The role of high-density lipoproteins in the regulation of angiogenesis

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Abstract
Angiogenesis is important for postnatal physiological processes including tissue neovascularization in response to an ischaemic injury. Conversely, uncontrolled inflammatory-driven angiogenesis can accelerate atherosclerotic plaque and tumour growth. Angiogenesis-associated diseases are highly prevalent globally, with cardiovascular-related disorders and cancer being the leading causes of mortality worldwide. A vast amount of research has been conducted on the vasculoprotective effects of high-density lipoproteins (HDLs) and while current HDL-raising therapies to date have not yielded the desired benefits clinically, its role in angiogenesis is yet to be fully elucidated. Epidemiological studies report positive correlations between elevated HDL levels and improved prognosis in both ischaemia- and inflammatory-driven pathologies, in which angiogenesis plays a key role. This review focuses on current evidence from epidemiological and prospective studies, coupled with animal models and mechanistic studies that highlight the ability of HDL to conditionally regulate angiogenesis.

Keywords
High-density lipoprotein • Angiogenesis • Inflammation • Ischaemia

1. Introduction
High-density lipoproteins (HDLs) were first hailed as being atheroprotective when a landmark study (Framingham Heart Study) found that the risk of myocardial infarction (MI) increased by 25% for every 0.13 mmol/L decrement in serum HDL below median values.1 Similar inverse associations have subsequently been found for apolipoprotein (apo)A-I, the main protein constituent of HDL.2 – 4 Despite the overwhelming evidence for the atheroprotective effects of HDL, recent HDL-raising clinical trials have been unable to show cardiovascular benefit, even with increases in HDL cholesterol of 11–70%.5 – 8

There remains, however, a strong interest in HDL as decades of epidemiological and preclinical research have demonstrated that HDL exhibits a plethora of beneficial properties including anti-inflammatory, antioxidant, anti-thrombotic, endothelial protective, and its well-characterized role in reverse cholesterol transport. A continuing understanding of the vascular biological effects of HDL, outside of its effects on atherosclerosis, is still required to reveal novel vascular functions/mechanisms that may ultimately facilitate the translation of HDL for clinical benefit.

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is a prerequisite for development and plays a critical role in postnatal physiological processes such as wound healing and in tissue neovascularization in response to ischaemia. Despite the importance of angiogenesis for survival, an imbalance in its regulation can cause deleterious effects in inflammatory-driven diseases such as atherosclerosis and cancer, resulting in an acceleration of the disease progression. Epidemiological studies suggest that there is a positive correlation between HDL levels and improved prognosis in angiogenesis-associated diseases. Furthermore, newly emerging preclinical and cellular studies support a role of HDL in the regulation of angiogenesis.

2. HDLs in health and disease
HDLs are comprised of several distinct subpopulations that predominantly exist as spherical particles. The outer surface of HDL contains apolipoproteins and phospholipids, which surround a core of cholesterol esters and triglycerides. Apolipoprotein (apo)A-I is the main apolipoprotein constituent of HDL.2 – 4 Despite the overwhelming evidence for the atheroprotective effects of HDL, recent HDL-raising clinical trials have been unable to show cardiovascular benefit, even with increases in HDL cholesterol of 11–70%.5 – 8

These are the ATP-binding cassette transporters ABCA1 and ABCG1 and the cell membrane protein scavenger receptor-BI (SR-BI). Current data suggest that, in endothelial cells, ABCA1 and ABCG1 mediate the activation of intracellular signalling pathways primarily through the efflux of cholesterol and oxysterols to apoA-I/HDL.
An interaction between HDL and SR-BI initiates the greatest number of reported intracellular signalling pathways, and there is evidence that some of these are activated independently of cholesterol efflux.6

