HHS Public Access

Author manuscript

Curr Opin Hematol. Author manuscript; available in PMC 2022 April 17.

Published in final edited form as:

Curr Opin Hematol. 2020 May ; 27(3): 206–213. doi:10.1097/MOH.0000000000000580.

ANGPTL4, A MULTIFUNCTIONAL PROTEIN INVOLVED IN METABOLISM AND VASCULAR HOMEOSTASIS

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Abstract

- Purpose of review: Since the first discovery of Angiopoetin-like 4 (ANGPTL4) in 2000, the involvement of ANGPTL4 in different aspects of lipid metabolism and vascular biology has emerged as an important research field. In this review, we summarize the fundamental roles of ANGPTL4 in regulating metabolic and non-metabolic functions and their implication lipid metabolism and with several aspects vascular function and dysfunction.

- Recent findings: ANGPTL4 is a secreted glycoprotein with a physiological role in lipid metabolism and a predominant expression in adipose tissue and liver. ANGPTL4 inhibits the activity of lipoprotein lipase and thereby promotes an increase in circulating triglyceride levels. Therefore, ANGPTL4 has been highly scrutinized as a potential therapeutic target. Further involvement of ANGPTL4 has been shown to occur in tumorigenesis, angiogenesis, vascular permeability and stem cell regulation, which and open new opportunities of using ANGPTL4 as potential therapeutic targets for other pathophysiological conditions.

- Summary: Further determination of ANGPTL4 regulatory circuits and defining specific molecular events that mediate its biological effects remains key to future ANGPTL4-based therapeutic applications in different disease settings. Many new and unanticipated roles of ANGPTL4 in the control of cell-specific functions will assist clinicians and researchers in developing potential therapeutic applications.

Keywords

ANGPTL4; ATHEROSCLEROSIS; ANGIOGENESIS; LIPID METABOLISM

INTRODUCTION

ANGPTL4 is a member a family of 8 proteins from related gene products that are structurally similar to the angiopoietins (ANGPTs) and that play a role in a wide array of biological functions including the regulation of lipid and glucose metabolism, hematopoietic

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

stem cell expansion, chronic inflammation, angiogenesis, and vascular permeability [1–4]. ANGPTL4 was discovered simultaneously by three different screenings. A screen of novel PPAR gamma targets, a screen for novel fasting induced factors from liver, and a PCR screen to identify novel angiopoietin related proteins [5–7]. ANGPTL4 is highly expressed in ATs and liver in humans and mice and to a lesser extent in heart, muscles and other tissues. Its expression is under the regulation of several transcription factors including PPARs, glucocorticoid receptors, HIF1α and others [5, 6, 8, 9]. Expression of ANGPTL4 is regulated by nutritional (e.g. fasting) and different metabolic states (e.g. hypoxia) in a tissue dependent manner. As a multifunctional protein, ANGPTL4 regulates many metabolic and non-metabolic processes through its distinct N-terminal and C-terminal domains [1–4]. ANGPTL4 is a 50 kDa protein with a distinct ~15 kDa N-terminal coiled coil domain (nANGPTL4) and a ~35kDa C-terminal fibrinogen like domain (cANGPTL4) connected by linker region. It is proteolytically cleaved by proprotein convertases at their recognition motif in the linker region [10–12]. Before cleavage, ANGPTL4 forms oligomers mediated by disulfide bond forming cysteine residues in nANGPTL4. After cleavage, ANGPTL4 is released from the cells where nANGPTL4 remains oligomerized while cANGPTL4 dissociates into monomers. However, it remains unclear that the cleavage is a necessary step for its actions. The most well -recognize action of ANGPTL4 is the posttranscriptional regulation of lipoprotein lipase (LPL), function that is shared with other ANGPTL proteins (ANGPTL3, ANGPTL4 and ANGPTL8) [1, 4, 13, 14]. ANGPTL4 interacts directly with LPL inhibiting its dimerization and function [15, 16]. To this regard, the ability to bind and inhibit the LPL activity is limited to nANGPTL4 and full length ANGPTL4 while cANGPTL4 is reported to be involved in number of non-lipid metabolic related processes [4, 17]. However, these non-lipid metabolic related functions have also been described to be mediated by full-length ANGPTL4 [4, 18].

