



Review article

Angiogenesis in the atherosclerotic plaque

Caroline Camaré^{a,b,c}, Mélanie Pucelle^a, Anne Nègre-Salvayre^{a,*}, Robert Salvayre^{a,b,c}^a INSERM - I2MC, U-1048, 1 avenue Jean Poulhès, BP 84225, 31432 Toulouse cedex 4, France^b Université Paul Sabatier Toulouse III, Faculty of Medicine, Biochemistry Département, Toulouse, France^c CHU Toulouse, Rangueil, 1 avenue Jean Poulhès, TSA 50032, 31059 Toulouse Cedex 9, France

ARTICLE INFO

Keywords:

Neovascularization
Angiogenesis
VEGF
Atherosclerosis
Hypercholesterolemia

ABSTRACT

Atherosclerosis is a multifocal alteration of the vascular wall of medium and large arteries characterized by a local accumulation of cholesterol and non-resolving inflammation. Atherothrombotic complications are the leading cause of disability and mortality in western countries. Neovascularization in atherosclerotic lesions plays a major role in plaque growth and instability. The angiogenic process is mediated by classical angiogenic factors and by additional factors specific to atherosclerotic angiogenesis. In addition to its role in plaque progression, neovascularization may take part in plaque destabilization and thromboembolic events. Anti-angiogenic agents are effective to reduce atherosclerosis progression in various animal models. However, clinical trials with anti-angiogenic drugs, mainly anti-VEGF/VEGFR, used in anti-cancer therapy show cardiovascular adverse effects, and require additional investigations.

1. Introduction

Atherogenesis is a slowly progressive process characterized by multifocal structural alterations of the vascular wall of medium and large arteries, leading to the formation of atherosclerotic plaques. The pathogenic events of atherogenesis associate endothelial dysfunction and activation, monocyte/macrophage adhesion, activation and migration, local oxidative stress, lipid deposition, extracellular matrix (ECM) synthesis, smooth muscle cell (SMC) migration and proliferation and neovascularization of the plaque [1–3].

In atherosclerosis areas, the local specific conditions (relative anoxia, inflammation, oxidative stress) induce classical and non classical angiogenic factors that promote sprouting angiogenesis from preexisting vasa vasorum [4]. Neovascularization increases the local flow of nutrients and O₂, and may thereby promote plaque progression

and remodeling. However, the incomplete maturation and the fragility of neocapillaries promote intraplaque hemorrhages that may lead to plaque instability and rupture [5]. Clinical trials with anti-angiogenic drugs (except statins) are not yet successful [6–8].

2. The arterial wall and atherosclerotic lesions

2.1. Structure of normal arteries

In mammals, the arterial wall is constituted by three histological layers.

The intima (or tunica intima), the innermost layer in contact with the blood flow, is constituted by a monolayer of endothelial cells and a subendothelial connective tissue layer limited by the internal elastic lamina. Endothelial cells are joined by tight junctions that participate

Abbreviations: AC, adenylate cyclase; ADMA, asymmetric methylarginine; ABCG1, ATP-binding cassette sub-family G member 1; AIBP, ApoA-I binding protein; ApoE, apolipoprotein E; APOE, human ApoE gene; Apoe, animal ApoE gene; ApoE^{-/-} mice, homozygous ApoE-deficient mice; ARNT, aryl hydrocarbon nuclear translocator; COX, cyclooxygenase; CPT1A, carnitine palmitoyl transferase; Dll4, Delta-like-4; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FGF, basic fibroblast growth factor; FGFR, FGF receptor; H₂O₂, hydrogen peroxide; HDL, high density lipoprotein; HIF, Hypoxia inducible factor; HSP, heparan sulfate proteoglycans; Flt-1, fms-like tyrosine kinase-1; HUVEC, human umbilical vein endothelial cell; ICAM-1, InterCellular Adhesion Molecule-1; iNOS, inducible nitric oxide synthase; LDLs, low density lipoproteins; LOX, lipoxigenase; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; NADPH, H⁺, nicotinamide adenine dinucleotide phosphate; NRP-1, neuropilin-1; nNOS, neuronal nitric oxide synthase; NF-κB, nuclear factor kappaB; NO, nitric oxide; PAI-1, plasminogen activator inhibitor 1; nSMase2, neutral sphingomyelinase-2; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PHD, prolyl hydroxylase; PI3K, phosphoinositide 3 kinase; PLC, phospholipase C; PKC, protein kinase C; PUFA, polyunsaturated fatty acid; PPAR, peroxisome proliferator activated receptor; RCT, reverse cholesterol transport; ROCK, Rho associated protein kinase; RTKs, receptor tyrosine kinase; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; S1PR, S1P receptors; SK1, sphingosine kinase-1; SMC, smooth muscle cell; Spns2, S1P carry spinster homolog 2; SRC, Sarcoma tyrosine kinase; TGF-β, transforming growth factor-β; TGFβR, TGF-β receptor; TK, tyrosine kinase; TIMPs, Tissue Inhibitor of Metalloproteinases; TLR, Toll-like receptor; VCAM-1, Vascular Cell Adhesion Molecule-1; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor

* Correspondence to: UMR-1048 - CHU Rangueil, BP 84225, 31432 Toulouse Cedex 4, France.

E-mail addresses: anne.negre-salvayre@inserm.fr (A. Nègre-Salvayre), robert.salvayre@inserm.fr (R. Salvayre).<http://dx.doi.org/10.1016/j.redox.2017.01.007>

Received 29 November 2016; Received in revised form 4 January 2017; Accepted 5 January 2017

Available online 01 February 2017

2213-2317/© 2017 The Authors. Published by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

in intercellular cohesion and by gap junctions involved in intercellular electrochemical coupling. In the normal arterial wall, the subendothelial ECM is a thin layer of connective tissue that constitutes an adhesive scaffold required for the anchorage-dependent survival of endothelial cells, a reservoir of growth factors and a transducer of physical and biochemical changes of the microenvironment. Intimal thickening is one of the earliest stages of atherosclerosis.

The intermediate layer, tunica media, is mainly constituted by SMC and ECM components, including elastin, collagen and proteoglycans. It is separated from the tunica intima by the internal elastic lamina and from the adventitia by the external elastic lamina. In medium-sized muscular arteries, the media is mainly constituted by smooth muscle cells (SMC) surrounded by ECM and its thickness is correlated with the diameter of arteries. The media of the aorta consists of concentric musculoelastic layers that serve to the biomechanical properties (viscoelasticity) of the aortic wall.

The adventitia (tunica adventitia) is the outer layer of the vessel. It is constituted by fibroblasts and a loose connective tissue that contains vasa vasorum. Vasa vasorum are derived from the same vessel or a neighboring vessel (artery or vein), run along the arterial wall and penetrate into the adventitia of arteries where they supply oxygen and nutrients to the vascular wall. In the thoracic aorta, these microvessels penetrate up to 2/3 of the external media, while the intima and the inner part of the media are nourished by diffusion from the vascular lumen. In intimal hyperplasia and atherosclerotic plaque, the vasa vasorum network is extended and penetrates the media and the pathological intima.

2.2. The atherosclerotic plaque

Atherosclerosis is a multifocal slowly progressive process affecting the intima of medium-sized and large arteries [1]. This chronic metabolic and inflammatory process is characterized by the formation of plaques constituted by a cholesterol-rich core (atheroma) surrounded by a fibrous cap (sclerosis). The histological classification describes the progression of lesions: types I and II are early lesions (intimal thickening and fatty streaks), whereas types III to VI lesions correspond to advanced lesions (fibro-lipidic, calcified and complicated plaques) [9–11].

The initial trigger of atherogenesis results apparently from the hemodynamic stress, i.e. turbulent blood flow, which elicits endothelial cell activation in atherosclerosis prone areas (arterial bifurcations) [1]. The activated endothelium exhibits an increased permeability, generates reactive oxygen species (ROS) and expresses inflammatory adhesion proteins and chemokines. The endothelium permeability allows an influx of plasma components, in the subendothelial area, where lipoproteins undergo various modifications, including oxidation. Chemokines and adhesion proteins promote the recruitment of leukocytes. Monocytes take up modified lipoproteins, accumulate lipids (mainly cholesterol esters) and are converted into macrophagic foam cells that form fatty streaks [1,2]. These early lesions may rapidly grow in case of hypercholesterolemia, or may regress if the LDL-cholesterol and other pro-atherogenic factors decrease [12].

Atherogenesis starts early in infancy, and evolves slowly over decades, leading progressively to the formation of plaques characterized by a lipid-rich core surrounded by a fibrous cap constituted by ECM proteins secreted by proliferating SMC in the intima and myofibroblasts. Cholesterol deposition, associated with a local inflammatory response and the secretion of pro-inflammatory cytokines, promotes the progression of atheromatous plaques [3]. Moreover, neoangiogenesis may play a role in plaque growth and complication, as suggested by the study of neovascularization in atherosclerotic lesions and in ruptured plaques associated with thrombotic events [5,13].

3. Angiogenesis in the atherosclerotic plaque

Neovascularisation in 'arteritis' was reported in the late 19th century by Koester [14]. Recent observations confirmed the presence of neocapillaries in atherosclerotic plaques [15,16] and suggested that neoangiogenesis may play a role in the progression of atherosclerotic plaque and complications [13,17]. The vasa vasorum density is higher in atherosclerotic prone areas and is an early event in atherogenesis [18,19]. Moreover, adventitial delivery of adenoviruses encoding VEGF elicits neoangiogenesis and intimal hyperplasia [20], whereas inhibitors of angiogenesis attenuate plaque growth [21,22]. In humans, intraplaque angiogenesis with hemorrhages is mainly associated with thin-cap atheroma, macrophage infiltration and large necrotic cores (i.e. vulnerable plaques). Plaque neocapillaries are often leaky, thus may release intraplaque erythrocytes (hemorrhages). Moreover, intramural hemorrhages induced in rabbit atherosclerotic lesions are associated with increased erythrocyte fragments, iron deposits, foam cells and cholesterol crystal formation [23].

In intimal hyperplasia and atherosclerotic lesions, the predominant angiogenic mechanism is sprouting angiogenesis from pre-existing vasa vasorum [13,24–26]. The angiogenic process can be initiated by hypoxia that induces the expression and release of angiogenic factors (e.g. VEGF), and is down-regulated when the normoxia is restored [24,27]. In atherosclerotic plaques, the local O₂ diffusion from the arterial lumen could be insufficient because of intimal thickening and inflammation. Hypoxic and inflammatory conditions promote the release of angiogenic and inflammatory factors that stimulate sprouting angiogenesis from vasa vasorum [28,29]. This neovascularization allows to supply nutrients and promotes macrophage infiltration, vessel wall thickening, lipid deposition, inflammation and atherosclerotic lesion progression [30].

The initial steps evoked by angiogenic factors (mainly VEGF-A) and NO, involve a vasodilatation and an increased local vascular permeability, the proteolysis of basement membrane and of surrounding ECM and the disruption of cell contact. Migration and proliferation of endothelial cells lead to the formation of the angiogenic bud [31]. The leading cells of the bud, or 'tip cell', are characterized by their migratory behavior and their dynamic filopodia rich in VEGFR-2, which directs the sprouting towards this VEGF gradient [32,33]. Moreover, these cells secrete proteolytic enzymes that degrade the surrounding ECM, thereby facilitating bud expansion. Then, the contraction of cytosolic actin filaments pulls them towards the stimulus (VEGF) [34,35]. Cells following tip cells, named 'stalk cells', proliferate to support the sprout elongation [36]. To construct a structured vascular tube and avoid an anarchical mass migration of endothelial cells, a control system exists between tip cells and stalk cells. This control involves the Notch receptor of stalk cells and its ligand, Delta-like-4 (Dll4) induced by VEGF at the tip cells surface. The contact between neighboring receptor and ligand induces the proteolytic cleavage of the Notch receptor and the cytosolic fragment of Notch down regulates VEGFR-2 expression and induces the stalk cell phenotype [36]. Stalk cells do not develop filopodia, but their proliferation and stretching support the bud expansion [32,37,38]. When two tip cells meet, buds merge and a vascular lumen is created [39]. The new vessel is then stabilized by the interaction of endothelial cells with pericytes and SMC [31]. The synthesis of ECM and basal lamina are stabilized by protease inhibitors (such as TIMPs and PAI-1), which are induced by the shear stress. In contrast, the absence of vessel perfusion induces its regression [33]. Postnatal vasculogenesis, i.e. the formation of new blood vessels from circulating endothelial progenitor cells, seems to play only a minor role in plaque neovascularization [40].

4. Mechanisms of plaque neovascularization

In atherosclerotic areas, the relative hypoxia and the local inflammation may trigger intraplaque angiogenesis mediated by classical

angiogenic factors (e.g. VEGF), growth factors, S1P/S1PR, MMPs, lipids, lipoproteins (LDLs and HDLs) and oxidized lipids [33,41].

4.1. Classical factors involved in angiogenesis

Angiogenesis is a dynamic process regulated by a delicate balance between angiogenic and angiostatic factors, which lead finally to increase, stabilize or reduce the vascular network. A large number of factors, including hypoxic, hemodynamic, and metabolic parameters, may participate in the regulation of angiogenesis in atherosclerotic areas [28,30,42,43]. We describe here the potential role of the classical angiogenic factors, including HIF, VEGF/VEGFR, Angiopoietin-1,2/Tie2 receptor, growth/angiogenic factors (PDGF/PDGFR, b-FGF/FGFR, TGF- β /TGF β R, EGF/EGFR), S1P/S1PR, and metalloproteinases (MMPs) [33].

4.1.1. Hypoxia/HIF

In mammalian tissues, hypoxia is one of the most potent angiogenic stimuli that up-regulates the expression of a variety of genes involved in angiogenesis and erythropoiesis, finally leading to increase O₂ delivery and facilitate metabolic adaptation to hypoxia [44].

The transcription factor HIF (Hypoxia-Inducible Factor) is a crucial regulator of the adaptive response to hypoxic conditions [45,46]. HIF is a heterodimer constituted of HIF-1 α , a cytosolic subunit regulated by O₂ concentration (hypoxia-inducible) and HIF-1 β (also known as ARNT, aryl hydrocarbon nuclear translocator) a constitutively expressed nuclear subunit [47].

HIF-1 α is mainly regulated post-translationally through an O₂-dependent proteolytic pathway. In normoxia, HIF-1 α is hydroxylated by the O₂-dependent prolyl hydroxylase (PHD) on a specific proline residue that is required for pVHL (von Hippel–Lindau tumor protein)-dependent ubiquitination and subsequent proteasomal degradation. In contrast, under hypoxic conditions, the activity of the O₂-dependent prolyl hydroxylase is reduced, thus HIF-1 α hydroxylation is low, its proteasomal degradation is reduced and its transactivation activity is maintained [44,48,49]. The half-life and activity of HIF-1 α are regulated by phosphorylation *via* the PI3K/Akt and MAP kinase pathways which promote the contact with its coactivators, and by various additional post-translational modifications, such as acetylation, hydroxylation on asparagine and by S-nitrosylation. In addition, HIF-1 α could be transcriptionally regulated in a NF- κ B-dependent manner [50].

The nuclear localization signal allows the nuclear translocation of HIF-1 α that forms a complex with HIF-1 β and p300/BP, which binds to the Hypoxia-Response Element (HRE) and transactivates many target genes including VEGF, VEGFR, angiopoietin-2 and NO synthase [44,48,51,52].

In atherosclerotic plaques, HIF activation is induced by the local relative hypoxia resulting from an insufficient O₂ diffusion in the thickened intima, and from an increased O₂ demand due to the local inflammatory response [28,29,53]. Interestingly in a model of arterial injury in ApoE^{-/-} mice, the local overexpression of HIF increased the size of atherosclerotic lesions, while the inhibition of the HIF-pathway by a dominant-negative mutant reduced the expression of VEGF-A, VEGFR1 and VEGFR2 and neointimal hyperplasia [54]. However the role of HIF in atherogenesis is more complex, since in LDLR^{-/-} mice, the genetic manipulation or the use of pharmacological inhibitors reducing prolyl hydroxylase activity (thus rising HIF-1 α expression) decreased atherosclerosis progression, as well as blood cholesterol and circulating monocytes [55,56]. Conversely, the overexpression of prolyl hydroxylase-3 increased atherosclerosis in ApoE^{-/-} mice [57].

4.1.2. VEGF (Vascular endothelial growth factor) / VEGFR (VEGF Receptor)

4.1.2.1. VEGF family. A diffusible angiogenic factor was discovered in

cancer cell culture in 1968 [58,59], and named tumor angiogenesis factor [60], vascular permeability factor [61,62], vascular endothelial growth factor [63], vascular endothelial cell mitogen or vasculotropin [64]. In fact, it is a single factor now referred to as VEGF (or VEGF-A), encoded by the *VEGFA* gene [65]. In humans, 5 homolog genes (*VEGFA*, *VEGFB*, *VEGFV*, *VEGFD*, and *PGF*) constitute the *VEGF* family, which belongs to the *VEGF/PDGF* superfamily [66] that appeared early in the evolution in the common ancestor of Eumetazoan [67].