While healthier people have higher HDL levels7 and confounding is a potential concern when drawing conclusions about the functions of HDL from population data, several epidemiological and prospective studies have demonstrated associations between HDL and diseases associated with angiogenesis. HDL has potent atheroprotective properties,8 with a strong inverse relationship reported between plasma HDL levels and atherosclerosis in multiple vascular beds9 and improved plaque stability.10–12 Plaque macrophages express numerous pro-angiogenic growth factors and cytokines that promote plaque neovascularization, which subsequently accelerate plaque growth13,14 and increase plaque instability/rupture.15 The inhibitory actions of HDL on plaque size and inflammation may indicate a causal relationship between HDL and the attenuation of inflammatory plaque angiogenesis. Furthermore, there is a striking inverse relationship between serum HDL and the risk of acute coronary events including MI15 as well as an improved prognosis following MI.16,17 This suggests a potential causal relationship between HDL and improved coronary collateralization in the context of ischaemia. In addition to the well-established link between plasma HDL and cardiovascular disease, an increasing body of epidemiological data demonstrates an inverse association between serum HDL and the incidence of a variety of malignancies.20–25

To date, the vascular biological effects of HDL have been attributed to its well-characterized role in reverse cholesterol transport as well as its anti-inflammatory and antioxidant properties.26 However, given that HDL has anti-atherosclerotic effects, improves prognosis following MI, and suppresses tumour growth and metastasis, it strongly suggests that HDL plays a role in the regulation of angiogenesis. More specifically, it appears that the regulation of angiogenesis by HDL may be dependent on the pathophysiological context such that it is able to inhibit inflammatory-driven pathological angiogenesis, which is associated with atherosclerosis and cancer, but yet augment ischaemia-mediated physiological angiogenesis that is critical for tissue regeneration following ischaemia or wounding. These associations and causal relationships identified in the literature are supported by an increasing amount of preclinical and in vitro data in relevant models of angiogenesis.

3. Ischaemia-mediated angiogenesis and HDL

Physiological ischaemia-mediated angiogenesis is triggered when there is a chronic imbalance in tissue oxygen supply vs. demand following injury or vessel occlusions, which restricts the supply of blood. The ensuing hypoxia induces neovascularization of the ischaemic tissue. In atheroocclusive disease, this response is a key stimulus for the formation of a coronary collateral circulation,27 which is an important factor for survival following MI28 and is causally related to improved prognosis in the context of stable chronic coronary disease.18 Epidemiological studies have established a strong inverse association between the risk of MI and serum HDL levels.1 For example, the Framingham Heart Study demonstrated that the risk for MI increases by ~25% for every 0.13 mmol/L decrement in serum HDL below median values. Furthermore, prospective studies show that elevated serum HDL is associated with improved survival and prognosis following MI.29,29 This indicates that, in the context of tissue ischaemia following an infarction, HDL may accelerate coronary collateral formation and extend survival time post-MI. Consistent with this, preclinical studies also find that apoA-I/HDL promote functions associated with physiological angiogenesis. For example, adenoviral overexpression and infusions of apoA-I induce increases in the number of circulating endothelial progenitor cells (EPCs),30,31 which are critical for neovascularization, and enhance re-endothelialization in mice32,33 and also suggest that apoA-I/HDL may be involved in vasculogenesis. Furthermore, in vitro studies show that HDL stimulates EC migration, a key angiogenic function.34 Studies by our group and others have also reported that intravenous infusions of apoA-I or reconstituted discoidal HDL (rHDL) promote neovascularization and increase gastrocnemius capillary density in the murine hindlimb ischaemia model.35,36 Recent work has identified a role of the scavenger receptor SR-BI in modulating angiogenesis in vivo using the hind limb ischaemia model.36 The augmentation of neovascularization following infusions of rHDL in wild-type mice was attenuated in SR-BI−/− mice. In vitro EC tubulogenesis assays have also revealed that lentiviral-mediated shRNA knockdown of SR-BI suppresses rHDL-induced tubule formation in the context of hypoxia.37 Moreover, infusion of apoA-I into mice transplanted with SR-BI−/− bone marrow-derived cells attenuated apoA-I-induced increases in circulating EPCs.30 Few studies have investigated the exact components of HDL that may be responsible for their regulation of angiogenesis in the context of ischaemia. However, Tan et al.38 found that lipid-free apoA-I, but not the phospholipid phosphatidylcholine (PLPC, the two components of rHDL), was able to induce in vitro EC tubule formation. Sphingosine-1-phosphate (S1P), which associates with HDL (although at relatively low levels of 2–5% of total HDL protein), also promotes tubulogenesis through the Gi/Ras/ERK pathway.38

