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Review Article

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Angiopoietin-like 4: a decade of research

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Synopsis

The past decade has seen a rapid development and increasing recognition of ANGPTL4 (angiopoietin-like 4) as a remarkably multifaceted protein that is involved in many metabolic and non-metabolic conditions. ANGPTL4 has been recognised as a central player in various aspects of energy homoeostasis, at least in part, via the inhibitory interaction between the coiled-coil domain of ANGPTL4 and LPL (lipoprotein lipase). The fibrinogen-like domain of ANGPTL4 interacts and activates specific integrins to facilitate wound healing, modulates vascular permeability, and regulates ROS (reactive oxygen species) level to promote tumorigenesis. The present review summarizes these landmark findings about ANGPTL4 and highlights several important implications for future clinical practice. Importantly, these implications have also raised many questions that are in urgent need of further investigations, particularly the transcription regulation of ANGPTL4 expression, and the post-translation cleavage and modifications of ANGPTL4. The research findings over the past decade have laid the foundation for a better mechanistic understanding of the new scientific discoveries on the diverse roles of ANGPTL4.

Key words: angiopoietin, homoeostasis, lipoprotein lipase, peroxisome-proliferator-activated receptor, reactive oxygen species, triacylglycerol

INTRODUCTION

In 2000, three independent research groups simultaneously identified a new protein that was similar to members of the ANG (angiopoietin) family. Kim et al. [1] identified the hepatic fibrinogen/ANG-related protein while performing a degenerative PCR screen for proteins homologous to the ANG family. During a screen for novel target genes of the nuclear receptor PPARα (peroxisome-proliferator-activated receptor α), Kersten et al. [2] isolated a fasting-induced adipose factor from the liver. Similarly, Yoon et al. [3] discovered a PPARγ/ANG-related protein as a downstream target of PPARα and PPARγ during pre-adipocyte differentiation. The HUGO Gene Nomenclature Committee has now named the gene encoding this protein *ANGPTL4* (ANG-like 4). Shortly after its discovery, the ANGPTL4 protein was classified as an adipokine due to its predominant expression in adipose tissues and liver; thus, it was believed to be involved in lipid metabolism. From intensive studies that have been carried out over the past decade, increasing recognition of this protein as a remarkably dynamic entity that is involved in a myriad of physiological and pathological conditions has occurred. The functions of this protein extend far beyond what was previously predicted and now include energy homoeostasis, wound repair, tumorigenesis, angiogenesis and redox regulation. The present review summarizes our current knowledge of ANGPTL4 and aims to identify other prospective research areas that will eventually serve the ultimate goals of applying new techniques in biomedicine and improving human health.

STRUCTURE AND EXPRESSION PATTERN OF ANGPTL4

The ANGPTL proteins belong to a superfamily of angiogenic-regulating, secreted proteins that bear the highest similarity to members of the ANG family. All members of the ANG-like family (ANGPTL1–7) have been found in both humans and mice, except for ANGPTL5, which is a human orthologue. The human *ANGPTL4* gene is well conserved among different species and shares ~77% and 99% amino acid sequence similarity with mouse and chimpanzee respectively. The human *ANGPTL4* gene is located on chromosome 19p13.3, has seven exons and encodes a 406-amino-acid glycoprotein with

Abbreviations used: AMPK, AMP-activated protein kinase; ANG, angiopoietin; ANGPTL4, ANG-like 4; AP-1, activator protein 1; ECM, extracellular matrix; HDL, high-density lipoprotein; LPL, lipoprotein lipase; NEFA, non-esterified fatty acid; PPAR, peroxisome-proliferator-activated receptor; PKC, protein kinase C; RNAi, RNA interference; ROS, reactive oxygen species; TAG, triacylglycerol; TGFβ, transforming growth factor β.

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a molecular mass of ~45–65 kDa. Like other members of this family, ANGPTL4 has a secretory signal peptide and contains a predicted N-terminal coiled-coil quaternary structure and a large, C-terminal fibrinogen-like domain. ANGPTL4 has three potential N-glycosylation sites and appears to be sialylated [4].