– VEGF-A is an endothelial specific growth factor, with a signal peptide for secretion, a heparin-binding site and a highly conserved cystine-knot domain involved in the binding of VEGF to their receptors [68]. The *VEGFA* gene gives rise to multiple VEGF-A isoforms, designated by VEGFxxx (xxx indicating the number of amino acid residues, e.g. VEGF121, VEGF145, VEGF165, VEGF189, VEGF206), which are generated by alternative exon splicing [69,70] and by various post-transcriptional mechanisms (e.g. alternative initiation codons, IRES, upstream ORF, alternative in-frame translation, miRNA) [71]. Most cell types express simultaneously several isoforms, mainly VEGF165 and VEGF121 [70,72]. The angiogenic effect of VEGF-A is mediated by VEGFR2 (see below). A group of additional isoforms, named VEGFxxx_b, generated by alternative splicing in exon 8, differ from VEGFxxx by 6 amino acids at the C-terminal end. For instance, VEGF165_b binds to VEGFR-2, but not to the neuropilin-1, thus triggers an incomplete cell signaling, and acts rather as a competitor that inhibits the angiogenic effect of VEGF165 [73].

The expression of VEGF-A is upregulated by hypoxia, inflammation, wound-healing and other pathological processes, through a transcriptional regulation mediated by various transcription factors, including HIF1 and sp1 [74,75].

VEGF-A is a potent angiogenic inducer that plays a crucial role in angiogenesis throughout life and is absolutely required for embryonic development, since single allele inactivation (*Vegfa*^{-/-}) is lethal at days E11-E12 [76,77].

The role of VEGF-A in atherosclerosis is potentially dual, since it induces both beneficial and detrimental effects [78]. VEGF-A protects endothelial cells by inducing the expression of anti-apoptotic proteins and NO production [70]. It acts as a mitogen promoting re-endothelialization [79], thereby preventing or repairing the endothelial injury that can initiate atherogenesis [1]. However, VEGF-A also increases endothelium permeability [61], adhesion protein expression [80], monocyte chemoattractant protein-1 (MCP-1) [81], thus promotes monocyte adhesion, transendothelial migration and activation [82]. Moreover, it may induce pro-atherogenic changes in lipoproteins [83]. In human coronaries, VEGF and its receptors are not detected in normal coronary segments, but are expressed in atherosclerotic areas, more specifically in endothelial cells of microcapillaries, in macrophages and in partially differentiated SMC [84].

The effects of VEGF in various animal models of atherosclerosis are sometimes apparently contradictory. VEGF-A enhances atherosclerosis progression in various animal models, e.g. cholesterol-fed rabbits and ApoE^{-/-} mice, a rat model of long-term inhibition of nitric oxide synthesis, a rabbit carotid artery collar model of intimal hyperplasia with adventitial delivery of adenoviruses encoding VEGF, in ApoE^{-/-} mice with systemic adenoviral gene transfer of hVEGF-A for one month [20,83,85,86]. But VEGF gene transfer has no effect in hypercholesterolemic LDLR^{-/-} apoB48^{-/-} mice [87] and in LDLR^{-/-} and LDLR^{-/-} ApoB100/100 mice [83].

In humans, angiogenic growth factors (proteins or genes) have been utilized in clinical trials, but so far no significant therapeutic efficacy has been demonstrated [88,89]. Moreover, VEGF inhibitors, used for the treatment of cancer, increased (moderately) the risk of myocardial infarction and arterial thromboembolism [90].

- VEGF-B is encoded by the *VEGFB* gene that generates two isoforms in various tissues by alternative splicing [91,92]. VEGF-B167 contains a C-terminal heparin-binding domain allowing its binding to heparan sulfate of ECM, whereas VEGF-B186 is devoid of this domain. The two isoforms are simultaneously expressed, the highest expression being observed in the heart, skeletal muscle, adipose tissue, and blood vessels [93]. VEGF-B binds specifically to VEGFR-1 and its coreceptor NRP-1 (neuropilin-1), but not to VEGFR-2 and VEGFR-3. VEGF-B is dispensable for embryonic angiogenesis, since *Vegfb*^{-/-} mice are viable, although they exhibit heart anomalies and impaired recovery from cardiac ischemia [94]. VEGF-B exhibits only weak (if any) angiogenic effect on cultured endothelial cells. *In vivo*, it is implicated in the maintenance of blood vessels and may participate in the reparative angiogenesis (mediated by Akt and eNOS) in ischemic myocardium. Moreover, the VEGF-B/VEGFR1 pathway stimulates fatty acid uptake by endothelial cells [95] and acts on adipose tissue by increasing capillary density (*via* an activation of the VEGF-A/VEGFR2 pathway), by stimulating adipose tissue metabolism, and by reducing obesity-associated inflammation [96]. In addition, transgenic expression or AAV-mediated gene transfer of VEGF-B induces cardiac hypertrophy and improves coronary vascularization without increasing vascular permeability or inflammation, in contrast to the other members of the VEGF family [93].
- PlGF

The Placental Growth Factor (PlGF), encoded by the *PGF* gene, exists as four isoforms, PlGF-1 to -4, in humans [93,97,98]. PlGF-1 and PlGF-3 are diffusible isoforms, whereas PlGF-2 and PlGF-4 have heparin binding domains [98]. PlGF has some similarities (homology, receptor) with VEGF-B, but their biological effects are different [93]. PlGF is expressed abundantly in the placenta. Its expression is low in other tissues under physiological condition, but it may rise under diseased conditions (hypoxia, oxidative stress). PlGF binds to VEGFR1 (FLT1) and sFLT1 but not to VEGFR2 (in contrast to VEGF-B that binds to VEGFR1 and VEGFR2). PlGF-2 can also bind to neuropilin (NRP-1). PlGF (at low concentration) promotes survival, migration and activation of vascular cells (endothelial cells and pericytes) and other cell types (e.g. macrophages, dendritic cells, fibroblasts, tumor cells). Indeed, PlGF may potentiate the effect of VEGF-A by displacing VEGF-A from VEGFR1 towards VEGFR2, or by inducing heterodimerization PlGF/VEGF-A and VEGFR1/VEGFR2, or by stimulating VEGF-A secretion [98,99]. *Pgf*^{-/-} mice (like *Vegfb*^{-/-} mice) are viable, thus indicating that PlGF is dispensable for embryonic development and post-natal life. However, *Pgf*^{-/-} mice exhibit impaired angiogenesis and capillary permeability during ischemia, inflammation, wound healing and cancer [100].

In atherosclerosis, PlGF has a pro-atherogenic role, as shown by increased atherosclerotic lesions in hypercholesterolemic rabbits treated by a local adenoviral delivery of PlGF2 and by the decrease of macrophage content in atherosclerotic lesions from PlGF/ApoE^{-/-} mice [98,101].
- VEGF-C and VEGF-D, encoded by the *VEGFC* and *VEGFD* genes respectively, are synthesized as inactive precursors that are activated by proteolytic maturation [102]. The mature cleaved VEGF-C and VEGF-D have an affinity for VEGFR-3 and VEGFR-2 in humans and are involved in lymphangiogenesis and angiogenesis. However, in mice, VEGF-D binds only to VEGFR-3 [103].

Vegfc^{-/-} mice lack lymphatic vasculature resulting in prenatal death after E15, while heterozygous *Vegfc*^{+/-} mice are viable, but have lymphatic hypoplasia and lymphedema [104]. In contrast, *Vegfd*^{-/-} mice are viable and have almost normal lymphatic vessels [105]. *Vegfc*^{-/-}*Vegfd*^{-/-} double-knockout mice exhibit the same phenotype than *Vegfc*^{-/-} mice (lymphangiogenesis defect leading to embryonic

death after E15), but, unexpectedly, differ from *Vegfr3*^{-/-} mice that die at E10 from a severe vascular defect [106,107]. Conversely, transgenic overexpression or adenoviral expression of VEGF-C and VEGF-D induces lymphangiogenesis through VEGFR-3 signaling and angiogenesis, permeability and inflammation through VEGFR-2 [103,108].

In atherosclerotic lesions, lymphatic vessels are present, but their potential pro- or anti-atherogenic role is still debated. In a model of carotid hyperplasia in rabbits, adventitial delivery of adenoviruses encoding VEGF-D increased the intimal thickening, whereas VEGF-C was inefficient [20]. In a model of LDLR/apoB48-deficient hypercholesterolemic mice, systemic adenovirus-mediated gene transfer of VEGF-A, -B, -C, or -D showed no proatherogenic effect [87].

Recently, it has been shown that lymphatic vessels are required for the HDL-mediated reverse cholesterol transport, and that administration of VEGF-C into the footpad of ApoE^{-/-} mice improves the local lymphatic function and reverse cholesterol transport [109]. Similarly, lymphatic insufficiency is associated with defective reverse cholesterol transport and increased atherosclerosis in hypercholesterolemic mice or expressing soluble VEGFR3 or treated with anti-VEGFR3 antibodies [110,111].

These data suggest that the maintenance of the lymphatic system is important for the reverse cholesterol transport mediated by HDL, and finally for the anti-atherogenic effect of these lipoproteins [110–112].

4.1.2.2. Receptors. A family of VEGF receptors (VEGFR) and co-receptors mediates the biological effects of VEGF isoforms [113–116]. VEGFRs are transmembrane receptor tyrosine kinases (RTKs) constituted by an extracellular ligand-binding region, a short transmembrane segment and a cytoplasmic region containing the tyrosine kinase (TK) domain and several tyrosine residues serving as phosphorylation sites. In humans, the VEGFR family is constituted by 3 members, VEGFR1 or Flt-1 (fms-like tyrosine kinase-1), VEGFR-2 or KDR (human kinase insert domain containing receptor)/Flk-1 (murine fetal liver kinase-1) and VEGFR-3 or Flt-4 (fms-like tyrosine kinase-4), encoded by genes *FLT1*, *KDR* and *FLT4*, respectively. [70,117,118]. The binding of the ligand to a functional VEGFR triggers receptor dimerization, activation of the tyrosine kinase, that phosphorylates specific tyrosine residues of the cytoplasmic domain. Phosphotyrosine residues allow the binding of SH2- or PTB domains of enzymes and adaptor proteins, thereby triggering intracellular signaling [118–120].

The receptors display differences in their tissue specific expression, signaling and biological effects [116,120].

Besides VEGFRs, two transmembrane proteins, neuropilins NRP-1 and NRP-2, act as co-receptors (devoid of tyrosine kinase activity) for some isoforms of VEGF, and modify the affinity of the ligands to their respective receptors. NRP-1 is associated with VEGFR-1 or VEGFR-2, and NRP-2 with VEGFR-2 or VEGFR-3 [116,117].

Some VEGF isoforms have an heparin-binding domain allowing their binding to heparan sulfate proteoglycans (HSP) that may compete for the binding of VEGF to VEGFR. VEGF bound to HSP is released during ECM degradation and can then bind to VEGFR [119,120].

- VEGFR-1 (FLT-1). In humans, the *FLT1* gene gives rise by alternative splicing to multiple isoforms, including a full-length transmembrane receptor tyrosine kinase VEGFR-1 (or FLT-1) and short soluble isoforms denoted sVEGFR-1 or sFLT1. VEGFR-1 is expressed on vascular endothelial cells, smooth muscle cells and other cell types including macrophages, hematopoietic cells, neuronal cells and placenta. It is implicated in the regulation of cell survival, cell migration, angiogenesis and cancer cell invasion. VEGFR-1 can form homodimers and heterodimers with VEGFR-2. VEGFR-1 binds to VEGF-A (with high affinity), VEGF-B and PlGF, but the resulting signaling is weak and ultimately VEGFR-1 acts as a negative regulator of angiogenesis [118,121]. This negative regulatory func-

tion is required for embryo development, since *Vegfr1*^{-/-} mice die at embryonic day 8.5 with excessive endothelial cell proliferation [122]. Interestingly, the VEGFR1-TK(-/-) mice (homozygous mice with inactive TK domain of VEGFR1) are healthy, thus suggesting that the anti-angiogenic activity is not TK-dependent but rather results from a competition between VEGFR1 and VEGFR2 for VEGF-A [123].

However, the tyrosine kinase of VEGFR1 plays a role in VEGF-A-induced macrophage chemotaxis, since VEGFR1-TK(-/-) macrophages do not migrate toward VEGF-A [124].

Several experimental data suggest that VEGFR1 may play a role in atherosclerosis. In a murine model of intimal hyperplasia, the loss of TK activity in VEGFR1-TK(-/-) reduces the extent of lesions, thus suggesting that VEGFR-1 (with active tyrosine kinase) could mediate a pro-atherogenic signaling [124]. The binding of PlGF and VEGF-B to VEGFR-1 induces the release of VEGF-A, which binds to VEGFR2 and exerts a pro-angiogenic and atherogenic effect [100]. The PlGF adenoviral gene transfer increases the size of atherosclerotic lesions in ApoE^{-/-} mice and in hypercholesterolemic rabbits [101]. Moreover, LDLs trigger co-endocytosis of LDLR and VEGFR-1, and ubiquitination-mediated proteasomal degradation of VEGFR-1 that may enhance the angiogenic effect of VEGFR-2 in atherosclerotic lesions [125]. The soluble sVEGFR-1 (or sFLT-1), a splice variant encoding only the extracellular domain, acts as a decoy that competes with VEGFR-2 for VEGF-A, thereby reducing the angiogenic activity of VEGF-A. In a murine model of neointimal formation, sFlt-1 gene overexpression has an anti-atherogenic effect [124]. In humans, increased circulating sFlt-1 is associated with pre-eclampsia and peripartum cardiomyopathy [120,126].

– VEGFR-2 (KDR/Flk-1) binds to VEGF-A, VEGF-C and VEGF-D and plays a major role in angiogenesis [70,118,120]. VEGF-A binds to VEGFR-2 and VEGFR-1 with high affinity, but these VEGFRs trigger different cell signaling, because the tyrosine kinase activity of VEGFR-2 (and subsequent autophosphorylation) is much higher than that of VEGFR-1 [70,118,120]. The simultaneous presence of the two receptors on endothelial cell regulates the angiogenic signaling triggered by VEGF [117,119,120]. VEGFR-2 is expressed in growing vessels, and is required for angiogenesis during embryonic development, since VEGFR2^{-/-} mice die at E8.5 from impaired development of endothelial and hematopoietic cells [127], a phenotype similar to that of the VEGFA^{-/-} mice [117]. The angiogenic effect mediated by VEGFR-2 involves a complex signaling including PLCγ/PKC, PI3K/Akt, ERK1/ERK2, SRC, YES1, CBL, FAK1, Ras [116,117,120].

The atherogenic role of VEGFR-2, is still debated, because it exhibits a pro-atherogenic effect in some animal models [20,83,85,86], but has no effect in other models [83,87]. In ApoE^{-/-} mice, a pan-VEGFR inhibitor, PTK787/ZK222584, is pro-atherogenic [128]. However, vaccination against VEGF2 reduces atherosclerosis in ApoE^{-/-} and LDLR^{-/-} mice [129,130].

– VEGFR3 (Flt-4), encoded by the FLT4 gene in humans, exists as two transmembrane RTK isoforms that binds to VEGF-C and VEGF-D and a secreted isoform that acts as a decoy receptor for VEGFC/VEGFD. Ligand binding triggers homodimerization of the transmembrane VEGFR3, autophosphorylation and subsequent signaling through Ras and PI3K-Akt [117]. The secreted isoform may function as a decoy receptor for VEGFC and/or VEGFD, and plays an important role as negative regulator of VEGFC-mediated lymphangiogenesis and angiogenesis.

VEGFR-3 is required for the embryonic development of the cardiovascular and lymphatic systems. The VEGFR3^{-/-} mice die at E10.5 because of defective cardiovascular remodeling [106]. In addition, VEGFR3 plays a role in the migration and survival of lymphatic endothelial cells.

The role of VEGFR3 in atherosclerosis has been investigated in a

murine model of aorta transplant associated with anti-VEGFR3 antibody to block lymphatic regrowth. The reverse cholesterol transport was inhibited by anti-VEGFR3 antibodies [110]. In the same way, transgenic mice with lymphatic insufficiency and hypercholesterolemia (expressing the sVEGFR3 competitor or heterozygous VEGFR3-deficient Chy mutation, crossed with LDLR^{-/-}/ApoB100/100), exhibited defective reverse cholesterol transport, increased level of atherogenic lipoproteins and more extended atherosclerotic lesions [111]. This suggests that the lymphatic system plays a role for the reverse cholesterol transport and thereby is anti-atherogenic [112].

4.1.3. Angiopoietin-1 and 2/Tie2 receptor

The system of angiopoietins and their Tie2 receptor is involved in the stabilization-destabilization of vessels. Angiopoietins (Angpt) and related angiopoietin-like proteins (Angptl) are encoded by homologous genes *ANGPT*-1 to 4, and *ANGPTL*-1 to 7. Receptors specific for angiopoietins, Tie2 (tyrosine kinase with immunoglobulin and EGF homology domains), encoded by the *TEK* gene [132,133] and Tie1, are classical RTK constituted by an extracellular domain which contains the binding site for angiopoietins, a short transmembrane domain and an intracellular domain with the tyrosine kinase site.

Angiopoietin-1 (Angpt-1) is a glycoprotein secreted by periendothelial cells (pericytes and SMCs). It has a paracrine action on its receptor Tie2 located on endothelial cells [134]. Tetramers of angiopoietin-1 induce Tie2 tetramerization, autophosphorylation and intracellular signaling that promotes cell survival and tightens endothelial junctions and interactions with pericytes, thereby stabilizing vessels, reducing vascular permeability and promoting anti-inflammatory effect. Angiopoietin-2 binds to Tie2, reduces Tie2 aggregation, and acts rather as an antagonist of Angiopoietin-1 to Tie2 [135]. Angiopoietin-2 reduces contacts between endothelial cells, ECM and pericytes, thus increases the vascular permeability and makes endothelial cells more accessible to growth factors. This may facilitate angiogenesis by angiogenic agents (e.g. VEGF), or promote vascular regression in the absence of angiogenic factors. Finally, the angiogenic/antiangiogenic balance, vascular morphogenesis, maintenance and remodeling are dependent on cooperation between Tie2 and VEGFR systems [136–140].