The mechanisms that involved the regulation of lipid metabolism by ANGPTL4 are well characterized and are summarized in the present review together with its association cardiometabolic disorders including atherosclerosis and T2D. This review will also explore other work describing the role of ANGPTL4 in additional processes that are not directly related with its lipid metabolism-mediated function since the molecular signaling events that explain their mechanisms of action are still uncertain.

Metabolic functions of ANGPTL4

ANGPTL4 in lipid metabolism, diabetes and metabolic disorders—Given its ability to inhibit LPL, ANGPTL4 plays a vital role in lipid metabolism [19]. Different mechanisms have been proposed for ANGPTL4 mediated inhibition of LPL activity. Initial reports suggested that nANGPTL4 irreversibly inhibits LPL activity by converting it from an active dimeric state to inactive monomers [15, 20]. Later studies demonstrate that ANGPTL4 can bind and inactivate LPL complexed with GPIHBP1, and the inactivation leads to dissociation of GPIHBP1 [21]. ANGPTL4 promotes the unfolding of the hydrolase domain in LPL and inactivates irreversibly [22, 23]. Interestingly, disruption of GPIHBP1 also disrupts LPL activity and causes hypertriglyceridemia, depletion of both ANGPTL4 and GPIHBP1 noticeably reduces hypertriglyceridemia suggesting a GPIHBP1-independent clearance of TAGs [24, 25]. More recently, it has been suggested that ANGPTL4 specifically

inhibits LPL by binding the lipid domain and then preventing substrate catalysis at the active site [26]. In addition to regulating its activity in the endothelial surface, in adipocytes ANGPTL4 also stimulates intracellular cleavage and degradation of LPL suggesting that ANGPTL4 can alter both the stability and activity of LPL [27, 28].

Early work showed that overexpression of ANGPTL4 decreases LPL activity and increases circulating TAG levels. Conversely, ANGPTL4 deficient mice exhibit increased plasma LPL activity, increased TAG clearance and decreased plasma TAG levels [29]. Consistent with this, humanized mice treated with antibodies against ANGPTL4 display reduced circulating TAGs [30]. Human studies have also uncovered the essential role of ANGPTL4 in metabolic disorders. ANGPTL4 levels correlate positively with age and body mass but correlate negatively with circulating high-density lipoprotein (HDL) cholesterol [31]. Remarkably, serum ANGPTL4 levels are not positively associated with plasma TAGs levels [32–34]. While ANGPTL4 levels correlate positively with fasting glucose levels and other metabolic parameters, data concerning type-2 diabetes (T2D) and circulating ANGPTL4 are inconsistent [35, 36]. To this end, one study demonstrated a two-fold elevation in ANGPTL4 serum levels in patients with T2D while another study had opposite findings [35, 37].

Human genetic studies of various coding variants of ANGPTL4 have corroborated many of the findings in mice. A low frequency missense variant of ANGPTL4 (E40K) is associated with reduced plasma TAG levels [38]. The E40K mutation hinders with the stabilization of N-terminal and full-length ANGPTL4 oligomers, which are necessary for ANGPTL4-mediated inhibition of LPL. Thus, this mutation uncovers beneficial metabolic outcomes on different lipid parameters, including lower plasma TAGs and higher HDL-C levels compared to non-carriers. This mutation also confers protection against coronary arterial diseases (CAD) and metabolic disorders. The lipid lowering effects of ANGPTL4 are also observed in other variants including T266M [39].

Unlike the clear lipid lowering effects of E40K mutation, the association of E40K mutation with T2D was not evident until lately. In this regard, the participants of the DiscovEHR human genetics study showed that carriers of E40K have lower fasting glucose levels and have less chances of developing T2D [40]. Similarly, the Million Veteran Program also shows that E40K is associated with reduced risk for T2D [41, 42].