The native full-length ANGPTL4 can form higher-order structures via intermolecular disulfide bonds [5]. The N-terminal region of ANGPTL4 (herein designated as nANGPTL4) is responsible for its assembly into either dimeric or tetrameric structures [6], and oligomerization of ANGPTL4 is important for its function as an LPL (lipoprotein lipase) inhibitor [5–7]. The full-length ANGPTL4 protein undergoes proteolytic processing by proprotein convertases at the linker region, releasing the nANGPTL4 and the monomeric C-terminal portion of ANGPTL4 (cANGPTL4) [6,8,9]. The cleavage of ANGPTL4 appears to be tissue-dependent. In human, the liver secretes the cleaved nANGPTL4, whereas adipose tissue secretes the full-length form [10]. *In vitro* experiments showed that several proprotein convertases, including PCSK3 (proprotein convertase subtilisin/kexin type3), furin and PC5/6 (proprotein convertase 5/6), cleaved human ANGPTL4 via recognition of the amino acid sequence $R^{161}RKR^{164}$ [5,9]. However, the expression of these proprotein convertases in various tissues during physiopathological conditions and whether they are responsible for the *in vivo* processing of full-length ANGPTL4 is still unknown.

Most members of the ANGPTL family are currently considered orphan ligands because information regarding their potential binding partners is lacking. Notably, none of these ANGPTL proteins binds to TIE 1/2 tyrosine kinase receptors, suggesting that they may exert distinct functions from other ANG proteins, which play important roles in angiogenesis and haemopoiesis by signalling through TIE receptors [11,12]. ANGPTL4 shares only ~30% amino acid sequence similarity with ANGPTL3, its closest member within the family. Studies showed that ANGPTL4 and ANGPTL3 inhibit LPL in a non-redundant way [13]. The oligomerization of the ANGPTL4 protein determines the intrinsic ability of ANGPTL4 to inhibit LPL activity [5,7], thus the proteolytic cleavage of ANGPTL4 by proprotein convertases may influence this function subsidiarily [9]. Full-length ANGPTL4 also binds to heparin sulfate proteoglycans, but the domains for these interactions remain to be determined. Recently, the cANGPTL4 protein was shown to interact with integrins $\beta 1$ and $\beta 5$ and their cognate ECM (extracellular matrix) proteins [14,15]; however, binding of ANGPTL4 to matrix proteins did not interfere with the recognition of those matrix proteins by their cognate integrins.

In mice, the ANGPTL4 protein is most highly expressed in white and brown adipose tissues and to a much lesser extent in other tissues such as ovary, heart, liver, skeletal muscle and intestine [3,16]. In humans, ANGPTL4 is ubiquitously expressed at higher levels in the liver, adipose tissue, blood plasma, placenta, small intestine and heart [16,17], albeit with drastic inter-individual variations in the level of expression. Both the full-length and truncated forms of ANGPTL4 are found in blood plasma, but the baseline expression levels of this protein vary greatly among different individuals [10,18]. The expression of ANGPTL4 can be induced by numerous stimuli. Under fasting conditions, most, but not all [19], studies indicate that the expression of ANGPTL4 is strongly up-regulated in the following organs/tissues: adipose tissue, liver, heart, plasma and skeletal muscle [2,20,21]; this is mediated by glucocorticoid, whose circulating levels are increased during fasting [22]. In addition to fasting, plasma ANGPTL4 levels are also increased by chronic caloric restriction or NEFAs [non-esterified fatty acids (free fatty acids)] [17]. The fasting- and NEFA-induced increase in ANGPTL4 is mediated by the nuclear hormone receptors PPARs, which transcriptionally stimulate ANGPTL4 expression via a functional PPAR-response element in the human and mouse *ANGPTL4* genes [10,23,24]. Hypoxic conditions also elevate the expression of ANGPTL4 in cadiomyocytes, adipocytes, articular chondrocytes, endothelial cells and brain [25–30]. Synthetic agonists for PPARs raise plasma ANGPTL4 levels in humans and mice as well [2,3,10,17,31,32]. TGFβ (transforming growth factor β), a critical cytokine, can also stimulate ANGPTL4 expression in primary tumour cells to prime metastasis [33]. Interestingly, a recent finding suggested that TGFβ and PPARβ/δ could either synergistically or antagonistically stimulate ANGPTL4 expression, depending on cellular contexts [23,24].

ANGPTL4 IN ENERGY HOMOEOSTASIS

Food intake

ANGPTL4 is expressed in the mouse hypothalamus, cortex and pituitary gland, where it is subject to a number of regulatory influences, including hypoxia [26]. Discrepant findings exist on whether ANGPTL4 expression in the hypothalamus is induced by fasting [20,34]; however, recent data have suggested that ANGPTL4 is involved in regulating food intake and energy expenditure [34]. Whereas central administration of ANGPTL4 lowered food intake and body weight gain and enhanced energy expenditure, deletion of ANGPTL4 had the opposite effect. These data indicate that ANGPTL4 acts as an anorexigenic factor, possibly via inhibition of hypothalamic AMPK (AMP-activated protein kinase) activity [34].