Genetic models gave information on the crucial role of angiopoietins and Tie receptors during development. *Angpt1*^{-/-} mice and Tie2-deficient mice have the same lethal phenotype and die around E11.5 from defects in vasculogenesis associated with abnormal hematopoiesis and heart endocardium [141,142]. In transgenic mice overexpressing angiopoietin-1 in the skin, vessels are larger and more numerous than in control mice [143].

Angpt2^{-/-} mice are normal at birth, but exhibit post-natal anomalies of angiogenesis and lymphangiogenesis dependent on the genetic background. All *Angpt2*^{-/-} newborn mice develop chylous ascites and die in the 2nd week on the 129/J genetic background, while the postnatal mortality is less than 10% on C57Bl/6 genetic background [144]. Tie1^{-/-} mice die between E13.5 and birth because of loss of integrity of vessels leading to widespread edema [145,146].

In the field of atherosclerosis, it has been suggested that angiopoietin-1/Tie2 exhibits anti-inflammatory and anti-atherogenic effects, since this system maintains a quiescent endothelial phenotype and reduces vascular permeability, ICAM-1, VCAM-1, and E-selectin expression and leukocyte adhesion [138,139]. Consistently, angiopoietin-1 protects against the development of cardiac allograft arteriosclerosis [147]. However, angiopoietin-1 could also stimulate the migration of monocytes and neutrophils, thereby worsening the local inflammatory response [148].

Angiopoietin-2 acts as an antagonist of angiopoietin-1 and promotes vascular permeability, angiogenesis and leukocyte recruitment, thus may play a proatherogenic role. This is supported by the anti-atherogenic effect of anti-angiopoietin-2 blocking antibodies in hypercholesterolemic LDLR^{-/-} apoB^{100/100} mice [149]. Moreover, in

human carotid atherosclerotic plaques, high levels of angiotensin-2 are associated with increased MMP2 activity, high microvessel density and plaque complications (intraplaque hemorrhages, plaque rupture) [150]. In contrast, a single systemic administration of angiotensin-2 adenovirus reduces LDL oxidation and macrophage accumulation in the plaque and decreases the size of atherosclerotic lesions in ApoE^{-/-} mice, *via* NO production [148].

Vaccination against Tie2 reduces carotid and aortic atherosclerosis in LDLR^{-/-} mice [151]. Likewise, decreased Tie1 expression in heterozygous Tie1^{+/-}, ApoE^{-/-} mice is associated with reduced atherosclerosis [152].

Angiotensin-like protein 2 (angptl2), which contributes to vascular inflammation, may be pro-atherogenic. Indeed, in murine models, systemic administration of angptl2 strongly increases the formation of atherosclerotic lesions in LDLR^{-/-}.ApoB^{100/100} mice [153], whereas angptl2 deletion reduces atherosclerosis progression in Angptl2^{-/-}, ApoE^{-/-} mice [154].

4.1.4. NO/NOS

Various Nitric Oxide Synthases (NOS) are expressed in vascular cells, and generate NO that plays a crucial role in vascular biology. In humans, the NOS family includes 3 genes encoding neuronal NOS (nNOS encoded by *NOS1*), inducible NOS (iNOS encoded by *NOS2*) and endothelial NOS (eNOS, encoded by *NOS3*). iNOS and nNOS are cytosolic enzymes, while eNOS is membrane-bound. The constitutively expressed eNOS and nNOS are calcium-dependent, while iNOS is inducible and calcium-independent.

NOS oxidize L-arginine by using O₂ and NADPH, H⁺, to generate NO and L-citrulline. NO produced by eNOS in the endothelium induces the relaxation of arterial smooth muscle [155–157]. The activity of eNOS is regulated by various extracellular stimuli, such as shear stress, hormones and growth factors (e.g. insulin, adiponectin, VEGF, angiotensin II) and other mediators (e.g. thrombin, bradykinine, catecholamines, serotonin, ADP) that bind to their respective receptors and trigger intracellular signaling. Several signaling pathways, such as calcium/camodulin kinase II, PI3K/Akt, PKA and ERK1/2, take part in the regulation of eNOS. NO elicits SMC relaxation through the guanylate cyclase/cGMP/PKG pathway, which decreases intracellular calcium by inhibiting the calcium entry and release from intracellular stores. The mechanism involves the activation of hyperpolarizing (thus relaxing) K⁺ channels, and myosin light chain dephosphorylation (through activation of cGMP-dependent protein kinase that activates myosin light chain phosphatase). NO can induce S-nitrosylation that enhances the relaxation, but also eNOS retro-inhibition. In addition, NO may regulate platelet aggregation, leukocyte adhesion, local inflammatory response, endothelial apoptosis, SMC proliferation, progenitor cell mobilization and angiogenesis [158–161].

Several genetic murine models, either NOS-deficient or overexpressing NOS, have been generated to investigate the (patho)physiological role of NOS, especially in atherogenesis.

nNOS deficient mice (*Nos1*^{-/-}) are viable, exhibit stomach enlargement due to pyloric spasm, are aggressive, but show no obvious cerebral anomaly. These mice are more resistant to cerebral ischemia, but exhibit increased neointimal formation and constrictive remodeling in a carotid ligation model [162,163].

In eNOS deficient mice (*Nos3*^{-/-}), the defect of vascular relaxation (endothelial dysfunction) is associated with high blood pressure, increased neointima hyperplasia in response to arterial injury [164,165]. In ApoE^{-/-} mice, hypercholesterolemia is associated with eNOS dysfunction and impaired angiogenesis, possibly mediated by ADMA. This was reversed by L-arginine administration [166].

iNOS deficient mice (*Nos2*^{-/-}) show normal development, but they exhibit an altered immune response to some intracellular pathogens and endotoxic shock [167–169]. In contrast, transgenic mice overexpressing iNOS in cardiomyocytes, exhibit cardiac hypertrophy and dilation associated with an increased production of peroxynitrite,

inflammatory cells infiltrate and fibrosis, leading to cardiomyopathy, bradyarrhythmia, and sudden death [170].

Interestingly, the deletion of eNOS in eNOS-KO, ApoE^{-/-} mice and of nNOS in nNOS-KO, ApoE^{-/-} increases atherosclerotic lesions [165,171,172]. The overexpression of eNOS in the endothelium of ApoE^{-/-} mice reduces blood pressure, plasma cholesterol and atherosclerosis [173]. These data suggest an atheroprotective role of eNOS and nNOS. In contrast, in iNOS-KO/ApoE^{-/-} mice, the level of plasma lipoperoxides and the size of atherosclerotic lesions is reduced, suggesting a pro-atherogenic role of iNOS [174].

The triple KO *Nos1*^{-/-}, *Nos2*^{-/-}, *Nos3*^{-/-} mice develop lipid-rich atherosclerotic lesions in the aorta, arteriosclerosis (intimal and medial thickening and perivascular fibrosis) in coronary and renal arteries, and spontaneous myocardial infarction, thus suggesting an atheroprotective role of the whole NOS system [175,176]. The atheroprotective effect of eNOS was confirmed in ApoE^{-/-} mice by the protective effect of a pharmacological eNOS inducer (AVE9488) [177]. Moreover, blocking the binding of eNOS to caveolin-1 by a specific competitor peptide reduces atherosclerosis in diabetic mice [178]. In addition to its vasodilator effect and its role in the regulation of arterial blood pressure, NO synthesized by endothelial cells could be involved in vascular remodeling and angiogenesis [179,180]. eNOS regulates endothelial cell proliferation and sprouting, and pericytes recruitment for blood vessel stabilization [181]. NO-induced vasodilatation and a cross talk between eNOS and the VEGF pathway may explain the angiogenic potential of NO [182]. The role of NO is also supported by the effect of L-NAME that blocks both NO production and angiogenesis [183]. However, the conditions prevailing in atherosclerotic plaque (hypoxia, inflammation) may promote iNOS overexpression [184], which could generate harmful oxidative products rendering neovessels leaky and inducing plaque instability [185].

4.1.5. PDGF/PDGFR

Growth factors of the PDGF family, particularly PDGF-B and its receptor PDGFRβ are involved in angiogenesis. Indeed, new vessels are stabilized by the recruitment of peri-endothelial cells (smooth muscle cells and pericytes). The PDGF-B/PDGFRβ system plays a critical role in the recruitment of periendothelial mural cells, as shown by abnormal angiogenesis in *PDGFB*^{-/-} and in *PDGFRβ*^{-/-} mice embryos, in which abnormal capillary permeability and rupture of microaneurysms in late embryogenesis cause edemas and lethal hemorrhages. This suggests that PDGF-B secreted by endothelial cells binds to PDGFRβ of pericytes and vascular SMC, attracts them and induces their proliferation. Moreover, PDGF signaling in vascular SMC is regulated by neuropilin-1 and 2 [186]. It may be noted that the earliest stages of differentiation and migration of peri-endothelial progenitor cells to the angiogenic site require TGF-β-like factor synthesized by endothelial cells [33]. Finally, pericytes contribute to the stability of the new capillaries [187–190].

In atherogenesis, PDGF-B/PDGFRβ may promote SMC proliferation and ECM synthesis during the formation of intimal hyperplasia and fibrous cap of atherosclerotic lesions [1]. This has been confirmed by the atheroprotective role of Imatinib, a PDGFR-TK inhibitor, in a model of diabetic ApoE^{-/-} mice [191]. However, only few data are available on the role of PDGF-B in angiogenesis in atherosclerotic lesions. Recent studies on human stable and unstable carotid plaques show a negative correlation between plasma PDGF level and neovessel density [192,193].

4.1.6. TGF-β

TGF-β (TGF-β1 isoform) and its receptors type I (TGFβRI or ALK5) and type II (TGFβRII or TGFBR2) are involved in vascular assembly during embryogenesis and in the maintenance of the vascular wall integrity. TGF-β can be synthesized by endothelial cells and perivascular cells, for example during healing process [194]. TGF-β can induce VEGF synthesis by endothelial cells and perivascular inflammatory

cells, and may participate in angiogenesis [195]. TGF- β also induces the synthesis of PDGF-B by endothelial cells and the expression of PDGFR β in perivascular fibroblasts and SMC. TGF- β promotes the differentiation of SMC and pericytes co-cultured with endothelial cells [194,196]. However, *in vitro* the effect of TGF- β on endothelial cells is biphasic in a dose-dependent manner. At low concentration (300 pg/ml), it increases both adhesion and anchorage-dependent migration of endothelial cells, but these effects are inhibited by higher TGF- β concentration (1 ng/ml) [194]. This dual effect may result from the induction of VEGF by TGF- β 1 and a cross-talk between the signaling pathways activated by these growth factors [197]. Thus, TGF- β may play a role in angiogenesis and in the maturation and stabilization of neo-vessels, but it may also exhibit an anti-angiogenic effect, which depends on its local concentration and its interaction with other growth factors.

4.1.7. b-FGF

Basic Fibroblast growth factor (b-FGF or FGF-2) is involved in angiogenesis through its tyrosine kinase receptor FGFR1, which is highly expressed in the endothelium, and signals for endothelial cell proliferation, migration, tubulogenesis and secretion of proteases. FGFR2, which is less expressed in the endothelium, may play a role in cell motility. VEGF and b-FGF act synergistically to stimulate the proliferation and migration of endothelial cells, pericytes and SMC, and to drive the assembly of the endothelium during angiogenesis [198]. However, unexpectedly, in the presence of TGF- β 1, FGF2 may up-regulate VEGF expression in endothelial cells, thereby triggering p38(MAPK) activation and apoptotic signaling [197].

4.1.8. EGFR

EGFR is a tyrosine kinase receptor present at the plasma membrane of various cell types involved in atherogenesis, including fibroblasts, SMC, endothelial cells and macrophages [199]. In endothelial cells, EGFR plays a role in migration and angiogenesis via the activation of PI3K/Akt/eNOS pathway [200]. EGFR overexpression in endothelial cells of canine mammary tumors is associated with an increased microcapillary density and metastatic potential [201]. Conversely, the irreversible inhibition of EGFR blocks HUVEC proliferation and angiogenesis [202]. In a cellular model of colorectal cancer, the phosphorylation of EGFR is associated with the stabilization of HIF via MAPK activation, but this single activation is not sufficient for the induction of VEGF synthesis by the cells, suggesting that EGFR acts synergistically with other signaling pathways to induce the transcription of pro-angiogenic factors [203].

4.1.9. S1P/S1PR

S1P is a sphingolipid mediator, which is generated by the phosphorylation of sphingosine by sphingosine kinase-1 (SK1) at the inner leaflet of the plasma membrane [204]. S1P is secreted in the extracellular medium by ABC family transporters [205] and by the Spns2 carrier (S1P carry spinster homolog 2) [206].

The biological effect of secreted S1P is mediated through its binding to specific G-protein coupled receptors. These receptors form a specific family of seven transmembrane domain G-coupled receptors (S1PRs), which comprises five members, S1P1 to S1P5, that activate various signaling pathways [31,207–210]. The cardiovascular system expresses mainly S1P1, S1P2 and S1P3, which are involved in cytoskeletal remodeling, adhesive and junctional changes, cell migration, proliferation, survival and angiogenesis [211].

S1P1 (or EDG-1), encoded by *S1PR1* gene, is expressed in many cell types, particularly in cardiomyocytes and endothelial cells, where it plays a role in the development of the cardiovascular system [212,213]. S1P1 is coupled to a Gi protein that inhibits adenylate cyclase (AC) and stimulates MAPK and PLC/PI3K/Akt-induced signaling responses, such as eNOS activation, SMC relaxation, vasodilatation, permeability, migration, proliferation and tubulogenesis of endothelial cells [214].

S1P1 controls the trafficking of N-cadherin of endothelial cells and strengthens contacts between endothelial cells and pericytes [31]. S1P1 can transactivate VEGFR2, causing Akt activation and eNOS phosphorylation [207,215]. In turn, VEGFR2 may activate SK1, S1P generation and S1P1 expression [207].

S1P2 or EDG-5, encoded by *S1PR2*, is associated mainly with G12/13, and with Gi and Gq [216]. S1P2 is involved in the development and maintenance of the cardiovascular system. G12/13 protein activates the Rho/ROK pathway that is involved in cytoskeleton remodeling, particularly in the formation of stress fibers, thereby causing the disruption of adherens junctions and increasing endothelial permeability [217]. The Rho/ROK pathway is also involved in PI3K/Akt inhibition, which blocks cell migration and proliferation, sensitizes cells to apoptotic signals and inhibits angiogenesis, vascular remodeling and tissue repair [212].

S1P3 or EDG-3, encoded by *S1PR3*, is coupled to Gi/o, Gq and G12/13 proteins. This receptor seems to share some properties with S1P1 and other with S1P2. S1P3 is expressed in various cell types and can regulate cell migration, proliferation and survival through a Gi/Gq-dependent activation of PLC/PI3K/Akt [218]. It also regulates the contraction of vascular SMCs through a calcium-dependent mechanism [212]. In HUVEC, it is involved in the organization of the cytoskeleton and the assembly of adherens junctions [212]. It activates via Gi, the Ras/MAPK/ERK1/2 and p38MAPK pathways involved in the endothelial cell proliferation and migration [219].

4.2. Angiogenic factors in atherosclerosis

Angiogenesis in the atherosclerotic plaque involves classical angiogenic mechanisms that are implicated in adaptive angiogenesis under physiological conditions and specific factors generated in atherosclerotic areas. For instance, local hypoxia upregulates HIF-1 α and VEGF, which triggers angiogenic signaling and endothelial sprouting. If the O₂ supply is restored, HIF-1 α is degraded, which reduces VEGF production and subsequent angiogenic signaling. In addition, angiopoietin-1, PDGF and TGF- β inhibit angiogenesis and stabilize the neovessels. If the stabilization step is lacking, neovessels are leaky and may regress [31]. In atherosclerotic lesions, various persisting stimuli may induce a sustained angiogenic signaling, which leads to sprouting without resolution phase and stabilization of neo-vessels [13,30,220].

4.2.1. Lipids, oxidized lipids and oxidized lipoproteins of the plaque

In human atherosclerosis, neovessels are formed in early lesions, in which lipid accumulation is associated with inflammatory cells [13,221,222]. In aortic advanced plaques, the neovessel density is higher in lipid-rich inflammatory lesions than in fibrocalcific plaques [13,41]. This suggests that atheromatous lipids may stimulate angiogenesis either directly or indirectly by inducing the release of angiogenic factors [223].

4.2.1.1. Cholesterol, rafts and caveolae. Cholesterol is a structural component of animal cellular membranes that is required for normal cellular function. The distribution of cholesterol in the plasma membrane is heterogeneous and depends on exchanges with intracellular cholesterol pools and extracellular lipoproteins [224–226]. Cholesterol-rich microdomains, such as rafts and caveolae, play a regulatory role in angiogenic signaling [227,228], as shown by the increased proliferation of endothelial cells occurring in caveolin-1 deficient mice [229]. In atherosclerosis, caveolin-1 plays apparently a complex role, with either pro- or anti-atherogenic effects depending on the cell type [230]. In *Cav1*^{-/-} ApoE^{-/-} mice, the deficiency of caveolin-1 is associated with a decrease of atherosclerotic areas [231]. In a porcine model of atherosclerosis, hypercholesterolemia was associated with a dense and disorganized angiogenic sprouting in coronary atherosclerotic areas, contrasting with the organized vasa vasorum structure of normal vessels [19]. However, the relationship between

cholesterolemia and angiogenesis is more complex, since in various animal models, such as Watanabe heritable hyperlipidemic rabbits, ApoE^{-/-} mice, and Yucatan miniswine fed with high cholesterol diet, the angiogenic response to ischemia is decreased concomitantly with reduced VEGF/VEGFR2 signaling, oxidative stress, NO/NOS dysfunction and endostatin expression [166,232–237].