As described above, the impact of ANGPTL4 in lipoprotein metabolism is well established. However, its potential role in glucose metabolism in mice is controversial. ANGPTL4 overexpression in mice has been reported to have different effects on glucose metabolism. These effects range from no effect on glucose metabolism, decrease glucose levels, improve glucose tolerance while also inducing hyperlipidemia, and impairment of glucose tolerance [37, 43–45]. These discrepancies are likely due to differences in the degree and/or site of overexpression of ANGPTL4. In contrast, the studies performed with ANGPTL4 deficient mice seem to be more consistent. ANGPTL4 is located within the glucocorticoid ceramide cascade, whereby depletion of ANGPTL4 decreases ceramide production in liver, which is responsible for insulin resistance [46]. ANGPTL4 deficiency, specifically in ATs, results in increased plasma and adipose LPL activity and enhanced clearance of FA into the AT [47]. Concomitantly, ANGPTL4 deficient mice fed a high fat diet (HFD) showed an increase in

lipolysis and fatty acid oxidation (FAO) in the. This effect was linked to increased PGC1α and UCP-1 activity and a decrease in fatty acid synthase activity [47], which results in a reduced net accumulation of TAGs and diacylglycerols and increased insulin sensitivity in peripheral metabolic tissues including liver and muscle [47]. Further reports support the improvement in glucose metabolism in ANGPTL4 deficient mice and suggest that effects in gut microbiota could participate in this process [40, 48]. Of note, conditional deletion of ANGPTL4 in brown adipose tissues (BAT) also increases both plasma and BAT LPL activity and redirects the shuttling of FAs to BAT, leading to an enhancement in fat tolerance, FAO, thermogenesis during acute cold exposure, and improvement in glucose tolerance in short-term HFD feeding [49].

Overall, these studies have established ANGPTL4 as an important protein associated with metabolic diseases and underscore the potential therapeutic applications.

ANGPTL4 in heart disease—A number of genetic studies have suggested that elevated plasma TAGs are associated to increased risk factors for cardiovascular disease. Several large-scale genetic analyses have shown that the E40K mutation in humans results in reduced circulating TAG levels, increased HDL-C levels and thus conferring protection against CAD. In addition, E40K and T266M polymorphisms predict CAD risk in patients with T2D [50]. Plasma ANGPTL4 levels have been described to have prognostic value for future cardiovascular events indicating the potential of ANGPTL4 as biomarker for CAD [51]. Interestingly, the magnitude of the reduction for CAD as a result of inactivating mutations may be greater than would be expected from circulating TAG effects alone. This suggest that inactivating mutations in the ANGPTL4 locus are cardioprotective via unexplored mechanisms. Indeed, the reduced risk of developing CAD in individuals with E40K mutation is not mediated by decrease in plasma cholesterol levels. Studies using global knockout or transgenic overexpression mouse models suggest both pro- and anti-atherogenic roles of ANGPTL4 [52, 53]. Unfortunately, despite the antiatherogenic effects of ANGPTL4 depletion, the therapeutic potential of inhibitors has been compromised owing to unexpected side effects. ANGPTL4-deficient mice or monkeys, generated through germline deletion or through the use of monoclonal antibodies against ANGPTL4, exhibit severe metabolic and systemic abnormalities upon high-fat diet (HFD) feeding [30, 54], including peritonitis, ascites, anorexia, mesenteric lymphadenopathy and accumulation of lipid-enriched foamy macrophages in mesenteric lymph nodes [29, 30, 54, 55]. Accordingly, transgenic overexpression of ANGPTL4 in ApoE*3-Leiden mice attenuates atherosclerosis primarily by suppressing lipid uptake in macrophages [53]. Conversely, and consistent with this observation, $Ldlr^{-/-}$ mice transplanted with bone marrow from $Angptl4^{-/-}$ mice developed bigger atherosclerotic plaques [55].