Gut microbiota

ANGPTL4 has been presented as a putative factor linking the intestinal microbiota to fat storage in adipose tissue [35]. Introduction of microbiota into germ-free mice suppresses *ANGPTL4* mRNA in the small intestine [35,36]; however, it is expected that different bacterial strains differentially modulate ANGPTL4 expression. In a colonic cell line, various probiotic bacteria, including *Lactobacillus paracasei* ssp. *paracasei* F19, caused pronounced up-regulation of *ANGPTL4* mRNA [37], and this up-regulation was reproduced by factors secreted from *L. paracasei* ssp. *paracasei* F19.

Lipid metabolism

The demonstration of the regulation of ANGPTL4 by PPARs provided the first clue towards a potential role in lipid metabolism. Abundant evidence now indicates that ANGPTL4 suppresses the clearance of circulating TAGs (triacylglycerols), which is achieved by inhibiting LPL, the enzyme that hydrolyses TAGs in lipoprotein particles. Accordingly, deletion of ANGPTL4 causes lowering of circulating TAGs, whereas ANGPTL4 overexpression leads to an increase in TAGs. There is limited evidence that ANGPTL4 also inhibits hepatic lipase, a TAG hydrolase specifically expressed in liver and involved in HDL (high-density lipoprotein) and lipoprotein remnant metabolism. ANGPTL4 inhibits LPL, at least partially, by promoting the conversion of active LPL dimers into inactive LPL monomers. The primary region responsible for blocking LPL is located within 12 highly conserved amino acids that are near the N-terminus of ANGPTL4. Mutating three polar amino acid residues within this region abolished the ability of ANGPTL4 to inhibit LPL [7]. Moreover, inhibition of LPL activity by ANGPTL4 was suppressed by the LPL-anchor and -transport protein GPIHBP1 (glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1), perhaps by stabilizing the LPL dimers [38].

Transcription of *ANGPTL4* in numerous cell types is extremely sensitive to fatty acid stimulation [17,39,40], and the induction of ANGPTL4 by fatty acids and oral lipid overload probably serves to protect a cell against cellular lipid overload by reducing extracellular TAG hydrolysis and consequent fatty acid uptake. In cardiomyocytes, this mechanism prevents lipotoxicity and oxidative stress [39], whereas, in macrophages, it protects against lipid-induced macrophage activation. Failure of this mechanism in mesenteric lymph nodes leads to excessive lipolytic release of fatty acids from lymph chylomicrons, macrophage foam cell formation, endoplasmic recticulum stress and marked inflammation that becomes systemic.

In addition to inhibiting LPL-dependent extracellular lipolysis, ANGPTL4 also stimulates intracellular hydrolysis, as was observed in adipocytes and myocytes [41]. Accordingly, ablation of the *ANGPTL4* gene is associated with a blunted increase in plasma NEFA during fasting. The exact mechanism behind the intracellular lipolytic effect of ANGPTL4 remains unclear. Concurrent inhibition and activation of extracellular and intracellular lipolysis respectively may reduce fat storage and increase lipid availability from adipose tissue during fasting. Interestingly, one recent study using monozygotic twins shows that the expression of ANGPTL4 in adipose tissue is positively correlated with the adipose tissue hormone sensitive lipase, supporting the notion that ANGPTL4 could promote adipose tissue lipolysis in human [42].

Glucose homoeostasis

Adenoviral-mediated overexpression of ANGPTL4 was shown to greatly lower circulating glucose levels and improve glucose tolerance, which was suggested to be mediated by decreased hepatic glucose production [32]. In contrast, whole-body ANGPTL4 transgenic mice of two different backgrounds exhibited a worsening of glucose tolerance after prolonged high-fat feeding [16]. Clamp studies indicated that in fasted mice, transgenic ANGPTL4 overexpression was associated with impaired glucose utilization and insulin resistance in the periphery, but higher insulin-mediated suppression of glucose production in the liver [21]. No changes in plasma glucose levels were observed in ANGPTL4$^{-/-}$ mice that were fed standard chow [43]. Unfortunately, interpretation of the effects of deleting ANGPTL4 from animals with high fat-induced obesity and insulin resistance is compromised by the occurrence of a severe systemic inflammatory response. Thus published data on the impact of ANGPTL4 are inconsistent.