Mechanistic studies have reported that HDL-induced EC migration and re-endothelialization are dependent on the same kinase signalling proteins required for physiological angiogenesis including phosphatidylinositol 3-kinase (PI3K), Akt, and mitogen-activated protein kinase (MAPK), all of which are known to drive the hypoxia-inducible factor-1α (HIF-1α)/vascular endothelial growth factor (VEGF) pathway.39,40 The transcription factor HIF-1α is the pivotal driver of physiological angiogenesis. Recent in vitro studies have revealed that, under hypoxic conditions, rHDL augments the protein levels of HIF-1α above levels following stimulation by hypoxia alone. This highlighted a key role of HDL in the regulation of hypoxia-driven angiogenesis. While most transcription factors are regulated transcriptionally, the stability and transcriptional activity of HIF-1α is regulated post-translationally. In aerobic conditions, prolyl hydroxylase domain proteins (PHD1–3) hydroxylate the proline residues on HIF-1α, which allows the von Hippel–Lindau ubiquitin ligase complex to target HIF-1α for ubiquitination and proteasomal degradation.41–44 Conversely, a decrease in intracellular [O2] triggers the activation of the PI3K/Akt signalling pathway resulting in the induction of gene transcription of the ubiquitin ligases Siah1 and Siah2, which target and promote the degradation of PHDs, leading to HIF-1α stabilization.45 Consistent with its role in hypoxia-driven angiogenesis, our group recently reported that HDL modulates the post-translational regulation of HIF-1α in a way that promotes stabilization.46 First, HDL was found to induce the expression of the ubiquitin ligases, Siah1 and Siah2, and suppressed the prolyl hydroxylases, PHD2 and PHD3. siRNA knockdown of Siah1 and Siah2 confirmed the importance of both Sias in mediating the effects of HDL in hypoxia-driven angiogenesis by inhibiting in vitro matrigel tubulogenesis. Additional studies also determined that these effects were mediated by interaction with SR-BI and the PI3K/Akt signalling pathway.37 The components of
rHDL were individually tested in this study and found that Siah2 was up-regulated by both apoA-I and PLPC. PHD2 and PHD3 protein levels were suppressed by PLPC. The rHDL particle, in all tests, was found to be more regulatory than the individual components, but apoA-I and PLPC appear to also exert their own effects.

In hypoxic conditions, HIF-1α translocates into the nucleus, where it complexes with the HIF-β subunit, and binds to the hypoxia response element (HRE). This drives the expression of pro-angiogenic mediators, such as VEGF, angiopoietin, fibroblast growth factor (FGF), matrix metalloproteinases (MMPs), tissue inhibitors of MMPs, and stromal cell-derived factor-1α (SDF-1α), which directs the migration of EPCs to the site of ischaemia. In vitro studies have found that, in the context of hypoxia, rHDL is able to increase VEGF protein levels and the VEGF receptor, VEGFR2. Furthermore, in the murine hindlimb ischaemia model, infusions of apoA-I increased mRNA levels of VEGF and SDF-1α mRNA expression in ischaemic hindlimbs.

Endothelial nitric oxide synthase (eNOS) produces nitric oxide (NO) and both play key roles in the induction of angiogenesis in response to ischaemia. eNOS/NO are downstream of the HIF-1α/VEGF pathway and enhance EC proliferation, EPC mobilization and migration, and increased VEGF expression. In vitro and in vivo studies have found that HDL increases the phosphorylation of eNOS. eNOS has several phosphorylation sites including Ser-116, Ser-617, Ser-635, Ser-1179, and Thr-497, and a comprehensive in vitro study found that eNOS activity can be up-regulated by physiological concentrations of HDL and apoA-I through regulation of phosphorylation sites on the eNOS enzyme, specifically the prolonged increase in phosphorylation of Ser-116 and a transient increase at Ser-1179. This may be partially due to AMP-activated protein kinase (AMPK) activation, coupled with dephosphorylation of Thr-497 and are likely to all collectively be responsible for increased eNOS activity by HDL. HDL-induced eNOS activation is mediated by two key signalling pathways, namely PI3K/Akt and MAPK pathways. The link between HDL and eNOS phosphorylation was also confirmed when apoA-I was overexpressed transgenically in vivo in the Ldlr+/− mice fed a high cholesterol diet (HCD). ApoA-I transgene overexpression significantly increased eNOS dimer levels and NOS activity, suggesting that apoA-I and HDL maintain endothelial cell function in HCD-fed mice by preserving active eNOS dimer levels. Additional mechanistic in vitro and in vivo studies report that HDL can reverse the inhibitory interaction of eNOS with the cholesterol-binding protein caveolin 1, resulting in increased eNOS activity.