HDL may impair angiogenesis by endothelial progenitor cells, through activation of the Rho-associated kinase signaling [238]. A link has been clearly established between angiogenesis and the cholesterol content in the plasma membrane of endothelial cells, by manipulating the cholesterol efflux through the ABCG1/AIBP/HDL system. An increased cholesterol efflux reduces lipid rafts, VEGF/VEGFR2-mediated signaling and angiogenesis, whereas the down-regulation of cholesterol efflux by blocking the ABCG1/AIBP/HDL system promotes VEGF/VEGFR2-mediated signaling and angiogenesis [223].

These data suggest that, in atherosclerotic areas, angiogenesis is dependent on a delicate balance regulating membrane cholesterol and inflammation. Cholesterol accumulation may trigger inflammatory responses through TLR signaling and inflammasome activation [239] that can promote both angiogenesis and atherosclerosis (see *infra*).

4.2.1.2. Oxidized LDLs. The effects of oxLDLs on angiogenesis are multiple and somewhat puzzling, because both angiogenic and antiangiogenic responses have been reported. This may result, at least in part, from a dose-dependent biphasic effect of oxLDLs. Low oxLDL concentrations are angiogenic, whereas moderate concentrations are anti-angiogenic and higher doses are cytotoxic to endothelial cells [240–243].

Several signaling pathways are implicated in the angiogenic effect of low concentrations of oxLDLs and oxidized phospholipids. The binding of oxLDLs to LOX-1 triggers NADPH oxidase activation, intracellular ROS generation, p38-MAPK activation, VEGF synthesis and VEGFR-2 autophosphorylation [240] and the activation of the PI3K/Akt/eNOS pathway [241]. Oxidized lipids induce the expression of several genes involved in cell adhesion, migration and angiogenesis (e.g. HIF1 α /VEGF, VEGFR-2, PDGFR, NOTCH-1, and NRP-1), and the down-regulation of pro-apoptotic genes [242,244–246]. OxLDLs trigger a ROS-dependent activation of nSMase2 and SK1 and the subsequent generation of S1P that elicits the angiogenic response through S1PRs activation and VEGFR2 transactivation [243,247]. In addition, physiological concentrations of H₂O₂ increase the expression of S1P1 in bovine endothelial cells, activate the nSMase2/S1P pathway, and subsequently stimulate the activation of eNOS, ERK1/2 and cell proliferation [248,249].

In contrast, higher oxLDL concentrations exhibit an anti-angiogenic effect characterized by a decreased migration and proliferation, an increased apoptosis of endothelial cells and a reduced differentiation of endothelial progenitor cells [250,251]. Moreover, 7-ketocholesterol triggers local inflammatory and angiogenic responses, mediated by cytokines and VEGF [252]. Interestingly, HIF-1 α inhibition abrogates the proangiogenic effect of oxLDLs *in vivo* [245].

4.2.1.3. Fatty acids, oxidized polyunsaturated fatty acids and oxidized phospholipids. The mitochondrial metabolism of fatty acids in endothelial cell is involved in angiogenesis regulation, as shown by silencing carnitine palmitoyl transferase CPT1A that decreases mitochondrial fatty acid oxidation, depletes cell stores deoxyribonucleoside triphosphates and reduces endothelial cell proliferation and vessel sprouting [253].

Polyunsaturated fatty acids (PUFAs) from phospholipids and cholesteryl esters are highly susceptible to peroxidation that generates a variety of lipid peroxidation products (LPPs). Some LPPs are stable,

while others (e.g. reactive carbonyl compounds) can react with thiols or amino groups to form advanced lipid peroxidation end products (ALEs) [254]. Moreover, PUFAs are substrates of various oxygenases leading to the formation of a variety of icosanoids, some of them being mediators of angiogenesis [255].

Oxidized phospholipids exhibit pro-inflammatory [256] and pro-angiogenic properties [244]. OxPAPC (oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine) induces sprouting and tubulogenesis *in vitro* and angiogenesis *in vivo*, mediated by the induction of HIF/VEGF, COX-2 and interleukin-8 expression. These results suggest that oxidized phospholipids may promote intimal neovascularization in atherosclerotic arteries [244].

4.2.1.4. Aldehydes and reactive carbonyl compounds. Reactive carbonyl compounds resulting from late stages of PUFA peroxidation include aldehydes such as malondialdehyde (MDA), acrolein and 4-hydroxynonenal (4-HNE). These highly reactive compounds can react with cellular and ECM components and alter their structure and biological properties [254,257,258].

The formation of 4-HNE and acrolein adducts on EGFR and PDGFR can induce their activation [259–261]. However, long term modification of these receptors reduces their affinity for their ligands and inhibits tyrosine kinase activity [261–263]. The carbonyl stress is involved in endothelial dysfunction through structural modification and dysfunction of eNOS and VEGFR2 [264]. It could also reduce SK1 activation, which is involved in oxLDL-induced angiogenesis [243,247].

4.2.1.5. HDL-cholesterol. HDLs are involved in the reverse transport of cholesterol (RCT) and their level is inversely correlated with coronary artery disease. The reverse cholesterol transport is mediated by ABC transporters and AIBP (ApoA-I binding protein) that accelerate HDL-mediated cholesterol efflux. The depletion of cholesterol from plasma membrane disrupts cholesterol-rich microdomains and affects their signaling function [223]. For instance, AIBP/HDL-mediated cholesterol efflux inhibits VEGF-induced angiogenesis by reducing VEGFR2 localization in lipid rafts, VEGFR2 dimerization and downstream signaling [265].

HDLs may regulate angiogenesis through the transport of S1P. Low concentrations of HDLs are angiogenic *in vitro* and the release of free S1P from HDLs by endothelial lipase may be involved in neovascularization *in vivo* [266]. Inversely, high HDL concentration reduces angiogenesis by inhibiting the Akt/ERK pathway [267].

4.2.1.6. Lipids/PPAR-gamma. Alongside S1P signaling mediated via S1PR, S1P is also an intracellular ligand that binds to the nuclear receptor PPAR-gamma and enhances its association with its co-activator PGC1 β , thereby regulating the expression of genes involved in angiogenesis [268]. PPAR-gamma is highly expressed in early human atherosclerotic lesions compared to healthy aorta [41]. The synthetic PPAR-gamma ligand rosiglitazone induces VEGF-A production in vascular smooth muscle cells [269]. Moreover, various lipid mediators present inside the plaque (e.g. fatty acids, oxidized lipids, S1P and LPA) may activate PPAR-gamma of medial SMCs thereby enhancing VEGF-A expression and stimulating angiogenesis [5].

4.2.2. Reactive oxygen species (ROS) and oxidative stress

ROS are generated by several systems in the vascular wall, particularly NAD(P)H oxidase, lipoxygenase (LOX), cyclooxygenase (COX), endothelial NO synthase (eNOS), cytochrome P450, xanthine oxidase and mitochondrial respiratory chain. ROS are neutralized and

degraded by antioxidant systems (e.g. superoxide dismutases, glutathione peroxidases, catalase, peroxiredoxins, thioredoxins) and small antioxidants and ROS-scavengers from endogenous (e.g. glutathione, uric acid, bilirubin, coenzyme Q) and dietary origin (e.g. tocopherols, ascorbic acid, carotenoids, polyphenols).

Physiological concentrations of ROS are involved in cell metabolism and signal transduction, and take part in the regulation of various cellular functions, such as proliferation, migration and angiogenesis. In contrast, oxidative stress (ROS excess) resulting from an imbalance between ROS production and degradation, may lead to cellular dysfunction, accelerated senescence and apoptosis [270–273].

A moderate concentration of extracellular ROS triggers the expression of angiogenic factors. For instance, ROS generated during arterial injury may enhance the local expression of VEGF [274]. In addition, intracellular ROS generated in endothelial cells upon stimulation by growth factors, inflammatory cytokines or oxLDLs may trigger angiogenic signaling pathways involving p38MAPK, ERK1/2, NF- κ B and the nSMase2/SK1 pathway. Antioxidants block this signaling and the angiogenic response [240,243,271,275,276].

4.2.3. Inflammation

The local inflammatory state occurring in atherosclerotic lesions may play a role in angiogenesis *via* several mechanisms, such as endothelial cell activation, release of chemokines, cytokines, growth factors, lipid mediators, proteases and increased endothelial metabolic rate. The increased vascular permeability allows plasma component extravasation. Proteases released by cells degrade ECM, thereby facilitating the migration of leukocytes attracted by chemokines, and of endothelial cells stimulated by angiogenic factors [277–280]. Inflammation increases the local metabolic rate (as shown by a raised glucose uptake) and O₂ consumption, thereby leading to a local relative hypoxia that enhances the expression of hypoxia-inducible genes. For instance the expression of angiogenic factors is up-regulated by proinflammatory cytokines, thereby promoting angiogenesis [30].

When local hypoxia and inflammation decreases, the angiogenic signaling is down-regulated and neovessels may either regress or mature. TGF- β stimulates migration, differentiation and proliferation of mural cells, and pericytes establish direct cell-cell contact with endothelial cells. Moreover, TGF- β induces the production of ECM and the secretion of PAI-1 (plasminogen activator inhibitor) that inhibit the degradation of perivascular ECM [281].

4.2.4. Metalloproteases

Angiogenic endothelial cells express proteolytic enzymes that can degrade ECM, thereby facilitating cell migration and release of growth factors trapped in the ECM [31]. Various metalloproteases are involved in this process [282], including membrane-type matrix metalloproteinase-1 (MT1-MMP/MMP-14) [283] and some ADAM family proteins [35]. MT1-MMP is a membrane-anchored pericellular collagenase and the main collagenase responsible for endothelial cells sprouting [279]. MT1-MMP induces the activation of the diffusible matrix metalloproteinase MMP2. The local ECM degradation allows tip cell migration and capillary sprouting [284]. The activation of MT1-MMP and MMP2 by oxLDLs is involved in the activation of the sphingolipid pathway and cell proliferation [285]. Moreover, MT1-MMP cooperates with S1P, to induce endothelial cell proliferation and migration [286,287]. MT1-MMP expression by tip cells during early steps of angiogenesis is down-regulated by interactions between pericytes and endothelial cells during the maturation step of neovessels, [288,289].

In atherosclerotic plaques, intraplaque hypoxia may induce the activation of gelatinases (MMP-7 and MMP-9), thereby promoting fibrous cap degradation and plaque instability [290].

5. Consequences of angiogenesis on the evolution of the atherosclerotic plaque

5.1. Plaque growth

The neovascularization of the atherosclerotic areas supplies O₂, lipoproteins and other nutrients, which allow lipid core expansion, leukocyte influx, plaque growth and disease progression [30,222,291]. The increased endothelial permeability allows the entry of lipoproteins into the intima, and the progressive oxidation of LDLs by ROS generated by inflammatory cells. Oxidized lipids are less concentrated at the periphery of the plaque, where they may induce cell activation, migration, proliferation and angiogenesis. In contrast, in the central area of the plaque, lipids are more oxidized (because of low clearance and auto-oxidation), thus may induce toxic events that contribute to the expansion of the necrotic core, neovessel injury, intraplaque hemorrhages and plaque instability [13,291,292].

5.2. Plaque complications

5.2.1. Intraplaque hemorrhages

Intraplaque hemorrhages are associated with high density of microvessels within the atherosclerotic plaque. Such hemorrhages induce the formation of intraplaque clots and the deposition of iron, fibrin and erythrocyte components [293]. In atherosclerotic plaques from human carotids, hemorrhagic areas are surrounded by macrophages (CD68+) that are involved in the phagocytosis of red blood cells and iron. In addition, these macrophages can release VEGF that enhances the permeability of neovessels, which promotes red blood cell extravasation [30]. Moreover, as intraplaque neovessels are not or only partly covered by mural pericytes and SMC, they are fragile and prone to blood extravasation [30]. Around intraplaque hemorrhagic areas, fibrin and platelet CD41 are often detected by immunostaining, suggesting the occurrence of an intraplaque thrombotic process [291]. Moreover, intraplaque hemorrhages elicit local cholesterol crystal formation, ROS generation, oxidative stress, and protease activation that contribute to plaque growth, instability and rupture and finally to thromboembolic events [294].

5.2.2. Cholesterol crystal formation within the plaque

The core of the plaque is enriched by the influx of blood cells during bleeding events. The cholesterol-rich membranes of circulating cells, namely activated platelets, leukocytes and erythrocytes [295] may release free cholesterol within the hemorrhagic plaque. Free cholesterol can form cholesterol crystals, which can disrupt biological membranes, erode the fibrous cap and protrude into the lumen where they may cause embolism or thrombosis [296]. These cholesterol crystals could also trigger an inflammatory response within the arterial wall [297,298] and erode the newly formed microvessels in the plaque [41]. The presence of cholesterol crystals in the arterial wall is a factor of parietal thrombosis exacerbated by angiogenesis and intraplaque bleeding [299,300].

5.2.3. Oxidation within the plaque

Oxidative stress in atherosclerotic prone areas leads to the formation of oxidized lipoproteins and lipid peroxidation derivatives [301–303]. Moreover, in advanced atherosclerotic plaques, intraplaque microhemorrhages release hemoglobin, heme and iron that promote free radicals and ROS generation (e.g. through Fenton reaction), thereby inactivating nitric oxide and promoting lipid peroxidation [30]. This is supported by histological studies of coronary plaques, where glycophorin A, a red blood cell protein, colocalizes with CD163 (hemoglobin scavenger receptor)-positive macrophage and 4-HNE-protein adducts, suggesting that intraplaque hemorrhages are associated with oxidative stress in unstable plaques [304–306]. Protective systems may counterbalance the oxidative stress resulting from hemo-

globin release. The pro-oxidant effects of hemoglobin, heme and iron are limited by macrophagic cells that clear altered erythrocytes (e.g. exposing phosphatidylserines) and cell debris [307], hemoglobin-haptoglobin Hp1-1 complexes (deficient in Hp2-2 homozygous subjects) [308,309] and heme-hemopexin complexes [291].

Moreover, leukocytes present in the lesions contain oxidizing enzymes (NADPH oxidase, myeloperoxidase) that may oxidize the components of the plaque [310,311]. However, it may be noted that angiogenesis in lipid-rich areas of advanced plaques is often associated with oxidative and inflammatory markers, thus suggesting that angiogenesis promotes non-resolving inflammation [5,275].

5.2.4. Proteolysis within the plaque

In atherosclerotic lesions, various proteases are activated and take part in the degradation of ECM, thereby contributing to plaque remodeling and penetration of microvessels into the plaque. However, excessive degradation of the fibrous cap may promote plaque instability, erosion or rupture [290,312]. Studies on carotid endarterectomy have shown that plasminogen activators (tPA and uPA) and plasmin are often activated in complex unstable plaques. In addition, plasminogen activators and plasmin may in turn trigger the release of gelatinases MMP-2, MMP-7 and MMP-9 [290,312]. Moreover, in atherosclerotic lesions, neovessels may facilitate the influx of activated leukocytes, promoted by highly expressed endothelial P-selectin or by extravasation through highly permeable endothelium or by hemorrhages.

In human carotid plaques, hemorrhage markers (hemoglobin, plasminogen) are co-localized with leukocytic proteases (e.g. specific neutrophil lipocain/MMP-9 complex, myeloperoxidase, elastase) and anti-proteases (e.g. PAI-1, alpha-1 antitrypsin, thrombin inhibitors) [312,313].

5.2.5. Inflammatory cells

In lipid-rich atherosclerotic areas, angiogenesis is often associated with inflammatory cells that play a role in plaque instability and rupture. Conversely, neovascularization of atherosclerotic lesions favors the influx of leukocytes in the plaque. Monocytes/macrophages play a dual physiological role in tissues, where they act as a defense system that may release toxic compounds (ROS, enzymes), scavenge potentially harmful compounds and induce both pro-inflammatory and anti-inflammatory responses [314,315]. During atherogenesis, macrophagic cells play a major role in the local inflammation and lipid accumulation. Macrophages accumulate lipids and become foam cells that are unable to migrate and produce cytokines that take part in the local inflammatory burden [1–3]. Among the variety of inflammatory cytokines, the CXC chemokine interleukin-8 is present in human coronary atherosclerotic plaques and exhibits a potent angiogenic effect [316]. In addition, inflammatory cells release MMPs that degrade ECM and release matrix-bound VEGF [292]. Moreover, macrophages of atherosclerotic lesions secrete angiogenic factors, such as PDGF, FGF-2, TGF- β 1, PD-ECGF (platelet-derived endothelial cell growth factor), PAF, HB-EGF (heparin-binding epidermal growth factor-like growth factor) [292]. Finally, in inflammatory cells produce various factors that promote intraplaque neovascularization and neovessels induce in turn the influx of blood molecules and cells that promote plaque instability, hemorrhage and rupture.

6. Pro-angiogenic and antiangiogenic agents in animal models and clinical trials

Antiangiogenic molecules, endostatine and fumagillin [317] tested in ApoE^{-/-} mice induce a reduction of atherosclerotic lesions and macrophages infiltration [21]. A site-specific $\alpha_v\beta_3$ -integrin targeted delivery of nanoparticles containing the antiangiogenic drug fumagillin was effective to block endothelial cells proliferation and reduce intraplaque neovascularization in hypercholesterolemic rabbits [318].

Angiopoietin-2 blocking antibodies reduce neovascularization and fatty streak progression in LDLR^{-/-} apoB^{100/100} mice, but do not reduce the size of pre-existing atherosclerotic lesions [149]. In contrast, the overexpression of angiopoietin-2 reduces the size of atherosclerotic lesions in the aortic root of ApoE^{-/-} mice via inhibition of LDL oxidation [319].