Human genetic studies

Approximately 3% of people of European ancestry are carriers of an E40K variant of the *ANGPTL4* gene. The E40K mutation significantly impairs the ability of ANGPTL4 to inhibit LPL by destabilizing or impairing the production of ANGPTL4 [44]. Consistent with the role of ANGPTL4 as an LPL inhibitor, carriers of the E40K variant exhibit decreased plasma TAG levels [45] and, in some studies, higher HDL levels compared with non-carriers [46]. Surprisingly, a genome-wide association study showed that a common sequence variant at a locus near the *ANGPTL4* gene was associated with plasma HDL but not with TAG concentrations [47]. A more common coding variant, T266M, was not associated with plasma TAGs or CVD (cardiovascular disease) risk in a recent meta-analysis of five studies [44].

ANGPTL4 IN INFLAMMATION

Increased lipid uptake is typically associated with a marked stimulation of inflammation- and immunity-related genes. This induction can be inhibited by LPL inhibitors, indicating that the mechanism is LPL-dependent. ANGPTL4 takes on a dampening role with regard to the levels of inflammatory markers such as serum amyloid A and IL-6 (interleukin-6), as shown by the elevated levels of these inflammatory factors in ANGPTL4-knockout mice that have been fed a high-saturated-fat diet. These mice lived no longer than 25 weeks, and their fibrin exudates contained large amounts of foam cells and various leucocytes. In addition, epididymal adipose tissue displayed coagulation necrosis and steatitis by both lymphocytes and granulocytes. Focal infiltrates of neutrophils, eosinophils, macrophages and rod-shaped bacteria, as well as a rise in the expression of the Kupffer cell marker CD68, were observed in the liver. The enlarged mesenteric lymph nodes also displayed an abundance of multinucleated Touton giant cells that were formed from the fusion of lipid-filled tissue macrophages, which is an indication of lipid lymphadenopathy. Besides regulating inflammation, ANGPTL4 prevents the uptake of saturated fatty acids by macrophages. ANGPTL4 is induced by chyle, which is produced after fat intake, leading to protection of the mesenteric lymph nodes and resident macrophages from the pro-inflammatory effects of saturated fatty acids [48].

The inflammatory process is an important development for the maintenance of an optimal state of health because chronic or uncontrolled inflammation is life-threatening. Recently, ANGPTL4 was characterized as a positive acute phase protein, as its expression was stimulated in liver, heart, muscle and adipose tissue during the acute phase response [49]. However, a direct effect of ANGPTL4 on inflammatory pathways has yet to be demonstrated and deserves further investigation.

ANGPTL4 IN KIDNEY DISEASE

A recent study suggested an important role for ANGPTL4 in the kidney. It was shown that minimal change disease, a common cause of nephrotic syndrome in children, was associated with overproduction of ANGPTL4 in the glomerulus, which is the filtering unit of the kidney [4,50]. One key morphological feature of minimal change disease is the loss of the characteristic foot processes of the podocyte, a specialized cell type involved in filtration that is a site of production of ANGPTL4 in the kidney. Podocyte-specific overexpression of ANGPTL4 in rats resulted in the development of most of the characteristic features of this disease: large-scale selective proteinuria, loss of glomerular basement membrane charge and diffuse fusion of podocyte foot processes. Furthermore, Clement et al. [4] showed an increase in hyposialylated ANGPTL4 in a puromycin aminonucleoside-treated rat model of podocyte injury, suggesting that sialylated and hyposialylated glomerular ANGPTL4 might have different effects on the barrier function of the glomerular basement membrane. Of clinical relevance, the dietary sialic acid precursor *N*-acetyl-D-mannosamine caused an increase in sialylation of glomerular ANGPTL4 and a decrease in albuminuria in the same transgenic rats. Clearly, how ANGPTL4 interacts with other pathways that are implicated in minimal change disease needs to be investigated for the development of effective therapies. Given the hypertriglyceridaemic effect of ANGPTL4, it would be useful to determine if podocyte-secreted ANGPTL4 escapes into the circulation and contributes to the hypertriglyceridaemia component of nephrotic syndrome.