Recent published work has also demonstrated that ANGPTL4 expression has both pro- and anti-atherogenic effects depending on the cellular context [47, 55]. While germline deletion of ANGPTL4 resulted in reduced circulating TAGs and atherosclerosis but severe inflammation and metabolic complications [55] as outlined above, the hematopoietic cell-specific deletion of ANGPTL4 avoided these complications but resulted in increased atherosclerosis as a result of increased monocyte proliferation, enhanced lipid accumulation in macrophages and generation of foam cells and overall vascular inflammation [55].

In contrast, absence of ANGPTL4 in white adipose tissue (WAT) decreased circulating TAG and cholesterol levels, when subjected to a high fat diet (HFD) feeding, thus suggesting that ANGPTL4 depletion in white AT was antiatherogenic [47]. Interestingly, hypercholesterolemic adipose deficient ANGPTL4 mice developed smaller atherosclerotic lesions compared to WT mice due to a substantial decrease in circulating lipids, as well as a reduction in proinflammatory cytokines that resulted in reduced endothelial cell (EC) inflammation [47].

Non-metabolic functions of ANGPTL4

ANGPTL4 in angiogenesis and vascular permeability—ANGPTL4 has multifaceted functions and its physiological roles may be largely dependent on the pathological conditions. In addition to be highly expressed in AT and liver and to a lesser extent in heart and muscle, ANGPTL4 is also expressed in tumor cells, macrophages, placental tissue and the vascular endothelium. As previously described, the ability to bind and inhibit the LPL activity has been limited to nANGPTL4 and full length ANGPTL4 while cANGPTL4 has been involved in number of non-metabolic related processes. Indeed, given the similarity in structure with angiopoietins, ANGPTL4 obtained a great deal of attention in anoikis resistance, altered redox regulation, angiogenesis, and vascular permeability [4, 56]. However, the C-terminal domain of angiopoeitin-like proteins, originally described after the initial cloning as Angiopoietins and angiopoietin-related proteins (ARPs) [57–59], is shorter than the corresponding homologous regions within angiopoietins (ANGPT1 and ANGPT2); as a consequence, the C-terminal of these proteins cannot efficiently bind to the endothelial TIE1 or TIE2 receptors [7, 57–59], as such ANGPTL4 still remains as an orphan ligand. Interestingly, ARP1 and ARP2 were shown to induce EC sprouting [59] and ANGPTL3 (ARP3) was described to stimulate EC adhesion and migration and induce blood vessel formation in a rat corneal assay [57]. On the other hand, ANGPTL4 (ARP4) was described to inhibit proliferation, chemotactic activity, and tubule formation of ECs and diminish angiogenesis and vascular leakiness *in vivo*, using a mouse corneal model and Miles assay, respectively [58]. However, also early described as an apoptosis survival factor for ECs but without affecting DNA synthesis or sprout formation [7]. Although ANGPTL4 was describe to function as a potent antiangiogenic factor [58], other reports suggested proangiogenic actions for ANGPTL4 in arthritis, conventional renal cell carcinoma and breast cancer [60–62]. Gene expression profiling during collagen-induced arthritis indicated that *ANGPTL4* was one of the most highly expressed mRNAs early in disease [60] and using recombinant mouse ANGPTL4 promoted endothelial cell survival and formation of tubule-like structure. Similarly, in PPARγ enhanced ErbB2-induced mammary tumorigenesis *in vivo* and tumor angiogenesis through induction of the pro-angiogenic factor ANGPTL4 which was shown to promote vascular endothelial cell migration in vitro [62]. Moreover, *ANGPTL4* mRNA was present in very large amounts in tumoral cells from conventional renal cell carcinomas, ANGPTL4 was produced in the hypoxic areas surrounding necrotic regions [61]. Interestingly, expression ANGPTL4 was found to be induced by hypoxia in human ECs and when tested in the chicken chorioallantoic membrane assay, ANGPTL4 induced a strong pro-angiogenic response, independently of vascular endothelial growth factor [61]. In other pathophysiological settings, ANGPTL4 was also reported to have proangiogenic or antiagiogenic actions [18,