A role of both SR-BI and ABCG1 has been identified in the HDL-induced activation of eNOS and NO production. Yuhanna et al. was the first to describe the SR-BI dependence for HDL activation of eNOS. The PDZ domain of the C-terminus of SR-BI was found to be critical for HDL/SR-BI-induced eNOS activation as well as subsequent EC migration and vascular re-endothelialization. Another study demonstrated that HDL suppressed the binding of eNOS with caveolin 1 in an ABCG1-dependent manner.

HDL-associated S1P is found to activate eNOS through PI3K/Akt pathways and via the S1P1 receptor in a range of EC types. Paraoxonase 1 (PON1) is an antioxidant that is carried on HDL and has also been identified to play a key role in mediating HDL-induced eNOS expression.

Taken together, cellular, preclinical, and epidemiological studies strongly suggest that HDL augments ischaemia-mediated physiological angiogenesis. This is shown in Figure 1.

4. Inflammatory-driven angiogenesis and HDL

In contrast to ischaemia-induced neovascularization, pathological angiogenesis is driven by inflammation. This occurs via two major pathways: (i) a direct effect of inflammatory cytokines/chemokines on endothelial cell proliferation and migration and (ii) an indirect effect that involves the initial recruitment of macrophages to the inflamed site, which in turn secrete a host of pro-angiogenic factors including VEGF, FGF, bFGF, tumour necrosis factor-α (TNF-α), and granulocyte macrophage-colony stimulating factor. Macrophages are therefore potent stimulators of pathological angiogenesis, which also accelerate plaque growth/instability. Key inflammatory mediators including TNF-α and cytokines/chemokines such as monocyte chemoattractant protein-1 (MCP-1) have all been shown to directly induce angiogenesis both in vitro and in vivo. Epidemiological studies have found that there is a striking inverse relationship between HDL and acute coronary events, while others have reported an association between low serum HDL and susceptibility to plaque rupture. Furthermore, human intervention studies found rHDL infusions caused reductions in plaque lipid content and macrophage size.

