tissue remodeling, resulting in development and progression of lifestyle diseases and cancer.

Figure 1 Kadomatsu *et al.*

Figure 2 Kadomatsu *et al.*

A**Physiological response****Breakdown of physiological response**

Aging, UV, Chemical compounds, etc

B

Figure 3B Kadomatsu *et al.*

A

Figure 4A Kadomatsu *et al.*

B Figure 4B Kadomatsu *et al.*