ANGPTL4 IN WOUND HEALING

ANGPTL4 plays a novel role in keratinocyte migration during skin wound healing. Normal wound healing entails a continuum of events that include inflammation, re-epithelialization and matrix remodelling and involves a complex interplay among connective tissue formation, cellular activity and growth factor activation [51]. ECM components integrate each phase during wound healing, communicating with cells and growth factors in a dynamic reciprocal manner that eventually results in proper wound closure. Goh et al. [14,15] identified ANGPTL4 as a novel matricellular protein whose expression was up-regulated by PPARβ/δ in response to inflammation during wound healing. Full-length ANGPTL4 and cANGPTL4 proteins, which are produced by wound keratinocytes, co-ordinate cell–matrix communication in several ways during the re-epithelialization phases of wound healing. ANGPTL4 independently interacts with specific matrix proteins and integrins, and these proteins form a ternary complex when present together. First, ANGPTL4 specifically interacts with vitronectin and fibronectin in the wound bed and regulates the availability of the local ECM by delaying the degradation of those proteins by proteases. The delayed degradation of vitronectin and fibronectin has a direct impact on integrin-mediated signalling by altering the balance between substrate-anchored matrix proteins and soluble matrix protein fragments. This interaction does not interfere with integrin–matrix protein recognition, suggesting a separate binding site (or sites) for ANGPTL4 to stabilize the integrin-matrix protein complex. Secondly, ANGPTL4 specifically interacts with integrins $\beta1$ and $\beta5$ that reside on the surface of wound keratinocytes, which activate integrin-mediated intracellular signalling, allow for selective integrin recycling, enhance cell migration and accelerate the wound healing process [14,15]. Numerous intracellular signalling pathways, including FAK (focal adhesion kinase)-, Rho GTPase and 14-3-3σ-dependent signalling cascades that emanate from integrins to modulate cell migration are activated upon activation by ANGPTL4. ANGPTL4-deficient mice exhibit delayed wound re-epithelialization with impaired keratinocyte migration. Given the potential effect of ANGPTL4 on energy homoeostasis and inflammation, it would be useful to study the expression and effects of ANGPTL4 on the healing of diabetic wounds.

ANGPTL4 IN CELL DIFFERENTIATION

Our understanding of the role of ANGPTL4 in cell differentiation is in its infancy. Evidence suggested that ANGPTL4 may be involved in or associated with adipose differentiation and vascular tubule formation. During adipocyte differentiation, there is a dramatic early induction of the *ANGPTL4* transcript, and its expression level continues to increase post-differentiation [10,16,19]. However, the *in vivo* role of ANGPTL4 in adipocyte maturation is complicated as transgenic ANGPTL4 or knockout mice showed normal fat mass as a result from compensatory metabolic changes in adipose TAG metabolism [43]. The role of ANGPTL4 to stimulate endothelial cells remains controversial, with reports supporting either a pro- or anti-angiogenic functions (see the section on ANGPTL4 in angiogenesis and vascular permeability). A recent study showed that PPARβ/δ mediated ANGPTL4 to regulate PKCs (protein kinase Cs) and AP-1 (activator protein 1) transcription factor for epidermal differentiation, which is important for the maturation of the epidermis during the remodelling phase of wound healing [52]. ANGPTL4 stimulated the increase activation of c-Jun and JUNB, whereas it transcriptionally regulated the expression of PKCs. The expression of epidermal differentiation markers such as transglutaminase 1 and involucrin were induced

by ligand activated-PPARβ/δ and ANGPTL4, which is associated with increased AP-1 binding to the cognate promoter. Clearly, more future investigations into the ANGPTL4-stimulated transcriptional networks will be necessary for a better understanding of the precise role of ANGPTL4 in the differentiation of other cell types.

ANGPTL4 IN TUMORIGENESIS

Normal wound healing shares many similarities with the tumour microenvironment, and tumour invasion has been compared with 'wound healing gone awry.' Consistent with this notion, *ANGPTL4* has been identified as a prominent gene in a compact *in vivo* hypoxia signature that predicts a poor outcome in multiple tumour types [30,53]. *ANGPTL4* mRNA has been found to be up-regulated in the perinecrotic areas of many human tumours, clear cell renal carcinomas, oral tongue squamous cell carcinomas and human gastric cancers [54,55]. Tissue array analysis revealed an elevated ANGPTL4 expression in up to 40 known human epithelial tumour types, and its expression increased as tumours progressed from benign to metastatic states [56]. The elevated expression of ANGPTL4 in many cancers implicated a role of ANGPTL4 in tumour growth, and it was suggested to play a role in metastasis through lymphovascular invasion [57].