The transcription factor nuclear factor κB (NF-κB) is a pivotal promoter of pathological inflammatory-driven neovascularization. Under normal conditions, NF-κB is sequestered within the cytosol by the inhibitor of κB (IκB) proteins. Upon activation by inflammatory stimuli, the IκB kinase (IKK) enzyme complex is activated and phosphorylated, resulting in IκB phosphorylation. Of the three IKK subunits (α, β, and γ), the IKKβ subunit is primarily responsible for the phosphorylation of IκB. Upon phosphorylation, the IκB complex is targeted for ubiquitination and subsequent proteasomal degradation, freeing NF-κB to translocate into the nucleus where it binds to the NF-κB response element to initiate the transcription of MMPs and cell adhesion molecules (CAMs) including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). HDL has potent inhibitory effects on the activation of NF-κB. rHDL is found to inhibit each key step in the NF-κB activation pathway including IKK activity, the phosphorylation of IκB, and the translocation of NF-κB to the nucleus. This has been found in vitro studies in endothelial cells and monocytes, the important cell types in atherogenesis, and inflammatory-driven angiogenesis. Human intervention studies with infusions of rHDL have also reported a reduction in the expression of the inflammatory marker VCAM-1 in plaques and an attenuation of monocyte activation. Animal studies show that infusion of rHDL/apoA-I into rabbits with non-occlusive collars reduces aortic endothelial expression of cell adhesion markers VCAM-1 and ICAM-1, matrix metalloproteinases including MMP9, plaque macrophage content, and improves plaque stability. These observations were also seen in the atherosclerosis-prone apoE knockout mouse model, whereby apoA-I HDL raising in these mice suppresses the progression of atherosclerotic lesions and remodels them to a more stable phenotype. Furthermore, an apoA-I mimetic peptide (ETC-642) reduced VCAM-1 and RANTES (CCL5) expression.
Figure 1  Schematic representation of the role of HDL in ischaemia-mediated angiogenesis. (A) Under normoxic conditions, HDL stimulates EC migration and re-endothelialization via the MAPK signalling pathway. HDL also induces eNOS production, a key stimulator of NO production, that is critical for EC proliferation, EPC mobilization and function, VEGF induction, and angiogenesis. The actions of HDL on eNOS are mediated by its interaction with the ATP-binding cassette transporter ABCG1 and the interaction between HDL-associated S1P and the S1P₁ receptor. HDL suppresses the binding of eNOS with caveolin 1 (CAV1) in an ABCG1-dependent manner thereby augmenting eNOS levels. Additionally, HDL-associated S1P activates eNOS via the PI3K/Akt pathway through the S1P₁ receptor. (B) In hypoxia, HDL augments ischaemia-mediated angiogenesis via post-translational modulation of the key hypoxic transcription factor, HIF-1α. These effects are mediated through the receptor SR-BI to activate the PI3K/Akt pathway, which induces expression of the ubiquitin ligases (Siahs). The Siahs inhibit the expression of PHD2/3. This prevents the ubiquitination and degradation of HIF-1α, allowing it to accumulate, translocate to the nucleus, bind to HREs, and drive the transcription of key angiogenic factors (e.g., VEGF, VEGFR2, and CXCL12). These promote EC tubulogenesis, proliferation, and migration, key cellular processes required for angiogenesis.
of apoA-I/HDL. Silencing of ABCA1 in HCAECs attenuated the inhibitory effects of a ‘5A’ mimetic peptide on adhesion molecule expression. 5A is found to specifically interact with ABCA1 and promote cholesterol efflux, which may therefore play an important role in the anti-inflammatory properties of 5A.81 Another study identified that ECs deficient in ABCG1 are found to have elevated levels of inflammatory chemokines, ICAM-I and IL-6, as well as impaired cholesterol efflux. A role of SR-BI in the anti-inflammatory properties of HDL has also been identified. Silencing SR-BI in ECs abolished HDL-mediated induction of the anti-inflammatory protein 3β-hydroxy-steroid-Δ24 (DHCR24), which correlated with suppression of NF-κB and VCAM-1.77 This was found to be independent of cholesterol efflux.

The components of HDL that are important for its anti-inflammatory effects have not been entirely characterized; however, the phospholipid content of HDL appears to be important and this is dependent on the fatty acid composition of the phospholipid as to the extent at which rHDL can suppress inflammatory proteins such as VCAM-1.92 S1P is shown to inhibit TNF-α-induced E-selectin in ECs. HDL lysosphingolipids inhibit MCP-1 expression in rat aortic explants.83 Also, HDL isolated from PON11−/− mice failed to suppress VCAM-1, indicating a key role of PON1 in the anti-inflammatory properties of HDL.

The pro-angiogenic growth factor VEGF not only has HIF response elements (HREs) in its promoter region, but also NF-κB response elements. Its expression is therefore stimulated under inflammatory conditions. Similarly, VEGFR2 has NF-κB response elements in its promoter. Recent studies reported that, in contrast to the augmentation of VEGF/VEGFR2 in hypoxia, rHDL inhibited the expression of these proteins in the context of inflammation (TFN-α).34 Consistent with an inhibitory role of HDL in the context of inflammation, in vitro matrigel tubulogenesis assays found that following stimulation with either TFN-α or macrophage-conditioned media, rHDL inhibited tubule formation. There were also significant reductions in EC migration and proliferation following the same inflammatory stimuli. Furthermore, in the non-occlusive peri-arterial collar model of inflammatory-driven angiogenesis, infusions of apoA-I significantly inhibited neovessel formation, macrophage infiltration, and the expression of inflammatory proteins MCP-1 and TFN-α.34