Hypercholesterolemia is correlated with plaque angiogenesis and atherosclerosis progression [19]. Statin treatment attenuates neovascularization and atherosclerosis progression in experimental hypercholesterolemia [7,320].

Among clinical trials utilizing antiangiogenic agents in the treatment of cancer in humans, some of them have evaluated the impact of antiangiogenic agents in atherosclerosis. A recent study utilizing anti-VEGF in cancer therapy, points out hypertension as a major adverse effect [8]. In another study, intravitreal anti-VEGF therapy induced proteinuria and injury in renal allografts [321]. More generally, beside its role in angiogenesis and in the maintenance of microcirculation, VEGF signaling is also involved in compensatory responses and remodeling following heart stress or injury. The inhibition of this pathway by antiangiogenic therapy may generate endothelial dysfunction, reduce microvascular circulation, and induce various cardiovascular adverse effects, such as hypertension, left myocardial ischemia, cardiomyopathy, thromboembolic disease and thrombotic microangiopathy [6,322]. A meta-analysis of clinical trials utilizing VEGFRs inhibitors (in the treatment of cancer) showed a moderate increase of the arterial thromboembolism risk [131]. Finally, these studies show that antiangiogenic drugs used to date have adverse effects that probably preclude their use in long-term treatment of atherosclerosis. Thus, further investigations are required to discover novel antiangiogenic agents devoid of unacceptable adverse effects, and to evaluate their potential efficacy in preventing atherosclerotic plaque instability.

7. Conclusion

Angiogenesis involves a complex array of pro- and anti-angiogenic factors that trigger cell signaling and regulate migration, proliferation leading to capillary tube formation, remodeling of the surrounding ECM and stabilization of neovessels by pericytes. Within atherosclerotic plaques, many factors, including free radicals and oxidized lipids, may exert a biphasic role in this process. In early stages of atherosclerosis, the low inflammatory oxidative stress associated with a relative hypoxia in hyperplased intima, promotes neoangiogenesis from the adventitial vasa vasorum. In advanced atherosclerotic plaques, chronic inflammation, oxidized lipids and proteases may further promote angiogenesis, but these neocapillaries are leaky and are highly susceptible to injury by cytotoxic agents (e.g. oxidized lipids, oxidative stress) generated inside the plaque. This neocapillary injury may result in intraplaque hemorrhages, releasing blood cells, coagulation factors and proteases within the plaque. These events generate an accumulation of cholesterol and the formation of cholesterol crystals, fibrin deposition, release of hemoglobin, heme and iron ions promoting local oxidative stress, lipid peroxidation, and sustained inflammatory burden. Moreover, the activation of various proteases may degrade the fibrous cap, thereby inducing plaque instability and increasing the risk of plaque rupture, often associated with athero-thrombotic events. In experimental animal models, angiogenesis inhibitors have shown an efficacy to slow down the progression of atherosclerotic lesion formation. However, in humans, the use of anti-angiogenic agents in clinical trials for cancer therapy shows that the anti-angiogenic drugs currently available increase the risk of cardiovascular events in atherosclerotic patients.

Acknowledgements

This work was supported by Inserm (Institut National de la Santé et de la Recherche Médicale), ANR-Carina (ANR-12-BSV1-0016-01),

IdEx Emergence call-PALMA 2015, and Université Paul Sabatier Toulouse-3.

References

- [1] R. Ross, The pathogenesis of atherosclerosis: a perspective for the 1990s, *Nature* 362 (6423) (1993) 801–809.
- [2] A.J. Lusis, Atherosclerosis, *Nature* 407 (6801) (2000) 233–241.
- [3] P. Libby, Inflammation in atherosclerosis, *Nature* 420 (6917) (2002) 868–874.
- [4] J.B. Michel, O. Thauan, X. Houard, O. Meilhac, G. Caligiuri, A. Nicoletti, Topological determinants and consequences of adventitial responses to arterial wall injury, *Arterioscler Thromb. Vasc. Biol.* 27 (6) (2007) 1259–1268.
- [5] J.B. Michel, J.L. Martin-Ventura, A. Nicoletti, B. Ho-Tin-Noe, Pathology of human plaque vulnerability: mechanisms and consequences of intraplaque haemorrhages, *Atherosclerosis* 234 (2) (2014) 311–319.
- [6] C. Vavilavas, D. Lenihan, R. Kurzrock, A.M. Tsimberidou, Anti-vascular endothelial growth factor therapies and cardiovascular toxicity: what are the important clinical markers to target?, *Oncologist* 15 (2) (2010) 130–141.
- [7] J. Tian, S. Hu, Y. Sun, H. Yu, X. Han, W. Cheng, X. Ban, S. Zhang, B. Yu, I.K. Jang, Vasa vasorum and plaque progression, and responses to atorvastatin in a rabbit model of atherosclerosis: contrast-enhanced ultrasound imaging and intravascular ultrasound study, *Heart* 99 (1) (2013) 48–54.
- [8] V. Katsi, I. Zerdes, S. Manolakou, T. Makris, P. Nihoyannopoulos, D. Tousoulis, I. Kallikazaros, Anti-VEGF Anticancer Drugs: mind the Hypertension, *Recent Adv. Cardiovasc Drug Disco.* 9 (2) (2014) 63–72.
- [9] H.C. Stary, A.B. Chandler, R.E. Dinsmore, V. Fuster, S. Glagov, W. Insull Jr., M.E. Rosenfeld, C.J. Schwartz, W.D. Wagner, R.W. Wissler, A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, *Am. Heart Assoc. Circ.* 92 (5) (1995) 1355–1374.
- [10] F. Otsuka, M.C. Kramer, P. Woudstra, K. Yahagi, E. Ladich, A.V. Finn, R.J. de Winter, F.D. Kolodgie, T.N. Wight, H.R. Davis, M. Joner, R. Virmani, Natural progression of atherosclerosis from pathologic intimal thickening to late fibroatheroma in human coronary arteries: a pathology study, *Atherosclerosis* 241 (2) (2015) 772–782.
- [11] K. Yahagi, F.D. Kolodgie, F. Otsuka, A.V. Finn, H.R. Davis, M. Joner, R. Virmani, Pathophysiology of native coronary, vein graft, and in-stent atherosclerosis, *Nat. Rev. Cardiol.* 13 (2) (2016) 79–98.
- [12] W. Insull Jr., The pathology of atherosclerosis: plaque development and plaque responses to medical treatment, *Am. J. Med* 122 (1 Suppl) (2009) S3–S14.
- [13] P.R. Moreno, K.R. Purushothaman, M. Sirol, A.P. Levy, V. Fuster, Neovascularization in human atherosclerosis, *Circulation* 113 (18) (2006) 2245–2252.
- [14] W. Koester, *Endarteritis and arteritis*, *Berl. Klin. Woche.* 13 (1876) 454–455.
- [15] M.C. Winternitz, R.M. Thomas, P.M. LeCompte, *The Biology of Arteriosclerosis*, C. Thomas Pub, Springfield, IL, 1938.
- [16] E. Geiringer, Intimal vascularization and atherosclerosis, *J. Pathol. Bacteriol.* 63 (2) (1951) 201–211.
- [17] K. Sueishi, Y. Yonemitsu, K. Nakagawa, Y. Kaneda, M. Kumamoto, Y. Nakashima, Atherosclerosis and angiogenesis. Its pathophysiological significance in humans as well as in an animal model induced by the gene transfer of vascular endothelial growth factor, *Ann. NY Acad. Sci.* 811 (1997) 322–324.
- [18] E.L. Ritman, A. Lerman, The dynamic vasa vasorum, *Cardiovasc. Res.* 75 (4) (2007) 649–658.
- [19] H.M. Kwon, G. Sangiorgi, E.L. Ritman, C. McKenna, D.R. Holmes Jr., R.S. Schwartz, A. Lerman, Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia, *J. Clin. Invest.* 101 (8) (1998) 1551–1556.
- [20] S. Bhardwaj, H. Roy, T. Heikura, S. Yla-Herttuala, VEGF-A, VEGF-D and VEGF-D(DeltaNDeltaC) induced intimal hyperplasia in carotid arteries, *Eur. J. Clin. Invest.* 35 (11) (2005) 669–676.
- [21] K.S. Moulton, E. Heller, M.A. Konering, E. Flynn, W. Palinski, J. Folkman, Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice, *Circulation* 99 (13) (1999) 1726–1732.
- [22] M. Gossel, J. Herrmann, H. Tang, D. Versari, O. Galili, D. Mannheim, S.V. Rajkumar, L.O. Lerman, A. Lerman, Prevention of vasa vasorum neovascularization attenuates early neointima formation in experimental hypercholesterolemia, *Basic Res Cardiol.* 104 (6) (2009) 695–706.
- [23] F.D. Kolodgie, H.K. Gold, A.P. Burke, D.R. Fowler, H.S. Kruth, D.K. Weber, A. Farb, L.J. Guerrero, M. Hayase, R. Kutys, J. Narula, A.V. Finn, R. Virmani, Intraplaque hemorrhage and progression of coronary atheroma, *N. Engl. J. Med.* 349 (24) (2003) 2316–2325.
- [24] E.M. Conway, D. Collen, P. Carmeliet, Molecular mechanisms of blood vessel growth, *Cardiovasc. Res.* 49 (3) (2001) 507–521.
- [25] M. Simons, Angiogenesis: where do we stand now?, *Circulation* 111 (12) (2005) 1556–1566.
- [26] K. Tanaka, D. Nagata, Y. Hirata, Y. Tabata, R. Nagai, M. Sata, Augmented angiogenesis in adventitia promotes growth of atherosclerotic plaque in apolipoprotein E-deficient mice, *Atherosclerosis* 215 (2) (2011) 366–373.
- [27] P. Carmeliet, Angiogenesis in health and disease, *Nat. Med* 9 (6) (2003) 653–660.
- [28] J.C. Sluimer, M.J. Daemen, Novel concepts in atherogenesis: angiogenesis and hypoxia in atherosclerosis, *J. Pathol.* 218 (1) (2009) 7–29.
- [29] E. Marsch, J.C. Sluimer, M.J. Daemen, Hypoxia in atherosclerosis and inflammation, *Curr. Opin. Lipido.* 24 (5) (2013) 393–400.
- [30] P.R. Moreno, M. Purushothaman, K.R. Purushothaman, Plaque neovascularization: defense mechanisms, betrayal, or a war in progress, *Ann. NY Acad. Sci.* 1254 (2012) 7–17.
- [31] M. Potente, H. Gerhardt, P. Carmeliet, Basic and therapeutic aspects of angiogenesis, *Cell* 146 (6) (2011) 873–887.
- [32] A. Horowitz, M. Simons, Branching morphogenesis, *Circ. Res.* 103 (8) (2008) 784–795.
- [33] P. Carmeliet, R.K. Jain, Molecular mechanisms and clinical applications of angiogenesis, *Nature* 473 (7347) (2011) 298–307.
- [34] J.V. Small, T. Stradal, E. Vignal, K. Rottner, The lamellipodium: where motility begins, *Trends Cell Biol.* 12 (3) (2002) 112–120.
- [35] V.W. van Hinsbergh, P. Koolwijk, Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead, *Cardiovasc. Res.* 78 (2) (2008) 203–212.
- [36] B. Blanco, H. Gerhardt, VEGF and Notch in tip and stalk cell selection, *Cold Spring Harb. Perspect. Med.* 3 (1) (2013) a006569.
- [37] S. Suchting, C. Freitas, F. le Noble, R. Benedito, C. Breant, A. Duarte, A. Eichmann, The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching, *Proc. Natl. Acad. Sci. USA* 104 (9) (2007) 3225–3230.
- [38] P. Carmeliet, F. De Smet, S. Loges, M. Mazzone, Branching morphogenesis and angiogenesis candidates: tip cells lead the way, *Nat. Rev. Clin. Oncol.* 6 (6) (2009) 315–326.
- [39] H. Habeck, A.M. Vogel, S. Schulte-Merker, Getting connected, *Dev. Cell* 5 (5) (2003) 669–670.
- [40] C. Urbich, S. Dimmeler, Endothelial progenitor cells functional characterization, *Trends Cardiovasc. Med.* 14 (8) (2004) 318–322.
- [41] B. Ho-Tin-Noe, J.B. Michel, Initiation of angiogenesis in atherosclerosis: smooth muscle cells as mediators of the angiogenic response to atheroma formation, *Trends Cardiovasc. Med.* 21 (7) (2011) 183–187.
- [42] C. Cheng, I. Chrift, G. Pasterkamp, H.J. Duckers, Biological mechanisms of microvessel formation in advanced atherosclerosis: the big five, *Trends Cardiovasc. Med.* 23 (5) (2013) 153–164.
- [43] G. Eelen, P. de Zeeuw, M. Simons, P. Carmeliet, Endothelial cell metabolism in normal and diseased vasculature, *Circ. Res.* 116 (7) (2015) 1231–1244.
- [44] C.W. Pugh, P.J. Ratcliffe, Regulation of angiogenesis by hypoxia: role of the HIF system, *Nat. Med.* 9 (6) (2003) 677–684.
- [45] G.L. Semenza, G.L. Wang, A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation, *Mol. Cell Biol.* 12 (12) (1992) 5447–5454.
- [46] L.E. Huang, J. Gu, M. Schau, H.F. Bunn, Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway, *Proc. Natl. Acad. Sci. USA* 95 (14) (1998) 7987–7992.
- [47] R.H. Wenger, Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression, *FASEB J.* 16 (10) (2002) 1151–1162.
- [48] G.L. Semenza, Hydroxylation of HIF-1: oxygen sensing at the molecular level, *Physiol. (Bethesda)* 19 (2004) 176–182.
- [49] E. Berra, A. Ginouves, J. Poussegur, The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling, *EMBO Rep.* 7 (1) (2006) 41–45.
- [50] P. van Uden, N.S. Kenneth, S. Rocha, Regulation of hypoxia-inducible factor-1alpha by NF-kappaB, *Biochem. J.* 412 (3) (2008) 477–484.
- [51] R.H. Wenger, D.P. Stiehl, G. Camenisch, Integration of oxygen signaling at the consensus HRE, *Sci. STKE* 2005 (306) (2005) re12.
- [52] K. Hirota, G.L. Semenza, Regulation of angiogenesis by hypoxia-inducible factor 1, *Crit. Rev. Oncol. Hematol.* 59 (1) (2006) 15–26.
- [53] S. Frede, U. Berchner-Pfannschmidt, J. Fandrey, Regulation of hypoxia-inducible factors during inflammation, *Methods Enzym.* 435 (2007) 405–419.
- [54] M. Christoph, K. Ibrahim, K. Hesse, A. Augstein, A. Schmeisser, R.C. Braun-Dullaes, G. Simonis, C. Wunderlich, S. Quick, R.H. Strasser, D.M. Poitz, Local inhibition of hypoxia-inducible factor reduces neointima formation after arterial injury in ApoE^{-/-} mice, *Atherosclerosis* 233 (2) (2014) 641–647.
- [55] L. Rahtu-Korpela, J. Maatta, E.Y. Dimova, S. Horkko, H. Gylling, G. Walkinshaw, J. Hakkola, K.I. Kivirikko, J. Myllyharju, R. Serpi, P. Koivunen, Hypoxia-Inducible Factor Prolyl 4-Hydroxylase-2 Inhibition Protects Against Development of Atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* 36 (4) (2016) 608–617.
- [56] E. Marsch, J.A. Demandt, T.L. Theelen, B.M. Tullemans, K. Wouters, M.R. Boon, T.H. van Dijk, M.J. Gijbels, L.J. Dubois, S.J. Meex, M. Mazzone, G. Hung, E.A. Fisher, E.A. Biessen, M.J. Daemen, P.C. Rensen, P. Carmeliet, A.K. Groen, J.C. Sluimer, Deficiency of the oxygen sensor prolyl hydroxylase 1 attenuates hypercholesterolemia, atherosclerosis, and hyperglycaemia, *Eur. Heart J.* (2016).
- [57] H. Liu, Y. Xia, B. Li, J. Pan, M. Lv, X. Wang, F. An, Prolyl hydroxylase 3 overexpression accelerates the progression of atherosclerosis in ApoE^{-/-} mice, *Biochem. Biophys. Res. Commun.* 473 (1) (2016) 99–106.
- [58] M. Greenblatt, P. Shubi, Tumor angiogenesis: transfilter diffusion studies in the hamster by the transparent chamber technique, *J. Natl. Cancer Inst.* 41 (1) (1968) 111–124.
- [59] R.L. Ehrmann, M. Knöth, Choriocarcinoma. Transfilter stimulation of vasoproliferation in the hamster cheek pouch. Studied by light and electron microscopy, *J. Natl. Cancer Inst.* 41 (6) (1968) 1329–1341.
- [60] J. Folkman, E. Merler, C. Abernathy, G. Williams, Isolation of a tumor factor responsible for angiogenesis, *J. Exp. Med.* 133 (2) (1971) 275–288.
- [61] D.R. Senger, S.J. Galli, A.M. Dvorak, C.A. Perruzzi, V.S. Harvey, H.F. Dvorak, Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid, *Science* 219 (4587) (1983) 983–985.
- [62] P.J. Keck, S.D. Hauser, G. Krivi, K. Sanzo, T. Warren, J. Feder, D.T. Connolly, Vascular permeability factor, an endothelial cell mitogen related to PDGF, *Science*