Numerous studies have shown that tumours frequently exhibit elevated ROS (reactive oxygen species) and utilize a redox-based mechanism to escape from death by anoikis. The loss of dependence on integrin-mediated ECM contact for growth (or anoikis resistance) is an essential feature of tumour cells; yet how this resistance is acquired is a central problem in cancer biology. A recent study by Zhu et al. [56] identified tumour-derived ANGPTL4 as a novel player in redox cancer biology, and they found that it conferred anoikis resistance to tumours via autocrine adhesion mimicry. Tumour-derived ANGPTL4 directly interacts with β1 and β5 integrins, thus hijacking integrin-mediated signalling to maintain an elevated oncogenic O_2^-/H_2O_2 ratio. This action stimulates the redox-mediated activation of the Src machinery and, therefore, activates the downstream PI3K (phosphoinositide 3-kinase)/PKBα (protein kinase Bα) and ERK (extracellular-signal-regulated kinase) signalling cascade to promote cell survival and tumour growth. Of note, the suppression of ANGPTL4 by RNAi (RNA interference) modulates intracellular ROS generation to attenuate tumour growth that is associated with enhanced apoptosis *in vitro* and *in vivo*. Intriguingly, treatment with a mAb (monoclonal antibody; 11F6C4) against human cANGPTL4 significantly retards melanoma growth in a mouse model, reproducing the RNAi effects. Unlike another reported anti-ANGPTL4 antibody (14D12), which targets the N-terminus of the mouse ANGPTL4 protein [58], 11F6C4 targets an epitope that resides within the C-terminus of human ANGPTL4, and it does not affect the mitochondrial activities and glucose regulations of this protein (P. Zhu, M.J. Tan, H.C. Chong and N.S. Tan, unpublished work). Indeed, ANGPTL4 has been found to contribute to anoikis resistance in hepatoma cells [59]. When grown in a detached state, hepatoma cells form a synoikis-like multicellular aggregate that is resistant to inducers of apoptosis. Additionally, ANGPTL4 knockdown enhanced cell apoptosis and sensitized tumour cells to drug treatment, confirming that ANGPTL4 played a key role in anoikis resistance. These exciting findings suggest that anticancer strategies that focus on redox-based apoptosis induction in tumours may be clinically viable.

ANGPTL4 IN ANGIOGENESIS AND VASCULAR PERMEABILITY

Communication between tumour cells and the endothelium that encourages angiogenesis and vasculature permeability is crucial for the continued growth and metastasis of the tumour respectively. Angiogenesis, i.e. the formation of new blood vessels that originate from the existing vascular system, helps the tumour grow by feeding the cancer cells with essential nutrients and oxygen. Neovascularization also influences the dissemination of cancer cells throughout the entire body and eventually leads to metastasis. The vascularization level of a solid tumour is thought to be an excellent indicator of its metastatic potential. Despite major advances in the field of tumour angiogenesis, relatively little attention has been paid to the permeability of blood vessels in tumours. The leakiness of tumour vessels has been well documented in experimental tumour models and in human cancer, but the mechanism is poorly understood. Tumour metastasis depends on a state of increased vasculature leakiness and on the critical steps of intravasation and extravasation, which involve the directional migration of tumour cells across the disrupted endothelium.

Tumour-induced angiogenesis is mainly sustained by the production and secretion of angiogenic factors, e.g. vascular endothelial growth factor [60] and ANGs, which originate from tumour and stromal cells [61]. Given the similarity between ANGPTL and the ANG proteins, the potential involvement of ANGPTL4 in angiogenesis and vascular permeability has been a subject of much study. To date, the role of ANGPTL4 in angiogenesis and vascular leakiness remains controversial, and it is more likely that ANGPTL4 functions as a 'gatekeeper' that regulates vascular integrity in a context-dependent manner [62]. Early studies proposed an anti-angiogenic role of ANGPTL4; therefore, it could prevent metastasis by inhibiting vascular leakiness [63]. The ECM-bound, but not the soluble, form of ANGPTL4 inhibits endothelial cell adhesion, migration and sprouting [64]. Furthermore, ANGPTL4 was reported to prevent metastasis through inhibition of angiogenesis, tumour cell motility and invasion [64,65]. However, other reports have proposed that ANGPTL4 is pro-angiogenic and pro-metastatic. ANGPTL4 was found to significantly promote *in vitro* sprouting of vascular endothelial cells, and this finding was supported by work on a conventional renal cell carcinoma that proposed ANGPTL4 as a pro-angiogenic factor during ischaemia [55]. Notably,