63]. Topical application of recombinant ANGPTL4 accelerated wound reepithelialization in diabetic mice, in part, by improving angiogenesis via the modulation of the expression of several regulatory networks involved in cell migration, angiogenesis, and inflammation. ANGPTL4-induced nitric oxide (NO) production through an integrin/JAK/STAT3-mediated upregulation of inducible nitric oxide synthase (iNOS) expression in wound epithelia, and revealing a hitherto unknown mechanism by which ANGPTL4 regulated angiogenesis via keratinocyte-to-endothelial-cell communication [63]. In line with the initial identification of *ANGPTL4* gen as a hypoxia-induced target *in vitro* in the human microvascular EC line and in vivo in the vessels of ischemic tissues from peripheral artery disease[61], its expression was also increased in the vessels of a mouse model of hindlimb ischemia which also correlated with the accumulation of the full-length protein in ischemic tissues[18]. In response to hypoxia, endogenous ANGPTL4 accumulates in the subendothelial extracellular matrix (ECM), but despite the secreted protein undergoes proteolysis leading to truncated fragments present in the medium, only full-length ANGPTL4 was shown to interact with the ECM in a heparin/heparan sulfate proteoglycan dependent manner. Both matrix-associated and immobilized ANGPTL4 limited the formation of actin stress fibers and focal contacts in the adhering endothelial cells and inhibit their adhesion. Immobilized ANGPTL4 also decreases motility of endothelial cells and inhibits the sprouting and tube formation. Indicating that hypoxic ECs accumulate ANGPTL4 in the ECM and negatively regulates their angiogenic capacities through an autocrine pathway. Another study, revealed that through interaction with matrix proteins integrin β1 and integrin β5, the C-terminal fibrinogen-like domain of ANGPTL4 (cANGPTL4) facilitated cell migration and wound healing. All these studies, despite of the outcome observed, tested the paracrine actions of different forms of ANGPTL4 on ECs expressing ANGPTL4 and, therefore neglected the potential relative contribution of endothelial cell-intrinsic actions.

There are also reported opposite effects regarding its ability to promote metastasis and its effects on vascular permeability. ANGPTL4 was shown to remarkably prevent the metastatic process through the inhibition of vascular permeability, tumor cell motility, and invasiveness of melanoma cells [64]. However, ANGPTL4 was identified as one of the most highly expressed genes in distant metastases [65] and it is associated with breast cancer metastasis to the lungs [66]. In line with this, ANGPTL4 (full-length) was described to promote tumor metastasis by disrupting the integrity of vascular endothelial cell layers and facilitating the passage of breast cancer cells [64, 67]. Other work revealed that tumors produced high levels of cANGPTL4 and stimulated integrin-mediated signaling to maintain an elevated, oncogenic O_2^-:H_2O_2 ratio to confer anoikis resistance to tumor cells via autocrine adhesion mimicry [68]. Furthermore, tumor-derived cANGPTL4 instigated the disruption of endothelial continuity by directly interacting with integrin α5β1, VE-cadherin, and claudin-5 in a temporally sequential manner. Tumor-derived cANGPLT4 and recombinant human cANGPTL4 increased vascular permeability *in vitro* and *in vivo*. Using ANGPTL4-deficient and WT mice injected with either control or ANGPTL4-knockdown tumors confirmed that cANGPTL4 induced vascular leakiness and facilitated lung metastasis in mice. Mechanistically, the effect of cANGPTL4 in vascular permeability was described to be mediated through integrin α5β1-mediated PAK/Rac signaling that weakened EC–EC contacts and increased vascular leakiness [17]. Despite the plethora of evidence implicating

ANGPTL4 in cancer metastasis, the heterotypic role of ANGPTL4 in vascular integrity remains unclear. These seemingly conflicting results underscore the need to address the function of tumor microenvironment-derived ANGPTL4, while addressing its paracrine and/or autocrine role in ECs and clearly suggest a context-, cell type- and tissue-specific activity.