- 246 (4935) (1989) 1309–1312.
- [63] D.W. Leung, G. Cachianes, W.J. Kuang, D.V. Goeddel, N. Ferrara, Vascular endothelial growth factor is a secreted angiogenic mitogen, *Science* 246 (4935) (1989) 1306–1309.
- [64] J. Plouet, J. Schilling, D. Gospodarowicz, Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells, *EMBO J.* 8 (12) (1989) 3801–3806.
- [65] N. Ferrara, Vascular endothelial growth factor: basic science and clinical progress, *Endocr. Rev.* 25 (4) (2004) 581–611.
- [66] D.I. Holmes, I. Zachary, The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease, *Genome Biol.* 6 (2) (2005) 209.
- [67] Y.O. Kipryushina, K.V. Yakovlev, N.A. Odintsova, Vascular endothelial growth factors: a comparison between invertebrates and vertebrates, *Cytokine Growth Factor Rev.* 26 (6) (2015) 687–695.
- [68] U.A. Vitt, S.Y. Hsu, A.J. Hsueh, Evolution and classification of cystine knot-containing hormones and related extracellular signaling molecules, *Mol. Endocrinol.* 15 (5) (2001) 681–694.
- [69] E. Tischer, R. Mitchell, T. Hartman, M. Silva, D. Gospodarowicz, J.C. Fiddes, J.A. Abraham, The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing, *J. Biol. Chem.* 266 (18) (1991) 11947–11954.
- [70] N. Ferrara, H.P. Gerber, J. Lecouter, The biology of VEGF and its receptors, *Nat. Med.* 9 (6) (2003) 669–676.
- [71] T. Arcondeguy, E. Lacazette, S. Millevoy, H. Prats, C. Touriol, VEGF-A mRNA processing, stability and translation: a paradigm for intricate regulation of gene expression at the post-transcriptional level, *Nucleic Acids Res.* 41 (17) (2013) 7997–8010.
- [72] H. Takahashi, M. Shibuya, The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions, *Clin. Sci. (Lond.)* 109 (3) (2005) 227–241.
- [73] S.J. Harper, D.O. Bates, VEGF-A splicing: the key to anti-angiogenic therapeutics?, *Nat. Rev. Cancer* 8 (11) (2008) 880–887.
- [74] D. Shweiki, A. Itin, D. Soffer, E. Keshet, Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis, *Nature* 359 (6398) (1992) 843–845.
- [75] G. Pages, J. Pouyssegur, Transcriptional regulation of the Vascular Endothelial Growth Factor gene—a concert of activating factors, *Cardiovasc. Res.* 65 (3) (2005) 564–573.
- [76] P. Carmeliet, V. Ferreira, G. Breier, S. Pollefeyt, L. Kieckens, M. Gertsenstein, M. Fahrig, A. Vandenhoeck, K. Harpal, C. Eberhardt, C. Declercq, J. Pawling, L. Moons, D. Collen, W. Risau, A. Nagy, Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele, *Nature* 380 (6573) (1996) 435–439.
- [77] N. Ferrara, K. Carver-Moore, H. Chen, M. Dowd, L. Lu, K.S. O’Shea, L. Powell-Braxton, K.J. Hillan, M.W. Moore, Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene, *Nature* 380 (6573) (1996) 439–442.
- [78] P.W. Holm, R.H. Slart, C.J. Zeebregts, J.L. Hillebrands, R.A. Tio, Atherosclerotic plaque development and instability: a dual role for VEGF, *Ann. Med.* 41 (4) (2009) 257–264.
- [79] T. Asahara, D. Chen, Y. Tsurumi, M. Kearney, S. Rossow, J. Passeri, J.F. Symes, J.M. Isner, Accelerated restitution of endothelial integrity and endothelium-dependent function after phVEGF165 gene transfer, *Circulation* 94 (12) (1996) 3291–3302.
- [80] I. Kim, S.O. Moon, S.H. Kim, H.J. Kim, Y.S. Koh, G.Y. Koh, Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells, *J. Biol. Chem.* 276 (10) (2001) 7614–7620.
- [81] T. Marumo, V.B. Schini-Kerth, R. Busse, Vascular endothelial growth factor activates nuclear factor-kappaB and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells, *Diabetes* 48 (5) (1999) 1131–1137.
- [82] B. Barleon, S. Sozzani, D. Zhou, H.A. Weich, A. Mantovani, D. Marme, Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1, *Blood* 87 (8) (1996) 3336–3343.
- [83] S.E. Heinonen, A.M. Kivela, J. Huusko, M.H. Dijkstra, E. Gurzeler, P.I. Makinen, P. Leppanen, V.M. Olkkonen, U. Eriksson, M. Jauhiainen, S. Yla-Herttuala, The effects of VEGF-A on atherosclerosis, lipoprotein profile, and lipoprotein lipase in hyperlipidaemic mouse models, *Cardiovasc. Res.* 99 (4) (2013) 716–723.
- [84] M. Inoue, H. Itoh, M. Ueda, T. Naruko, A. Kojima, R. Komatsu, K. Doi, Y. Ogawa, N. Tamura, K. Takaya, T. Igaki, J. Yamashita, T.H. Chun, K. Matsusugu, A.E. Becker, K. Nakao, Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions: possible pathophysiological significance of VEGF in progression of atherosclerosis, *Circulation* 98 (20) (1998) 2108–2116.
- [85] F.L. Celletti, J.M. Waugh, P.G. Amabile, A. Brendolan, P.R. Hilfiker, M.D. Dake, Vascular endothelial growth factor enhances atherosclerotic plaque progression, *Nat. Med.* 7 (4) (2001) 425–429.
- [86] Q. Zhao, K. Egashira, S. Inoue, M. Usui, S. Kitamoto, W. Ni, M. Ishibashi, K. Hiasa Ki, T. Ichiki, M. Shibuya, A. Takeshita, Vascular endothelial growth factor is necessary in the development of arteriosclerosis by recruiting/activating monocytes in a rat model of long-term inhibition of nitric oxide synthesis, *Circulation* 105 (9) (2002) 1110–1115.
- [87] P. Leppanen, S. Koota, I. Kholova, J. Koponen, C. Fieber, U. Eriksson, K. Alitalo, S. Yla-Herttuala, Gene transfers of vascular endothelial growth factor-A, vascular endothelial growth factor-B, vascular endothelial growth factor-C, and vascular endothelial growth factor-D have no effects on atherosclerosis in hypercholesterolemic low-density lipoprotein-receptor/apolipoprotein B48-deficient mice, *Circulation* 112 (9) (2005) 1347–1352.
- [88] M. Giacca, S. Zaccagna, VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond, *Gene Ther.* 19 (6) (2012) 622–629.
- [89] G.M. Rubanyi, Angiogenic gene therapy for refractory angina, *Expert Opin. Biol. Ther.* 16 (3) (2016) 303–315.
- [90] L.I. Faruque, M. Lin, M. Battistella, N. Wiebe, T. Reiman, B. Hemmelgarn, C. Thomas, M. Tonelli, Systematic review of the risk of adverse outcomes associated with vascular endothelial growth factor inhibitors for the treatment of cancer, *PLoS One* 9 (7) (2014) e101145.
- [91] S. Grimmond, J. Lagercrantz, C. Drinkwater, G. Silins, S. Townson, P. Pollock, D. Gotley, E. Carson, S. Rakar, M. Nordenskjold, L. Ward, N. Hayward, G. Weber, Cloning and characterization of a novel human gene related to vascular endothelial growth factor, *Genome Res.* 6 (2) (1996) 124–131.
- [92] B. Olofsson, K. Pajusola, A. Kaipainen, G. von Euler, V. Joukov, O. Saksela, A. Orpana, R.F. Petterson, K. Alitalo, U. Eriksson, Vascular endothelial growth factor B, a novel growth factor for endothelial cells, *Proc. Natl. Acad. Sci. USA* 93 (6) (1996) 2576–2581.
- [93] M. Bry, R. Kivela, V.M. Leppanen, K. Alitalo, Vascular endothelial growth factor-B in physiology and disease, *Physiol. Rev.* 94 (3) (2014) 779–794.
- [94] D. Bellomo, J.P. Headrick, G.U. Silins, C.A. Paterson, P.S. Thomas, M. Gartside, A. Mould, M.M. Cahill, I.D. Tonks, S.M. Grimmond, S. Townson, C. Wells, M. Little, M.C. Cummings, N.K. Hayward, G.F. Kay, Mice lacking the vascular endothelial growth factor-B gene (Vegfb) have smaller hearts, dysfunctional coronary vasculature, and impaired recovery from cardiac ischemia, *Circ. Res.* 86 (2) (2000) E29–E35.
- [95] C.E. Hagberg, A. Falkevall, X. Wang, E. Larsson, J. Huusko, I. Nilsson, L.A. van Meeteren, E. Samen, L. Lu, M. Vanwildemeersch, J. Klar, G. Genove, K. Pietras, S. Stone-Elander, L. Claesson-Welsh, S. Yla-Herttuala, P. Lindahl, U. Eriksson, Vascular endothelial growth factor B controls endothelial fatty acid uptake, *Nature* 464 (7290) (2010) 917–921.
- [96] M.R. Robciuc, R. Kivela, I.M. Williams, J.F. de Boer, T.H. van Dijk, H. Elamaa, F. Tigist-Sahle, D. Molotkov, V.M. Leppanen, R. Kakela, L. Eklund, D.H. Wasserman, A.K. Groen, K. Alitalo, VEGFB/VEGFR1-Induced Expansion of Adipose Vasculature Counteracts Obesity and Related Metabolic Complications, *Cell Metab.* 23 (4) (2016) 712–724.
- [97] D. Maglione, V. Guerriero, G. Viglietto, P. Delli-Bovi, M.G. Persico, Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor, *Proc. Natl. Acad. Sci. USA* 88 (20) (1991) 9267–9271.
- [98] M. Dewerchin, P. Carmeliet, PlGF: a multitasking cytokine with disease-restricted activity, *Cold Spring Harb. Perspect. Med.* 2 (8) (2012).
- [99] S. De Falco, The discovery of placenta growth factor and its biological activity, *Exp. Mol. Med.* 44 (1) (2012) 1–9.
- [100] P. Carmeliet, L. Moons, A. Luttmann, V. Vincinti, V. Compennolle, M. De Mol, Y. Wu, F. Bono, L. Devy, H. Beck, D. Scholz, T. Acker, T. DiPalma, M. Dewerchin, A. Noel, I. Stalmans, A. Barra, S. Blacher, T. VandenDriessche, A. Ponten, U. Eriksson, K.H. Plate, J.M. Foidart, W. Schaper, D.S. Charnock-Jones, D.J. Hicklin, J.M. Herbert, D. Collen, M.G. Persico, Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions, *Nat. Med.* 7 (5) (2001) 575–583.
- [101] R. Khurana, L. Moons, S. Shafi, A. Luttmann, D. Collen, J.F. Martin, P. Carmeliet, I.C. Zachary, Placental growth factor promotes atherosclerotic intimal thickening and macrophage accumulation, *Circulation* 111 (21) (2005) 2828–2836.
- [102] V. Joukov, T. Sorsa, V. Kumar, M. Jeltsch, L. Claesson-Welsh, Y. Cao, O. Saksela, N. Kalkkinen, K. Alitalo, Proteolytic processing regulates receptor specificity and activity of VEGF-C, *EMBO J.* 16 (13) (1997) 3898–3911.
- [103] A. Dashkevich, C. Hagl, F. Beyersdorf, A.I. Nykanen, K.B. Lemstrom, VEGF Pathways in the Lymphatics of Healthy and Diseased Heart, *Microcirculation* 23 (1) (2016) 5–14.
- [104] M.J. Karkkainen, P. Haiko, K. Sainio, J. Partanen, J. Taipale, T.V. Petrova, M. Jeltsch, D.G. Jackson, M. Talikka, H. Rauvala, C. Betsholtz, K. Alitalo, Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins, *Nat. Immunol.* 5 (1) (2004) 74–80.
- [105] M.E. Baldwin, M.M. Halford, S. Roufail, R.A. Williams, M.L. Hibbs, D. Grail, H. Kubo, S.A. Stacker, M.G. Achen, Vascular endothelial growth factor D is dispensable for development of the lymphatic system, *Mol. Cell Biol.* 25 (6) (2005) 2441–2449.
- [106] D.J. Dumont, L. Jussila, J. Taipale, A. Lymboussaki, T. Mustonen, K. Pajusola, M. Breitman, K. Alitalo, Cardiovascular failure in mouse embryos deficient in VEGF receptor-3, *Science* 282 (5390) (1998) 946–949.
- [107] P. Haiko, T. Makinen, S. Kesitalo, J. Taipale, M.J. Karkkainen, M.E. Baldwin, S.A. Stacker, M.G. Achen, K. Alitalo, Deletion of vascular endothelial growth factor C (VEGF-C) and VEGF-D is not equivalent to VEGF receptor 3 deletion in mouse embryos, *Mol. Cell Biol.* 28 (15) (2008) 4843–4850.
- [108] M. Jeltsch, A. Kaipainen, V. Joukov, X. Meng, M. Lakso, H. Rauvala, M. Swartz, D. Fukumura, R.K. Jain, K. Alitalo, Hyperplasia of lymphatic vessels in VEGF-C transgenic mice, *Science* 276 (5317) (1997) 1423–1425.
- [109] H.Y. Lim, C.H. Thiam, K.P. Yeo, R. Bissoondial, C.S. Hii, K.C. McGrath, K.W. Tan, A. Heather, J.S. Alexander, V. Angeli, Lymphatic vessels are essential for the removal of cholesterol from peripheral tissues by SR-BI-mediated transport of HDL, *Cell Metab.* 17 (5) (2013) 671–684.
- [110] C. Martel, W. Li, B. Fulp, A.M. Platt, E.L. Gautier, M. Westerterp, R. Bittman, A.R. Tall, S.H. Chen, M.J. Thomas, D. Kreisler, M.A. Swartz, M.G. Sorci-Thomas, G.J. Randolph, Lymphatic vasculature mediates macrophage reverse cholesterol transport in mice, *J. Clin. Invest.* 123 (4) (2013) 1571–1579.
- [111] T. Vuorio, H. Nurmi, K. Moulton, J. Kurkipuro, M.R. Robciuc, M. Ohman, S.E. Heinonen, H. Samaranayake, T. Heikura, K. Alitalo, S. Yla-Herttuala, Lymphatic vessel insufficiency in hypercholesterolemic mice alters lipoprotein