A recent study identified that ABCA1 and ABCG1, via stimulation of cholesterol efflux, played a key role in apoA-I inhibition of angiogenesis.84 This was demonstrated in vitro in human umbilical vein endothelial cells and in vivo in zebra fish. The apoA-I-binding protein (AIBP) was also shown to play a role in the regulation of VEGFR2 via ABCA1/ABC1-mediated cholesterol efflux. The HDL/AIBP complex-induced cholesterol efflux caused a reduction in lipid rafts and disrupted dimerization of VEGFR2.84

The anti-inflammatory and anti-angiogenic effects of HDL are not just restricted to the suppression of the NF-κB pathway. As mentioned above, macrophages act as important orchestrators of inflammatory-driven angiogenesis. As part of the innate immune system, macrophages rely on several families of signaling receptors to detect tissue damage, one of which being the Toll-like receptor (TLR) family.85 TLR activation results in the expression of pro-inflammatory cytokines.86 ATF3 is a key inducible transcriptional regulator that is induced by TLR stimulation, but acts in a negative-feedback fashion to restrict excessive production of pro-inflammatory cytokines including TFN-α.87–89 HDL was recently reported to suppress TLR ligand-induced inflammation via increased activation of ATF3.90 Using a systems biology approach, ATF3 was found to be an HDL-inducible target gene in macrophages, and that the protective effects of HDL against TLR-induced inflammation were fully dependent on ATF3 in vitro and in vivo.91 ATF3 has been linked with inflammatory-driven angiogenesis.87,89 Moreover, one of the downstream repression targets of ATF3, Id1, regulates angiogenesis by modulating thrombospondin 1 and VEGF expression.91,92

A number of studies have investigated in detail how inflammatory disease can modify the HDL particle in terms of its composition, which in turn subsequently alters its function. For example, serum amyloid A (SAA) is found within HDL. There is some discrepancy in the literature as to the effect changes in HDL SAA content has on the anti-inflammatory properties of HDL. SAA content in HDL increases in coronary heart disease (CHD) patients84 and acute coronary syndrome (ACS) patients94 as well as patients with inflammatory diseases such as end-stage renal disease (ESRD).95 In ESRD and acute phase response patients, SAA-enriched HDL was found to have less potent anti-inflammatory properties.95,96 Conversely, in another study, SAA-enriched HDL from ACS patients inhibited VCAM-1 in ECs to the same extent as unmodified HDL.97 Another study found that HDL from coronary artery disease (CAD) and ACS patients was unable to suppress endothelial cell apoptosis in vitro and in vivo.98 Changes in HDL clusterin and apoC-III content in the HDL of CAD and ACS patients were also found to be directly related to the effects on apoptosis, whereby reduced clusterin and increased apoC-III content increased the pro-apoptotic protein t-bid and caspase-3 activation. In another study, HDL isolated from CAD and ACS patients failed to activate eNOS, NO production, or promote endothelial repair.57 This alteration in HDL function was attributed to a reduction in HDL-associated PON1 activity. eNOS inactivation was identified to be mediated via the lectin-type oxidized LDL receptor 1 (LOX-1) and subsequent activation of PKCβII.

Other changes in HDL composition under inflammatory conditions include increases in HDL triglyceride, which is found in CHD patients99 and type II diabetics.100 Furthermore, decreases in phospholipid content can be caused through psoriasis.101 Both an increase in HDL triglyceride and a decrease in phospholipid content reduce the cholesterol efflux capacity and anti-inflammatory effects of the modified HDL101. In summary, inflammatory diseases such as CHD, ESRD, type II diabetes, and psoriasis can cause multiple changes in HDL composition. These changes suppress the ability of HDL to exert anti-inflammatory and anti-apoptotic effects as well as induction of eNOS and endothelial repair. These are all key mechanisms that regulate angiogenesis. This strongly suggests that modified HDL generated in inflammatory disease is likely to affect its modulation of angiogenesis.