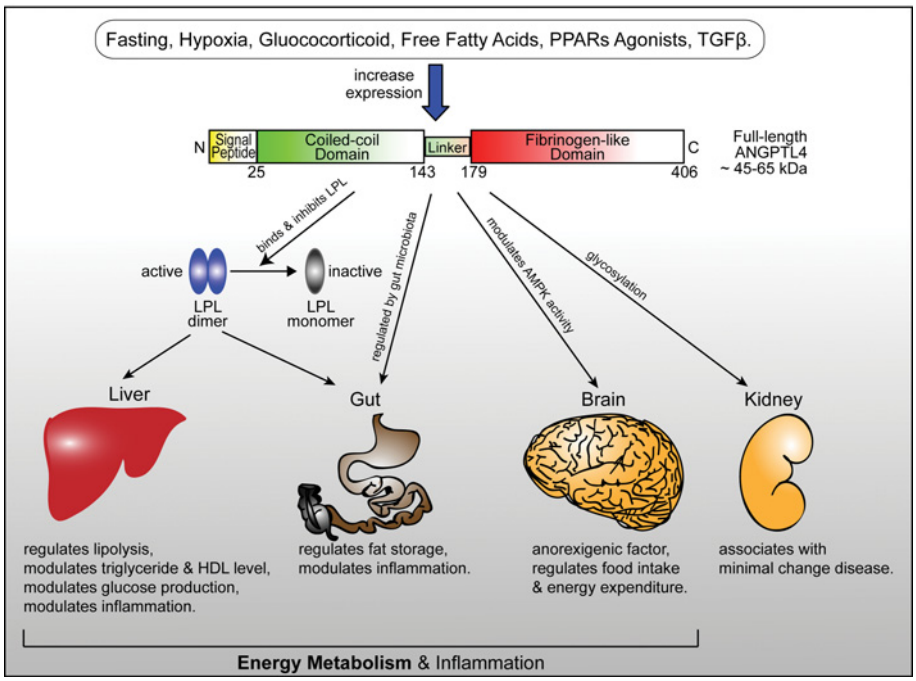

Figure 1 **Multiple roles of ANGPTL4 in energy homoeostasis**

Expression of ANGPTL4 is increased by various conditions and factors. ANGPTL4 is a secreted protein that can be proteolytically cleaved to release the N-terminal coiled-coil fragment (nANGPTL4) and the C-terminal fibrinogen-like fragment (cANGPTL4). ANGPTL4 is a central player in many aspects of energy homoeostasis. Studies have shown that nANGPTL4 binds and inhibits LPL to regulate lipolysis, glucose homoeostasis and fat storage as well as inflammation. ANGPTL4 has been implicated to modulate AMPK activity to regulate food intake and energy expenditure. Hyposialylated ANGPTL4 has also been shown to be associated with minimal change disease, which is a common cause of nephritic syndrome.

ANGPTL4 has been identified as one of the genes that can predict breast cancer to lung metastasis with the greatest frequency [66], and further studies indicated that TGFβ primes breast tumours for the seeding of lung metastasis through ANGPTL4 by modulating endothelial integrity to mediate lung metastasis seeding [59]. TGFβ-induced ANGPTL4 enhances the retention of cancer cells in the lungs, disrupts vascular endothelial cell–cell junctions, increases the permeability of the lung capillaries, and facilitates the *trans*-endothelial passage of tumour cells, thus promoting the vital steps of metastasis [33]. Until recently, there has been no further mechanistic understanding of ANGPTL4 on vascular leakiness. A recent study by Huang et al. [67] has provided new insight into the precise role of ANGPTL4 in this process. The report demonstrated that tumour-derived cANGPTL4 instigated the disruption of endothelial continuity by directly interacting with three novel binding partners: integrin $\alpha5\beta1$, VE-cadherin (vascular endothelial cadherin) and claudin-5, in a temporally sequential manner, thus facilitating metastasis. Indeed, the authors [67] showed that tumour cells and mice that were deficient in ANGPTL4 had reduced vascular permeability; furthermore, the mice had attenuated lung metastasis.

In all, growing evidence has led us to evaluate the potential of targeting ANGPTL4 as a therapeutic strategy in treatments that are aimed at various diseases such as diabetes, cancer and other vascular pathologies.