- levels and promotes atherogenesis, *Arterioscler Thromb. Vasc. Biol.* 34 (6) (2014) 1162–1170.
- [112] A. Aspelund, M.R. Robciuc, S. Karaman, T. Makinen, K. Alitalo, Lymphatic System in Cardiovascular Medicine, *Circ. Res.* 118 (3) (2016) 515–530.
- [113] C. de Vries, J.A. Escobedo, H. Ueno, K. Houck, N. Ferrara, L.T. Williams, The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor, *Science* 255 (5047) (1992) 989–991.
- [114] B.I. Terman, M. Dougher-Vermazen, M.E. Carrion, D. Dimitrov, D.C. Armellino, D. Gospodarowicz, P. Bohlen, Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor, *Biochem Biophys. Res. Commun.* 187 (3) (1992) 1579–1586.
- [115] S. Soker, S. Takashima, H.Q. Miao, G. Neufeld, M. Klagsbrun, Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor, *Cell* 92 (6) (1998) 735–745.
- [116] L. Claesson-Welsh, VEGF receptor signal transduction - A brief update, *Vasc. Pharm.* (2016).
- [117] S. Koch, L. Claesson-Welsh, Signal transduction by vascular endothelial growth factor receptors, *Cold Spring Harb. Perspect. Med.* 2 (7) (2012) a006502.
- [118] M. Shibuya, Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases, *J. Biochem.* 153 (1) (2013) 13–19.
- [119] A. Hoeben, B. Landuyt, M.S. Highley, H. Wildiers, A.T. Van Oosterom, E.A. De Bruijn, Vascular endothelial growth factor and angiogenesis, *Pharm. Rev.* 56 (4) (2004) 549–580.
- [120] M. Simons, E. Gordon, L. Claesson-Welsh, Mechanisms and regulation of endothelial VEGF receptor signalling, *Nat. Rev. Mol. Cell Biol.* (2016).
- [121] M.J. Cudmore, P.W. Hewett, S. Ahmad, K.Q. Wang, M. Cai, B. Al-Ani, T. Fujisawa, B. Ma, S. Sissaoui, W. Ramma, M.R. Miller, D.E. Newby, Y. Gu, B. Barleon, H. Weich, A. Ahmed, The role of heterodimerization between VEGFR-1 and VEGFR-2 in the regulation of endothelial cell homeostasis, *Nat. Commun.* 3 (2012) 972.
- [122] G.H. Fong, J. Rossant, M. Gertsenstein, M.L. Breitman, Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium, *Nature* 376 (6535) (1995) 66–70.
- [123] S. Hiratsuka, O. Minowa, J. Kuno, T. Noda, M. Shibuya, Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice, *Proc. Natl. Acad. Sci. USA* 95 (16) (1998) 9349–9354.
- [124] Q. Zhao, K. Egashira, K. Hiasa, M. Ishibashi, S. Inoue, K. Ohtani, C. Tan, M. Shibuya, A. Takeshita, K. Sunagawa, Essential role of vascular endothelial growth factor and Flt-1 signals in neointimal formation after periaortic injury, *Arterioscler Thromb. Vasc. Biol.* 24 (12) (2004) 2284–2289.
- [125] R. Usui, M. Shibuya, S. Ishibashi, Y. Maru, Ligand-independent activation of vascular endothelial growth factor receptor 1 by low-density lipoprotein, *EMBO Rep.* 8 (12) (2007) 1155–1161.
- [126] M. Shibuya, Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis, *Angiogenesis* 9 (4) (2006) 225–230 (discussion 231).
- [127] F. Shalaby, J. Rossant, T.P. Yamaguchi, M. Gertsenstein, X.F. Wu, M.L. Breitman, A.C. Schuh, Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice, *Nature* 376 (6535) (1995) 62–66.
- [128] S. Winnik, C. Lohmann, G. Siciliani, T. von Lukowicz, K. Kuschner, N. Kraenkel, C.E. Brokopp, F. Enseleit, S. Michels, F. Ruschitzka, T.F. Luscher, C.M. Matter, Systemic VEGF inhibition accelerates experimental atherosclerosis and disrupts endothelial homeostasis—implications for cardiovascular safety, *Int J. Cardiol.* 168 (3) (2013) 2453–2461.
- [129] R.J. Petrovan, C.D. Kaplan, R.A. Reisfeld, L.K. Curtiss, DNA vaccination against VEGF receptor 2 reduces atherosclerosis in LDL receptor-deficient mice, *Arterioscler Thromb. Vasc. Biol.* 27 (5) (2007) 1095–1100.
- [130] A.D. Hauer, G.H. van Puijvelde, N. Peterse, P. de Vos, V. van Weel, E.J. van Wanrooij, E.A. Biessen, P.H. Quax, A.G. Niethammer, R.A. Reisfeld, T.J. van Berkel, J. Kuiper, Vaccination against VEGFR2 attenuates initiation and progression of atherosclerosis, *Arterioscler Thromb. Vasc. Biol.* 27 (9) (2007) 2050–2057.
- [131] T.K. Choueiri, F.A. Schutz, Y. Je, J.E. Rosenberg, J. Bellmunt, Risk of arterial thromboembolic events with sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials, *J. Clin. Oncol.* 28 (13) (2010) 2280–2285.
- [132] D.J. Dumont, T.P. Yamaguchi, R.A. Conlon, J. Rossant, M.L. Breitman, tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors, *Oncogene* 7 (8) (1992) 1471–1480.
- [133] J. Partanen, E. Armstrong, T.P. Makela, J. Korhonen, M. Sandberg, R. Renkonen, S. Knuutila, K. Huebner, K. Alitalo, A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains, *Mol. Cell Biol.* 12 (4) (1992) 1698–1707.
- [134] S. Davis, T.H. Aldrich, P.F. Jones, A. Acheson, D.L. Compton, V. Jain, T.E. Ryan, J. Bruno, C. Radziejewski, P.C. Maisonpierre, G.D. Yancopoulos, Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning, *Cell* 87 (7) (1996) 1161–1169.
- [135] P.C. Maisonpierre, C. Suri, P.F. Jones, S. Bartunkova, S.J. Wiegand, C. Radziejewski, D. Compton, J. McClain, T.H. Aldrich, N. Papadopoulos, T.J. Daly, S. Davis, T.N. Sato, G.D. Yancopoulos, Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis, *Science* 277 (5322) (1997) 55–60.
- [136] D. Hanahan, Signaling vascular morphogenesis and maintenance, *Science* 277 (5322) (1997) 48–50.
- [137] G.D. Yancopoulos, S. Davis, N.W. Gale, J.S. Rudge, S.J. Wiegand, J. Holash, Vascular-specific growth factors and blood vessel formation, *Nature* 407 (6801) (2000) 242–248.
- [138] G. Thurston, Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis, *Cell Tissue Res.* 314 (1) (2003) 61–68.
- [139] H.G. Augustin, G.Y. Koh, G. Thurston, K. Alitalo, Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system, *Nat. Rev. Mol. Cell Biol.* 10 (3) (2009) 165–177.
- [140] A. Moss, The angiopoietin: tie 2 interaction: a potential target for future therapies in human vascular disease, *Cytokine Growth Factor Rev.* 24 (6) (2013) 579–592.
- [141] D.J. Dumont, G. Gradwohl, G.H. Fong, M.C. Puri, M. Gertsenstein, A. Auerbach, M.L. Breitman, Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo, *Genes Dev.* 8 (16) (1994) 1897–1909.
- [142] C. Suri, P.F. Jones, S. Patan, S. Bartunkova, P.C. Maisonpierre, S. Davis, T.N. Sato, G.D. Yancopoulos, Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis, *Cell* 87 (7) (1996) 1171–1180.
- [143] C. Suri, J. McClain, G. Thurston, D.M. McDonald, H. Zhou, E.H. Oldmixon, T.N. Sato, G.D. Yancopoulos, Increased vascularization in mice overexpressing angiopoietin-1, *Science* 282 (5388) (1998) 468–471.
- [144] N.W. Gale, G. Thurston, S.F. Hackett, R. Renard, Q. Wang, J. McClain, C. Martin, C. Witte, M.H. Witte, D. Jackson, C. Suri, P.A. Campochiaro, S.J. Wiegand, G.D. Yancopoulos, Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1, *Dev. Cell* 3 (3) (2002) 411–423.
- [145] M.C. Puri, J. Rossant, K. Alitalo, A. Bernstein, J. Partanen, The receptor tyrosine kinase TIE is required for integrity and survival of vascular endothelial cells, *EMBO J.* 14 (23) (1995) 5884–5891.
- [146] T.N. Sato, Y. Tozawa, U. Deutsch, K. Wolburg-Buchholz, Y. Fujiwara, M. Gendron-Maguire, T. Gridley, H. Wolburg, W. Risau, Y. Qin, Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation, *Nature* 376 (6535) (1995) 70–74.
- [147] A.I. Nykanen, R. Krebs, A. Saaristo, P. Turunen, K. Alitalo, S. Yla-Herttuala, P.K. Koskinen, K.B. Lemstrom, Angiopoietin-1 protects against the development of cardiac atherosclerosis, *Circulation* 107 (9) (2003) 1308–1314.
- [148] A. Ahmed, T. Fujisawa, Multiple roles of angiopoietins in atherosclerosis, *Curr. Opin. Lipidol.* 22 (5) (2011) 380–385.
- [149] T.L. Theelen, J.P. Lappalainen, J.C. Sluimer, E. Gurzeler, J.P. Cleutjens, M.J. Gijbels, E.A. Biessen, M.J. Daemen, K. Alitalo, S. Yla-Herttuala, Angiopoietin-2 blocking antibodies reduce early atherosclerotic plaque development in mice, *Atherosclerosis* 241 (2) (2015) 297–304.
- [150] S. Post, W. Peeters, E. Busser, D. Lamers, J.P. Sluijter, M.J. Goumans, R.A. de Weger, F.L. Moll, P.A. Doevendans, G. Pasterkamp, A. Vink, Balance between angiopoietin-1 and angiopoietin-2 is in favor of angiopoietin-2 in atherosclerotic plaques with high microvessel density, *J. Vasc. Res.* 45 (3) (2008) 244–250.
- [151] A.D. Hauer, K.L. Habets, E.J. van Wanrooij, P. de Vos, J. Krueger, R.A. Reisfeld, T.J. van Berkel, J. Kuiper, Vaccination against TIE2 reduces atherosclerosis, *Atherosclerosis* 204 (2) (2009) 365–371.
- [152] K.V. Woo, X. Qu, V.R. Babaev, M.F. Linton, R.J. Guzman, S. Fazio, H.S. Baldwin, TIE1 attenuation reduces murine atherosclerosis in a dose-dependent and shear stress-specific manner, *J. Clin. Invest* 121 (4) (2011) 1624–1635.
- [153] N. Farhat, N. Thorin-Trescases, M. Mamarbachi, L. Villeneuve, C. Yu, C. Martel, N. Duquette, M. Gayda, A. Nigam, M. Juneau, B.G. Allen, E. Thorin, Angiopoietin-like 2 promotes atherosclerosis in mice, *J. Am. Heart Assoc.* 2 (3) (2013) e000201.
- [154] E. Horio, T. Kadomatsu, K. Miyata, Y. Arai, K. Hosokawa, Y. Doi, T. Ninomiya, H. Horiguchi, M. Endo, M. Tabata, H. Tazume, Z. Tian, O. Takahashi, K. Terada, M. Takeya, H. Hao, N. Hirose, T. Minami, T. Suda, Y. Kiyohara, H. Ogawa, K. Kaikita, Y. Oike, Role of endothelial cell-derived angpt2 in vascular inflammation leading to endothelial dysfunction and atherosclerosis progression, *Arterioscler Thromb. Vasc. Biol.* 34 (4) (2014) 790–800.
- [155] R.F. Furchgott, J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, *Nature* 288 (5789) (1980) 373–376.
- [156] L.J. Ignarro, G.M. Buga, K.S. Wood, R.E. Byrns, G. Chaudhuri, Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide, *Proc. Natl. Acad. Sci. USA* 84 (24) (1987) 9265–9269.
- [157] R.M. Palmer, A.G. Ferrige, S. Moncada, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor, *Nature* 327 (6122) (1987) 524–526.
- [158] W.K. Alderton, C.E. Cooper, R.G. Knowles, Nitric oxide synthases: structure, function and inhibition, *Biochem. J.* 357 (Pt 3) (2001) 593–615.
- [159] J.P. Cooke, NO and angiogenesis, *Atheroscler. Suppl.* 4 (4) (2003) 53–60.
- [160] U. Forstermann, W.C. Sessa, Nitric oxide synthases: regulation and function, *Eur. Heart J.* 33 (7) (2012) 829–837 (837a–837d).
- [161] P.M. Vanhoutte, Y. Zhao, A. Xu, S.W. Leung, Thirty Years of Saying NO: sources, Fate, Actions, and Misfortunes of the Endothelium-Derived Vasodilator Mediator, *Circ. Res.* 119 (2) (2016) 375–396.
- [162] P.L. Huang, T.M. Dawson, D.S. Bredt, S.H. Snyder, M.C. Fishman, Targeted disruption of the neuronal nitric oxide synthase gene, *Cell* 75 (7) (1993) 1273–1286.
- [163] T. Morishita, M. Tsutsui, H. Shimokawa, M. Horiuchi, A. Tanimoto, O. Suda, H. Tasaki, P.L. Huang, Y. Sasaguri, N. Yanagihara, Y. Nakashima, Vasculoprotective roles of neuronal nitric oxide synthase, *FASEB J.* 16 (14) (2002) 1994–1996.
- [164] P.L. Huang, Z. Huang, H. Mashimo, K.D. Bloch, M.A. Moskowitz, J.A. Bevan, M.C. Fishman, Hypertension in mice lacking the gene for endothelial nitric oxide synthase, *Nature* 377 (6546) (1995) 239–242.