Taken together, there is a vast amount of evidence demonstrating that HDL/apoA-I exhibits anti-inflammatory and anti-angiogenic effects, in particular via suppression of NF-κB and macrophage activation. However, in diseases of inflammation, the composition of HDL can be modified which may have consequences for HDL regulation of angiogenesis. This is shown in Figure 2.

5. HDL and tumour angiogenesis

Tumour growth depends implicitly on new vasculature, as tumour cells become distant from nearby vessels.102,103 Furthermore, tumour neovessels accelerate tumour growth by allowing the passage of inflammatory cells that secrete pro-angiogenic factors.104 Rapid tumour expansion, through neovascularization, increases the likelihood of metastasis as tumours have poorly developed lymphatics so secreted tumour cells are shunted to the peripheral lymph nodes.105 Hypoxia is a common characteristic of locally advanced solid tumours that
Figure 2  Schematic representation of the role of HDL in inflammatory-driven angiogenesis. HDL inhibits inflammatory-driven angiogenesis via either direct effects on endothelial cells or indirect effects on macrophages. (A) In endothelial cells, the anti-inflammatory effects of HDL are mediated by the cell surface receptor SR-BI and subsequent inhibition of the NF-κB signalling pathway, the key promoter for pathological angiogenesis. This, in turn, results in the suppression of HIF-1α, TNF-α, VEGF, VEGFR2, and CCL2, causing a reduction in key inflammatory angiogenic processes including tubulogenesis, proliferation, and migration. Additionally, HDL inhibits the expression of the CAMs including VCAM-1 and ICAM-1, resulting in the suppression of macrophage recruitment. The ATP-binding cassette transporters ABCA1 and ABCG1 mediate the anti-inflammatory effects of apoA-I/HDL via stimulation of cholesterol efflux to reduce inflammation, disrupt lipid rafts, and subsequently reduce dimerization of VEGFR2. Furthermore, HDL-associated S1P inhibits TNF-α-induced E-selectin in endothelial cells resulting in the suppression of CCL2. (B) HDL also suppresses expression of the chemokine receptors CCR2 and CX3CR1 in plaque macrophages. The effects of HDL are not restricted to the NF-κB signalling pathway and also promotes the expression of ATF3, a key inducible transcription factor in macrophages known to suppress TLR ligand-induced inflammation, and thereby inhibits downstream TLR targets including TNF-α, Id1, VEGF, and thrombospondin 1 (THBS1). Finally, HDL reduces circulating levels of chemokines CCL2 and CCL5, preventing the recruitment of monocytes/macrophages to the atherosclerotic plaque.
has been linked with diminished therapeutic response and malignant progression.\textsuperscript{105} As the tumours grow, it rapidly outgrows the available blood supply, resulting in portions of the tumour with hypoxic regions.

Epidemiological studies have reported a strong inverse association between serum HDL levels and the incidence of breast,\textsuperscript{20–22,25} lung,\textsuperscript{23} and prostate cancer.\textsuperscript{24} Prospective studies also report that low serum HDL is associated with increased incidence of colorectal cancer.\textsuperscript{106} HDL levels were found to be lower in patients with metastatic tumours than in those without metastases,\textsuperscript{107} suggesting that HDL may have an impact on tumour metastasis. However, a study conducted on a cohort of type II diabetic patients found that this association could be partially attributed to reverse causation, whereby it appears that low HDL precedes the tumour but in reality the subclinical tumour precedes low HDL.\textsuperscript{108} However, very little is known about how HDL can exert anti-tumorigenic effects and whether inhibition of angiogenesis is one of its mechanisms of action.

Previous in vitro and in vivo studies have shown that HDL can augment angiogenesis in hypoxic conditions, but yet other studies show that, in the context of inflammation, HDL inhibits angiogenesis. Given that tumorigenesis is stimulated by both hypoxia and inflammation, the effects of HDL on tumour neovascularization and growth may be either neutral or varied depending on the cancer type.