ANGPTL4 has also been described to promote or limit vascular permeability in different pathophysiological conditions and through different mechanisms. Recent work shows that ANGPTL4 through binding to neuropilin 1 (NRP1) and NRP2 on ECs, leads to the activation of the RhoA/ROCK signaling pathway and breakdown of EC-EC junctions. Treatment with a soluble fragment of NRP1 prevented ANGPTL4 from binding to NRP1 and blocked ANGPTL4-induced activation of RhoA as well as EC permeability in vitro and retinal vascular leakage in diabetic animals in vivo [69]. On the other hand, a previous report showed that ANGPTL4 via αvβ3 interaction limited vascular permeability by increasing adherens and tight junctions integrity and thus affecting pericyte coverage, both leading to impaired angiogenesis and increased vascular leakage that were eventually caught up, suggesting a delay in vessel maturation [70]. Furthermore, in a model of oxygen-induced retinopathy, pathological neovascularization, which results from tissue hypoxia, was also strongly inhibited in ANGPTL4-deficient mice. This study therefore showed that ANGPTL4 tunes endothelial cell junction organization and pericyte coverage and controls vascular permeability and angiogenesis, both during development and in pathological conditions[70]. In another completely different setting, ANGPTL4 was identified as a Wnt signaling antagonist that binds to syndecans and forms a ternary complex with the Wnt co-receptor Lipoprotein receptor-related protein 6 (LRP6). This protein complex is internalized via clathrin-mediated endocytosis and degraded in lysosomes, leading to attenuation of Wnt/β-catenin signaling [71]. Although of potential relevance for the vascular endothelium, this interaction with syndecans [72] affecting EC signaling and functions has not been described.

The controversial role of ANGPTL4 in regulating controlling vascular permeability and angiogenesis seems to derive from the use of different experimental approaches that relied on the utilization of different forms of ANGPTL4 mostly *in vitro* systems, but, as well as to use of whole knockout animal models of ANGPTL4 to address the effect on the endothelium. Additionally, most of these studies only considered the paracrine actions of ANGPTL4 on ECs and neglected a potential cell-autonomous role of ANGPTL4 on ECs where it is also highly expressed.

ANGPTL4 in stem cell expansion—Members of the angiopoietin-like family of proteins, including ANGPTl4, have been shown to bind to paired immunoglobulin-like receptors, which supports ex vivo expansion of HSCs [73]. Remarkably, ANGPTL4 did not effectively stimulate expansion of HSCs. The effect of ANGPTL4 was the hypothesized to be more related to the commitment of progenitor cells rather than on immature self-renewing HSCs. ANGPTL4 was found upregulated in the BM during systemic inflammation and identified as novel cytokines enhancing myeloid cell regeneration [74]. Recombinant murine ANGPTL4 (rm ANGPTL4) stimulated the proliferation of myeloid colony-forming units (CFUs) *in vitro* and upon repeated *in vivo* injections, rmANGPTL4 increased BM progenitor cell frequency and this was paralleled by a relative increase in phenotypically

defined granulocyte-macrophage progenitors (GMPs). Furthermore, *in vivo* treatment with rmANGPTL4 resulted in elevated platelet counts in steady-state mice while allowing a significant acceleration of reconstitution of platelets after myelosuppressive therapy. The administration of rm ANGPTL4 increased the number of $CD61^{+}CD41^{low}$-expressing megakaryocytes (MK) in the BM of steady-state and in the spleen of transplanted mice. Furthermore, rmANGPTL4 improved the *in vitro* differentiation of immature MKs from hematopoietic stem and progenitor cells. Mechanistically, using a signal transducer and activator of transcription 3 (STAT3) reporter knock in model, we show that rmAngptl4 induces *de novo* STAT3 expression in immature MK which could be important for the effective expansion of MKs after myelosuppressive therapy [74].

In response to hypercholesterolemia, the BM and spleen overproduce pro-inflammatory Ly-$6C^{hi}$ monocytes that can infiltrate and preferentially accumulate and differentiate to macrophages in lesions [75]. In the context of atherosclerosis, ANGPTL4 deficiency in hematopoietic cells also promotes lipid accumulation in common myeloid progenitor (CMP) cells and leads to their increased proliferation and differentiation into leukocytes [55]. Overall, in hematopoietic cells, ANGPTL4 serves as a protective molecule that prevents lipid overloading and limits the proliferation of progenitor cells and generation of foam cells. Therefore, hematopoietic loss of ANGPTL4 increases atherosclerosis burden. This finding is consistent with a subsequent study that showed LPL deficiency reduces expression of master transcription factors (PU.1 and C/EBPα) and CSF (colony stimulating factors) and reduces proliferation and differentiation of progenitor cells into monocytes [76].

