Review

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# Diverse roles of ANGPTL2 in physiology and pathophysiology

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**Stresses based on aging and lifestyle can cause tissue damage. Repair of damage by tissue remodeling is often meditated 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 potently 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,

## 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.

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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 α5β1

ANGPTL2 enhances cell motility by activating Rac1, a Rho family GTPase, via integrin α5β1 [11]. In addition, ANGPTL2 signaling through integrin α5β1 promotes degradation of IκB, a factor that inhibits nuclear localization of nuclear factor κB (NFκB), thereby inducing expression of inflammation-related NFκB target genes [11]. It was recently shown that ANGPTL2 increases expression and activity of matrix metalloproteinases (MMPs) through integrin α5β1-mediated activation of p38 mitogen-activated protein kinase (MAPK) [16], leading to extracellular matrix (ECM) remodeling. Thus, ANGPTL2 signaling through integrin α5β1 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

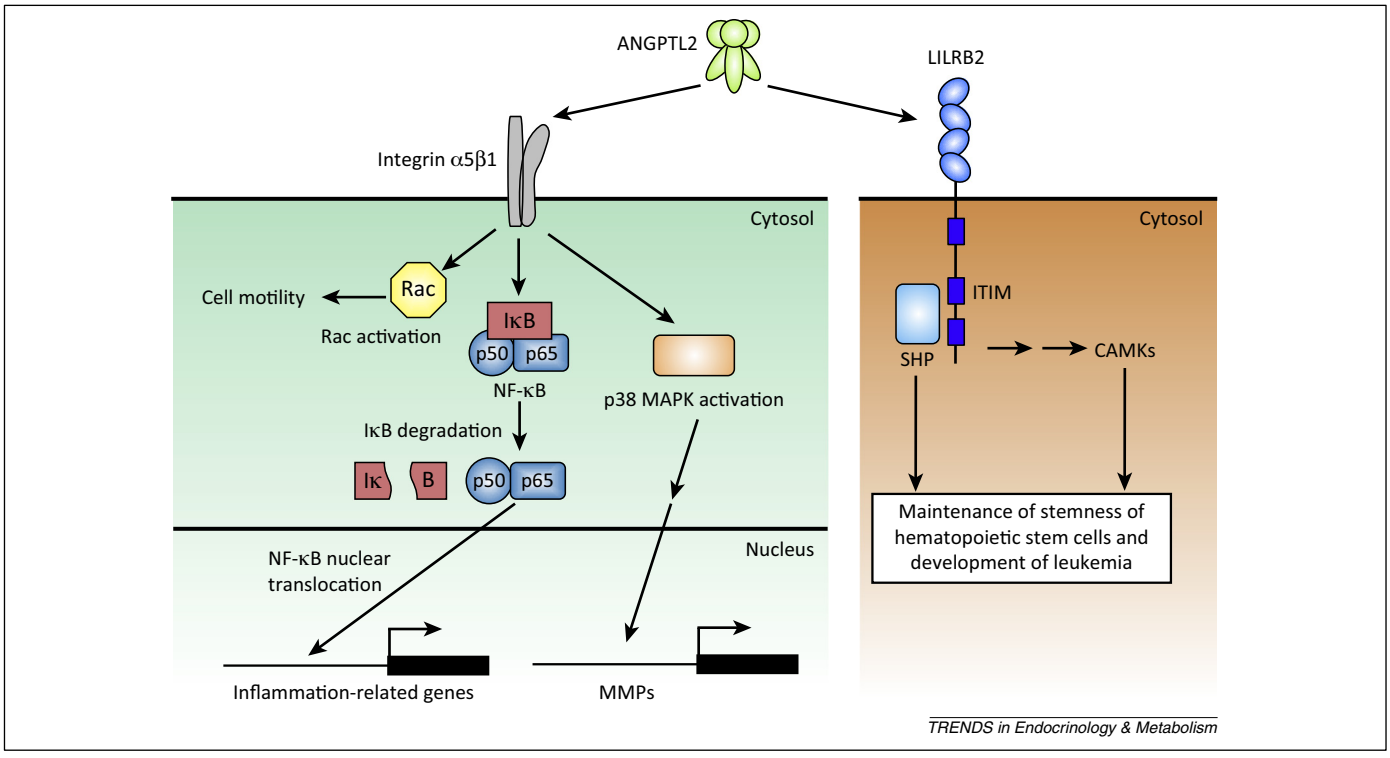


**Figure 1**. Angiopoietin-like protein 2 (ANGPTL2) downstream signaling. In integrin α5β1-expressing cells, such as endothelial and tumor cells, ANGPTL2 promotes cell motility by activating Rac. ANGPTL2 also induces expression and activity of matrix metalloproteinases (MMPs) via the integrin α5β1/p38 mitogen-activated protein kinase (MAPK) pathway, thereby promoting extracellular matrix (ECM) remodeling and tumor invasivity. ANGPTL2 also induces inflammation-related gene expression by promoting IκB degradation and nuclear localization of nuclear factor κB (NFκB). 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.