A study conducted in a mouse model of ovarian cancer showed that mice expressing the human apoA-I transgene had increased survival and decreased tumour development, when compared with wild-type littermates, following injection of mouse ovarian epithelial papillary serous adenocarcinoma cells (ID-8 cells).\textsuperscript{109} Quantitative analysis of tumour-associated blood vessels directly found a significant decrease in the number of vessels in the apoA-I transgenic mice compared with apoA-I knockout mice. The apoA-I transgenic animals also had reductions in tumour vessel area, length, density, and size.\textsuperscript{110} An apoA-I mimetic peptide (L-4F) delivered either subcutaneously or orally reduced the viability and proliferation of CT26 cells, a mouse colon adenocarcinoma cell line. L-4F also decreased CT26 cell-mediated tumour burden in BALB/c mice coupled with a significant decrease in CD31-positive neovessel expression in the L-4F-treated tumours.\textsuperscript{111}

Another study showed that the anti-tumorigenic effects of the apoA-I mimetic peptide L-5F are, in part, to the inhibition of VEGF/bFGF-mediated angiogenesis. Both microCT scanning and immunohistochemistry staining demonstrated that the quantity and size of vessels in tumour tissues were decreased in mice receiving the apoA-I mimetic peptide L-5F, compared with mice that did not receive L-5F peptide while the in vitro studies showed that L-5F inhibits VEGF/bFGF-induced proliferation, migration, invasion, and tube formation of ECs in a dose-dependent manner and that these effects are mediated by the inhibition of VEGFR\textsuperscript{2}.\textsuperscript{112}

Taken together, these studies suggest that HDL has anti-tumorigenic properties and there is evidence that this is via inhibition of angiogenesis. This is likely to depend on the type of cancer as some are more vascularized than others. Variations in the pathological stimulus driving the growth of different tumours, be it either inflammation or hypoxia, could also impact on the effectiveness of apoA-I/HDL on tumour neovascularization.

6. Evidence for the multifunctional regulation of angiogenesis by HDL

This review has highlighted the increasing evidence that HDL not only augments hypoxia-mediated angiogenesis but also inhibits inflammatory-driven neovascularization. This suggests that the regulation of angiogenesis by HDL is dependent on the pathophysiological context. One previous example of this was demonstrated in a study using the apoA-I mimetic peptide, D-4F. This peptide significantly increased the vascular expression and activity of haeme oxygenase-1 (HO-1).\textsuperscript{113} HO-1 is induced by hypoxia to facilitate angiogenesis in response to ischaemia, but conversely also inhibits leucocyte infiltration by suppressing cytokine expression and consequently reduces inflammation-mediated neovascularization.\textsuperscript{114} Our group recently confirmed these observations by directly comparing the effects of apoA-I/HDL on angiogenesis in both hypoxic/ischaemic and inflammatory conditions. This was tested using key in vitro angiogenic functional assays (proliferation, migration, and tubulogenesis) and in two pathophysiological relevant murine models.\textsuperscript{34}

Mechanistically, the key to the conditional regulation of angiogenesis by HDL may be VEGF. VEGF uniquely contains both HIF-1α and NF-κB response elements in its promoter region and is therefore stimulated under both hypoxic and inflammatory conditions. HDL stabilizes HIF-1α in hypoxia via modulation of its post-translation regulation, and in inflammation has striking inhibitory effects on each stage of the NF-κB activation pathway.

7. Conclusions and future directions

Angiogenesis is a complex process involving multiple cell types, growth factors, and signalling pathways and, depending on the disease pathophysiology, can have either beneficial or deleterious effects. Epidemiological and prospective studies have reported a strong association between HDL levels and the prognosis of a range of angiogenic diseases such as MI, atherosclerosis, and cancer. This review provides evidence that the effects of HDL extend beyond its cardioprotective effects and suggests that HDL conditionally regulates angiogenesis. This may be a valuable vascular biological property of HDL that if targeted correctly to both promote physiological angiogenesis and yet inhibit inflammatory-driven neovascularization could have great therapeutic potential. A greater understanding into the vascular biological effects of HDL may ultimately facilitate the development of novel treatments for therapeutic intervention in a host of angiogenic diseases.

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HDL and angiogenesis