Mesenchymal stem cell–mediated (MSC-mediated) regeneration therapy has been widely applied for cardiovascular disease [77, 78]. MSCs effect tissue repair largely via their paracrine factors and by activating endogenous progenitor cells, not only by cell replacement [78]. Inflammation is a normal response to pathological stress, and chronic degenerative disorders are usually associated with uncontrolled inflammation. In the setting of inflammation, it was recently reported that MSCs highly induced ANGPTL4 to suppress activation of inflammatory macrophages. ANGPTL4-deficient MSCs failed to inhibit the inflammatory macrophage phenotype [79]. In inflammation-related animal models, the injection of coculture medium or ANGPTL4 protein increased the anti-inflammatory macrophages in both peritonitis and myocardial infarction. In particular, cardiac function and pathology were markedly improved by ANGPTL4 treatment. Inflammatory mediators increased retinoic acid–related orphan receptor α (RORα)-mediated transcription of ANGPTL4 in MSCs and contribute to the anti-inflammatory activity of MSCs against macrophages under pathological conditions [79].

CONCLUDING REMARKS

There is an overall consensus that ANGPTL4 is a powerful regulator of lipid metabolism. At this point, there is sufficient evidence showing an undeniable association of ANGPTL4 with the development of cardiometabolic disorders. The large number of human genetic studies in recent years showing the association of loss of function mutations of ANGPTL4 with the risk of developing CVD and T2D intensified that interest. However, he severe inflammatory effects observed in mice and monkeys injected with antibodies against ANGPTL4 stopped

the therapeutic use of ANGPTL4 blocking. In the present review, we have highlighted the array and diversity of the functions attributed to ANGPTL4 that in turn are different across different tissues and cell types, thus suggesting that tissue-specific targeting of ANGPTL4 expression might be a more beneficial approach.

Notwithstanding the large amount of literature on ANGPTL4's functions, there are still too many open questions. For example, the subcellular localization of ANGPTL4 and how it is released from cells and if its processing is necessary for its actions is still unclear. Similarly, the molecular details of how ANGPTL4 interacts with its partners to regulate cell specific functions are not fully understood. For instance, how ANGPTL4 interacts to cell surface of ECs and binds to LPL needs further investigation. Moreover, the molecular mechanisms by which ANGPTL4 regulates other cellular functions including angiogenesis and vascular permeability and the distinction between autocrine vs paracrine regulation of these processes, as well as cell-autonomous actions of ANGPTL4 needs to be address in more detail.

Despite accumulating and compelling data supporting multiple roles for ANGPTL4 little is known about the molecular events that mediate its biological effects.

ACKNOWLEDGEMENTS

The authors apologize to the many scientists whose contributions to the field could not be acknowledged owing to space limitations.

FINANCIAL SUPPORT AND SPONSORSHIP

Fernández-Hernando and Suárez Laboratories are supported by the NHBLI (R35HL135820 to CF-H and R01HL105945 and R01HL135012 to YS) and the American Heart Association (16EIA27550005 to CF-H).

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Key points

- ANGPTL4 regulates circulating TAGs and lipid distribution across different tissues. This action is mediated via inhibition of LPL.
- Human genetic studies have shown that of function mutations in ANGPTL4 reduces TAGs and confers protection against CAD and T2D. As such, ANGPTL4 has been highly examined as a potential therapeutic target.
- Tissue specific targeting of ANGPTL4 could limit the adverse effects observed in systemic application of ANGPTL4 antibodies as ANGPTL4 seems to have distinct cell intrinsic and paracrine effects.
- In addition to the well-recognized effects on the regulation of lipid homeostasis, ANPTL4 has been described to regulate angiogenesis and vascular permeability.