CONCLUSIONS AND PERSPECTIVES

It has been more than a decade since the first identification of ANGPTL4, and now this protein is emerging as a central player in many aspects of energy homoeostasis (Figure 1). Recent findings have implicated ANGPTL4 in other non-metabolic, physiological and pathological situations, such as wound healing, oncogenesis and angiogenesis (Figure 2). Although these recent discoveries have generated renewed excitement and provided tremendous insight into the diverse roles of ANGPTL4, many questions remain unaddressed. While the role of ANGPTL4 in lipid metabolism can be directly or indirectly attributed to its inhibitory interaction with LPL, this mechanism is inadequate to explain other facets such as intracellular lipolysis. The disparate role of ANGPTL4 in glucose metabolism also remains to be clarified. Central to this question is the mechanism for the differential expression and cleavage of ANGPTL4, which generates the full-length ANGPTL4, nANGPTL4 and cANGPTL4 that are observed in various tissues. Thus far, the findings suggest that the post-translational processing of the ANGPTL4 may be cell/tissue context-specific and pathologically/physiologically dependent events. In addition to its role as an adipokine, ANGPTL4 has also been recognized as a novel matricellular protein, which affects cell–matrix communication and is important

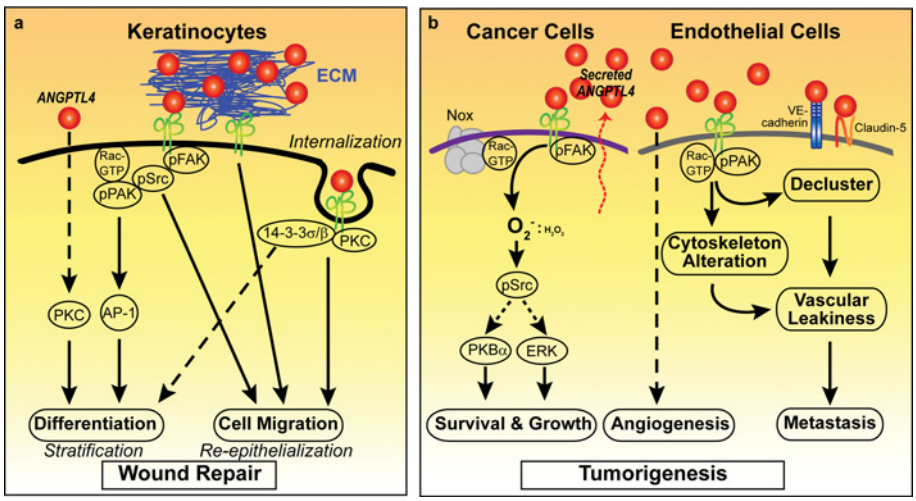

Figure 2 **Roles of ANGPTL4 in non-metabolic physiopathological situations**
(**a**) ANGPTL4 plays important roles in wound repair. ANGPTL4 produced by wound keratinocytes, co-ordinates cell–matrix communication to modulate keratinocyte migration during wound healing. ANGPTL4 also regulates PKCs and AP-1 for epidermal differentiation, facilitating the maturation of the epidermis during the remodelling phase of wound healing. (**b**) Cancer-derived cANGPTL4 plays multiple roles in tumour progression. In an autocrine manner, cANGPTL4 sustains cancer cell survival and promotes tumour growth. Cancer-derived cANGPTL4 can also act in a paracrine fashion on endothelial cells to promote angiogenesis and to increase vascular leakiness, thus promoting metastasis.

for wound healing, tumorigenesis and vascular integrity. Many early studies using adenovirus-mediated or other methodologies to overexpress ANGPTL4 in cells failed to clarify which protein forms were used in those experiments. Given recent findings, including our study showing that full-length and truncated forms of ANGPTL4 might exert a distinct array of roles in different contexts [56], all of the studies mentioned in the sections of this review need to be revisited. The discovery that ANGPTL4 can associate with matrix proteins and integrins to modulate integrin-mediated signalling is a pivotal step towards a better understanding of ANGPTL4-mediated signalling. However, the characterization of the full repertoire of ANGPTL4-interacting partners and the intracellular signalling cascade of these interactions awaits more extensive study. Taken together, the research findings from the last decade have laid the foundation for a better mechanistic understanding of and new scientific discoveries on the diverse roles of ANGPTL4.

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