- [165] P.J. Kuhlencordt, R. Gyurko, F. Han, M. Scherrer-Crosbie, T.H. Aretz, R. Hajjar, M.H. Picard, P.L. Huang, Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice, *Circulation* 104 (4) (2001) 448–454.
- [166] J.J. Jang, H.K. Ho, H.H. Kwan, L.F. Fajardo, J.P. Cooke, Angiogenesis is impaired by hypercholesterolemia: role of asymmetric dimethylarginine, *Circulation* 102 (12) (2000) 1414–1419.
- [167] J.D. MacMicking, C. Nathan, G. Hom, N. Chartrain, D.S. Fletcher, M. Trumbauer, K. Stevens, Q.W. Xie, K. Sokol, N. Hutchinson, et al., Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase, *Cell* 81 (4) (1995) 641–650.
- [168] X.Q. Wei, I.G. Charles, A. Smith, J. Ure, G.J. Feng, F.P. Huang, D. Xu, W. Muller, S. Monaco, F.Y. Liew, Altered immune responses in mice lacking inducible nitric oxide synthase, *Nature* 375 (6530) (1995) 408–411.
- [169] V.E. Laubach, E.G. Shesely, O. Smithies, P.A. Sherman, Mice lacking inducible nitric oxide synthase are not resistant to lipopolysaccharide-induced death, *Proc. Natl. Acad. Sci. USA* 92 (23) (1995) 10688–10692.
- [170] I.N. Mungro, R. Gros, X. You, A. Pirani, A. Azad, T. Csont, R. Schulz, J. Butany, D.J. Stewart, M. Husain, Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death, *J. Clin. Invest* 109 (6) (2002) 735–743.
- [171] P.J. Kuhlencordt, S. Hotten, J. Schodel, S. Rutzel, K. Hu, J. Widder, A. Marx, P.L. Huang, G. Ertl, Atheroprotective effects of neuronal nitric oxide synthase in apolipoprotein e knockout mice, *Arterioscler Thromb. Vasc. Biol.* 26 (7) (2006) 1539–1544.
- [172] V.W. Liu, P.L. Huang, Cardiovascular roles of nitric oxide: a review of insights from nitric oxide synthase gene disrupted mice, *Cardiovasc. Res.* 77 (1) (2008) 19–29.
- [173] R. van Haperen, M. de Waard, E. van Deel, B. Mees, M. Kutryk, T. van Aken, J. Hamming, F. Grosveld, D.J. Duncker, R. de Crom, Reduction of blood pressure, plasma cholesterol, and atherosclerosis by elevated endothelial nitric oxide, *J. Biol. Chem.* 277 (50) (2002) 48803–48807.
- [174] P.J. Kuhlencordt, J. Chen, F. Han, J. Astern, P.L. Huang, Genetic deficiency of inducible nitric oxide synthase reduces atherosclerosis and lowers plasma lipid peroxides in apolipoprotein E-knockout mice, *Circulation* 103 (25) (2001) 3099–3104.
- [175] S. Nakata, M. Tsutsui, H. Shimokawa, O. Suda, T. Morishita, K. Shibata, Y. Yatera, K. Sabanai, A. Tanimoto, M. Nagasaki, H. Tasaki, Y. Sasaguri, Y. Nakashima, Y. Otsuji, N. Yanagihara, Spontaneous myocardial infarction in mice lacking all nitric oxide synthase isoforms, *Circulation* 117 (17) (2008) 2211–2223.
- [176] H. Shimokawa, M. Tsutsui, Nitric oxide synthases in the pathogenesis of cardiovascular disease: lessons from genetically modified mice, *Pflug. Arch.* 459 (6) (2010) 959–967.
- [177] P. Wohlfart, H. Xu, A. Endlich, A. Habermeier, E.I. Closs, T. Hubschle, C. Mang, H. Strobel, T. Suzuki, H. Kleinert, U. Forstermann, H. Ruetten, H. Li, Antiatherosclerotic effects of small-molecular-weight compounds enhancing endothelial nitric-oxide synthase (eNOS) expression and preventing eNOS uncoupling, *J. Pharm. Exp. Ther.* 325 (2) (2008) 370–379.
- [178] A. Sharma, S. Sellers, N. Stefanovic, C. Leung, S.M. Tan, O. Huet, D.J. Granville, M.E. Cooper, J.B. de Haan, P. Bernatchez, Direct endothelial nitric oxide synthase activation provides atheroprotection in diabetes-accelerated atherosclerosis, *Diabetes* 64 (11) (2015) 3937–3950.
- [179] D. Fulton, J.P. Gratton, T.J. McCabe, J. Fontana, Y. Fujio, K. Walsh, T.F. Franke, A. Papapetropoulos, W.C. Sessa, Regulation of endothelium-derived nitric oxide production by the protein kinase Akt, *Nature* 399 (6736) (1999) 597–601.
- [180] S. Dimmeler, A.M. Zeiher, Akt takes center stage in angiogenesis signaling, *Circ. Res.* 86 (1) (2000) 4–5.
- [181] J.M. Ha, S.Y. Jin, H.S. Lee, H.K. Shin, D.H. Lee, S.H. Song, C.D. Kim, S.S. Bae, Regulation of retinal angiogenesis by endothelial nitric oxide synthase signaling pathway, *Korean J. Physiol. Pharm.* 20 (5) (2016) 533–538.
- [182] W.C. Chiu, T.J. Chiu, M.J. Chung, A.N. Chiang, beta2-Glycoprotein I Inhibits Vascular Endothelial Growth Factor-Induced Angiogenesis by Suppressing the Phosphorylation of Extracellular Signal-Regulated Kinase 1/2, Akt, and Endothelial Nitric Oxide Synthase, *PLoS One* 11 (8) (2016) e0161950.
- [183] Z. Chen, L. Zhang, J. Yu, L. Chen, B. Zhou, Identification of resveratrol derivative 3,3',4,4',5,5'-hexamethoxy-trans-stilbene as a novel pro-angiogenic small-molecule compound, *Eur. J. Pharm.* 791 (2016) 185–194.
- [184] T.A. Wihastuti, D. Sargowo, A. Tjokropawiro, N. Permatasari, M.A. Widodo, S. Soeharto, Vasa vasorum anti-angiogenesis through H(2)O(2), HIF-1alpha, NF-kappaB, and iNOS inhibition by mangosteen pericarp ethanolic extract (*Garcinia mangostana* Linn) in hypercholesterol-diet-given *Rattus norvegicus* Wistar strain, *Vasc. Health Risk Manag.* 10 (2014) 523–531.
- [185] J. Lee, E.H. Bae, S.K. Ma, S.W. Kim, Altered Nitric Oxide System in Cardiovascular and Renal Diseases, *Chonnam Med. J.* 52 (2) (2016) 81–90.
- [186] N. Kofler, M. Simons, The expanding role of neuropilin: regulation of transforming growth factor-beta and platelet-derived growth factor signaling in the vasculature, *Curr. Opin. Hematol.* 23 (3) (2016) 260–267.
- [187] P. Leveen, M. Pekny, S. Gebre-Medhin, B. Swolin, E. Larsson, C. Betsholtz, Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities, *Genes Dev.* 8 (16) (1994) 1875–1887.
- [188] P. Soriano, Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice, *Genes Dev.* 8 (16) (1994) 1888–1896.
- [189] P. Lindahl, B.R. Johansson, P. Leveen, C. Betsholtz, Pericyte loss and microaneurysm formation in PDGF-B-deficient mice, *Science* 277 (5323) (1997) 242–245.
- [190] M. Hellstrom, M. Kalen, P. Lindahl, A. Abramsson, C. Betsholtz, Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse, *Development* 126 (14) (1999) 3047–3055.
- [191] M. Lassila, T.J. Allen, Z. Cao, V. Thallas, K.A. Jandeleit-Dahm, R. Candido, M.E. Cooper, Imatinib attenuates diabetes-associated atherosclerosis, *Arterioscler Thromb. Vasc. Biol.* 24 (5) (2004) 935–942.
- [192] M. Chowdhury, J. Ghosh, M. Slevin, J.V. Smyth, M.Y. Alexander, F. Serracino-Inglott, A comparative study of carotid atherosclerotic plaque microvessel density and angiogenic growth factor expression in symptomatic versus asymptomatic patients, *Eur. J. Vasc. Endovasc. Surg.* 39 (4) (2010) 388–395.
- [193] J. Pelisek, G. Well, C. Reeps, M. Rudelius, A. Kuehnl, M. Culmes, H. Poppert, A. Zimmermann, H. Berger, H.H. Eckstein, Neovascularization and angiogenic factors in advanced human carotid artery stenosis, *Circ. J.* 76 (5) (2012) 1274–1282.
- [194] M.S. Pepper, Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity, *Cytokine Growth Factor Rev.* 8 (1) (1997) 21–43.
- [195] G. Ferrari, B.D. Cook, V. Terushkin, G. Pintucci, P. Mignatti, Transforming growth factor-beta 1 (TGF-beta1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis, *J. Cell Physiol.* 219 (2) (2009) 449–458.
- [196] K.K. Hirschi, S.A. Rohovsky, P.A. D'Amore, PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate, *J. Cell Biol.* 141 (3) (1998) 805–814.
- [197] G. Ferrari, G. Pintucci, G. Seghezzi, K. Hyman, A.C. Galloway, P. Mignatti, VEGF, a prosurvival factor, acts in concert with TGF-beta1 to induce endothelial cell apoptosis, *Proc. Natl. Acad. Sci. USA* 103 (46) (2006) 17260–17265.
- [198] S. Javerzat, P. Auguste, A. Bikfalvi, The role of fibroblast growth factors in vascular development, *Trends Mol. Med.* 8 (10) (2002) 483–489.
- [199] A.C. Dreux, D.J. Lamb, H. Modjtahedi, G.A. Ferns, The epidermal growth factor receptors and their family of ligands: their putative role in atherogenesis, *Atherosclerosis* 186 (1) (2006) 38–53.
- [200] V.B. Mehta, Y. Zhou, A. Radulescu, G.E. Besner, HB-EGF stimulates eNOS expression and nitric oxide production and promotes eNOS dependent angiogenesis, *Growth Factors* 26 (6) (2008) 301–315.
- [201] M.I. Carvalho, M.J. Guimaraes, I. Pires, J. Prada, R. Silva-Carvalho, C. Lopes, F.L. Queiroga, EGFR and microvessel density in canine malignant mammary tumours, *Res. Vet. Sci.* 95 (3) (2013) 1094–1099.
- [202] Y.B. Li, Z.Q. Wang, X. Yan, M.W. Chen, J.L. Bao, G.S. Wu, Z.M. Ge, D.M. Zhou, Y.T. Wang, R.T. Li, IC-4, a new irreversible EGFR inhibitor, exhibits prominent anti-tumor and anti-angiogenesis activities, *Cancer Lett.* 340 (1) (2013) 88–96.
- [203] T.L. Khong, N. Thairu, H. Larsen, P.M. Dawson, S. Kiriakidis, E.M. Paleolog, Identification of the angiogenic gene signature induced by EGF and hypoxia in colorectal cancer, *BMC Cancer* 13 (2013) 518.
- [204] H. Chan, S.M. Pitson, Post-translational regulation of sphingosine kinases, *Biochim Biophys. Acta* 1831 (1) (2013) 147–156.
- [205] P. Mitra, C.A. Oskeritzian, S.G. Payne, M.A. Beaven, S. Milstien, S. Spiegel, Role of ABCC1 in export of sphingosine-1-phosphate from mast cells, *Proc. Natl. Acad. Sci. USA* 103 (44) (2006) 16394–16399.
- [206] S. Fukuhara, S. Simmons, S. Kawamura, A. Inoue, Y. Orba, T. Tokudome, Y. Sunden, Y. Arai, K. Moriwaki, J. Ishida, A. Uemura, H. Kiyonari, T. Abe, A. Fukamizu, M. Hirashima, H. Sawa, J. Aoki, M. Ishii, N. Mochizuki, The sphingosine-1-phosphate transporter Spns2 expressed on endothelial cells regulates lymphocyte trafficking in mice, *J. Clin. Invest* 122 (4) (2012) 1416–1426.
- [207] S. Spiegel, S. Milstien, Sphingosine-1-phosphate: an enigmatic signalling lipid, *Nat. Rev. Mol. Cell Biol.* 4 (5) (2003) 397–407.
- [208] T.M. Leclercq, S.M. Pitson, Cellular signalling by sphingosine kinase and sphingosine 1-phosphate, *IUBMB Life* 58 (8) (2006) 467–472.
- [209] M. Maceyka, K.B. Harikumar, S. Milstien, S. Spiegel, Sphingosine-1-phosphate signaling and its role in disease, *Trends Cell Biol.* 22 (1) (2012) 50–60.
- [210] R.L. Proia, T. Hla, Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy, *J. Clin. Invest* 125 (4) (2015) 1379–1387.
- [211] M. Kono, Y. Mi, Y. Liu, T. Sasaki, M.L. Allende, Y.P. Wu, T. Yamashita, R.L. Proia, The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis, *J. Biol. Chem.* 279 (28) (2004) 29367–29373.
- [212] M.J. Kluk, T. Hla, Signaling of sphingosine-1-phosphate via the S1P/EDG-family of G-protein-coupled receptors, *Biochim Biophys. Acta* 1582 (1–3) (2002) 72–80.
- [213] D. Mazurais, P. Robert, B. Gout, I. Berrebi-Bertrand, M.P. Laville, T. Calmels, Cell type-specific localization of human cardiac S1P receptors, *J. Histochem. Cytochem.* 50 (5) (2002) 661–670.
- [214] K. Yonesu, T. Nakamura, Y. Mizuno, C. Suzuki, T. Nagayama, S. Satoh, F. Nara, A novel sphingosine-1-phosphate receptor 1 antagonist prevents the proliferation and relaxation of vascular endothelial cells by sphingosine-1-phosphate, *Biol. Pharm. Bull.* 33 (9) (2010) 1500–1505.
- [215] T. Tanimoto, Z.G. Jin, B.C. Berk, Transactivation of vascular endothelial growth factor (VEGF) receptor Flk-1/KDR is involved in sphingosine 1-phosphate-stimulated phosphorylation of Akt and endothelial nitric-oxide synthase (eNOS), *J. Biol. Chem.* 277 (45) (2002) 42997–43001.
- [216] A. Skoura, T. Hla, Regulation of vascular physiology and pathology by the S1P2 receptor subtype, *Cardiovasc. Res.* 82 (2) (2009) 221–228.
- [217] M. Adada, D. Canals, Y.A. Hannun, L.M. Obeid, Sphingosine-1-phosphate receptor 2, *FEBS J.* 280 (24) (2013) 6354–6366.
- [218] T. Shimizu, A. De Wispelaere, M. Winkler, T. D'Souza, J. Caylor, L. Chen, F. Dastvan, J. Deou, A. Cho, A. Larena-Avellaneda, M. Reidy, G. Daum, Sphingosine-1-phosphate receptor 3 promotes neointimal hyperplasia in mouse iliac-femoral arteries, *Arterioscler Thromb. Vasc. Biol.* 32 (4) (2012) 955–961.

- [219] T. Kimura, T. Watanabe, K. Sato, J. Kon, H. Tomura, K. Tamama, A. Kuwabara, T. Kanda, I. Kobayashi, H. Ohta, M. Ui, F. Okajima, Sphingosine 1-phosphate stimulates proliferation and migration of human endothelial cells possibly through the lipid receptors, Edg-1 and Edg-3, *Biochem. J.* 348 (Pt 1) (2000) 71–76.
- [220] P.R. Moreno, K.R. Purushothaman, E. Zias, J. Sanz, V. Fuster, Neovascularization in human atherosclerosis, *Curr. Mol. Med.* 6 (5) (2006) 457–477.
- [221] M. Jeziorska, D.E. Woolley, Neovascularization in early atherosclerotic lesions of human carotid arteries: its potential contribution to plaque development, *Hum. Pathol.* 30 (8) (1999) 919–925.
- [222] T.G. Kwon, L.O. Lerman, A. Lerman, The vasa vasorum in atherosclerosis: the vessel within the vascular wall, *J. Am. Coll. Cardiol.* 65 (23) (2015) 2478–2480.
- [223] L. Fang, S.H. Choi, J.S. Baek, C. Liu, F. Almazan, F. Ulrich, P. Wiesner, A. Taleb, E. Deer, J. Pattison, J. Torres-Vazquez, A.C. Li, Y.I. Miller, Control of angiogenesis by ALBP-mediated cholesterol efflux, *Nature* 498 (7452) (2013) 118–122.
- [224] J.L. Goldstein, M.S. Brown, A century of cholesterol and coronaries: from plaques to genes to statins, *Cell* 161 (1) (2015) 161–172.
- [225] R.E. Infante, M.L. Wang, A. Radhakrishnan, H.J. Kwon, M.S. Brown, J.L. Goldstein, NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes, *Proc. Natl. Acad. Sci. USA* 105 (40) (2008) 15287–15292.
- [226] R.S. Rosenson, H.B. Brewer Jr., W.S. Davidson, Z.A. Fayad, V. Fuster, J. Goldstein, M. Hellerstein, X.C. Jiang, M.C. Phillips, D.J. Rader, A.T. Remaley, G.H. Rothblat, A.R. Tall, L. Yvan-Charvet, Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport, *Circulation* 125 (15) (2012) 1905–1919.
- [227] C. Casalou, A. Costa, T. Carvalho, A.L. Gomes, Z. Zhu, Y. Wu, S. Dias, Cholesterol regulates VEGFR-1 (FLT-1) expression and signaling in acute leukemia cells, *Mol. Cancer Res.* 9 (2) (2011) 215–224.
- [228] J.F. Santibanez, F.J. Blanco, E.M. Garrido-Martin, F. Sanz-Rodriguez, M.A. del Pozo, C. Bernabeu, Caveolin-1 interacts and cooperates with the transforming growth factor-beta type I receptor ALK1 in endothelial caveolae, *Cardiovasc. Res.* 77 (4) (2008) 791–799.
- [229] B. Razani, J.A. Engelman, X.B. Wang, W. Schubert, X.L. Zhang, C.B. Marks, F. Macaluso, R.G. Russell, M. Li, R.G. Pestell, D. Di Vizio, H. Hou Jr., B. Kneitz, G. Lagaud, G.J. Christ, W. Edelmann, M.P. Lisanti, Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities, *J. Biol. Chem.* 276 (41) (2001) 38121–38138.
- [230] S. Pavlides, J.L. Gutierrez-Pajares, C. Danilo, M.P. Lisanti, P.G. Frank, Atherosclerosis, caveolae and caveolin-1, *Adv. Exp. Med. Biol.* 729 (2012) 127–144.
- [231] P.G. Frank, H. Lee, D.S. Park, N.N. Tandon, P.E. Scherer, M.P. Lisanti, Genetic ablation of caveolin-1 confers protection against atherosclerosis, *Arterioscler Thromb. Vasc. Biol.* 24 (1) (2004) 98–105.
- [232] E. Van Belle, A. Rivard, D. Chen, M. Silver, S. Bunting, N. Ferrara, J.F. Symes, C. Bauters, J.M. Isner, Hypercholesterolemia attenuates angiogenesis but does not preclude augmentation by angiogenic cytokines, *Circulation* 96 (8) (1997) 2667–2674.
- [233] T. Coffinhal, M. Silver, M. Kearney, A. Sullivan, B. Witzensbichler, M. Wagner, B. Annex, K. Peters, J.M. Isner, Impaired collateral vessel development associated with reduced expression of vascular endothelial growth factor in ApoE^{-/-} mice, *Circulation* 99 (24) (1999) 3188–3198.
- [234] M. Boodhwani, Y. Nakai, S. Mieno, P. Voisine, C. Bianchi, E.G. Araujo, J. Feng, K. Michael, J. Li, F.W. Sellke, Hypercholesterolemia impairs the myocardial angiogenic response in a swine model of chronic ischemia: role of endostatin and oxidative stress, *Ann. Thorac. Surg.* 81 (2) (2006) 634–641.
- [235] P. Haddad, S. Dussault, J. Groleau, J. Turgeon, F. Maingrette, A. Rivard, Nox2-derived reactive oxygen species contribute to hypercholesterolemia-induced inhibition of neovascularization: effects on endothelial progenitor cells and mature endothelial cells, *Arterioscler Thromb. Vasc. Biol.* 21 (2) (2011) 340–349.
- [236] F. Jin, N. Hagemann, U. Brockmeier, S.T. Schafer, A. Zechariah, D.M. Hermann, LDL attenuates VEGF-induced angiogenesis via mechanisms involving VEGFR2 internalization and degradation following endosome-trans-Golgi network trafficking, *Angiogenesis* 16 (2013), 2013, pp. 625–637.
- [237] A. Zechariah, A. ElAli, N. Hagemann, F. Jin, T.R. Doepfner, I. Helfrich, G. Mies, D.M. Hermann, Hyperlipidemia attenuates vascular endothelial growth factor-induced angiogenesis, impairs cerebral blood flow, and disturbs stroke recovery via decreased pericyte coverage of brain endothelial cells, *Arterioscler Thromb. Vasc. Biol.* 33 (7) (2013) 1561–1567.
- [238] C.Y. Huang, F.Y. Lin, C.M. Shih, H.K. Au, Y.J. Chang, H. Nakagami, R. Morishita, N.C. Chang, K.G. Shyu, J.W. Chen, Moderate to high concentrations of high-density lipoprotein from healthy subjects paradoxically impair human endothelial progenitor cells and related angiogenesis by activating Rho-associated kinase pathways, *Arterioscler Thromb. Vasc. Biol.* 32 (10) (2012) 2405–2417.
- [239] A.R. Tall, L. Yvan-Charvet, Cholesterol, inflammation and innate immunity, *Nat. Rev. Immunol.* 15 (2) (2015) 104–116.
- [240] A. Dandapat, C. Hu, L. Sun, J.L. Mehta, Small concentrations of oxLDL induce capillary tube formation from endothelial cells via LOX-1-dependent redox-sensitive pathway, *Arterioscler Thromb. Vasc. Biol.* 27 (11) (2007) 2435–2442.
- [241] S. Yu, S.L. Wong, C.W. Lau, Y. Huang, C.M. Yu, Oxidized LDL at low concentration promotes in-vitro angiogenesis and activates nitric oxide synthase through PI3K/Akt/eNOS pathway in human coronary artery endothelial cells, *Biochem Biophys. Res Commun.* 407 (1) (2011) 44–48.
- [242] M. Khaidakov, S. Mitra, X. Wang, Z. Ding, N. Bora, V. Lyzogubov, F. Romeo, S.A. Schichman, J.L. Mehta, Large impact of low concentration oxidized LDL on angiogenic potential of human endothelial cells: a microarray study, *PLoS One* 7 (10) (2012) e47421.
- [243] C. Camare, M. Trayssac, B. Garmy-Susini, E. Mucher, R. Sabbadini, R. Salvayre, A. Negre-Salvayre, Oxidized LDL-induced angiogenesis involves sphingosine 1-phosphate: prevention by anti-S1P antibody, *Br. J. Pharm.* 172 (1) (2015) 106–118.
- [244] V.N. Bochkov, M. Philippova, O. Oskolkova, A. Kadl, A. Furnkranz, E. Karabeg, T. Afonyushkin, F. Gruber, J. Breuss, A. Minchenko, D. Mechtcheriakova, P. Hohensinner, K. Rychli, J. Wojta, T. Resink, P. Erne, B.R. Binder, N. Leitinger, Oxidized phospholipids stimulate angiogenesis via autocrine mechanisms, implicating a novel role for lipid oxidation in the evolution of atherosclerotic lesions, *Circ. Res.* 99 (8) (2006) 900–908.
- [245] R. Hutter, W.S. Speidl, C. Valdiviezo, B. Sauter, R. Corti, V. Fuster, J.J. Badimon, Macrophages transmit potent proangiogenic effects of oxLDL in vitro and in vivo involving HIF-1alpha activation: a novel aspect of angiogenesis in atherosclerosis, *J. Cardiovasc. Transl. Res.* 6 (4) (2013) 558–569.
- [246] V.A. Shatrov, V.V. Sumbayev, J. Zhou, B. Brune, Oxidized low-density lipoprotein (oxLDL) triggers hypoxia-inducible factor-1alpha (HIF-1alpha) accumulation via redox-dependent mechanisms, *Blood* 101 (12) (2003) 4847–4849.
- [247] C. Camare, N. Auge, M. Pucelle, B. Saint-Lebes, M.H. Grazide, A. Negre-Salvayre, R. Salvayre, The neutral sphingomyelinase-2 is involved in angiogenic signaling triggered by oxidized LDL, *Free Radic. Biol. Med.* 93 (2016) 204–216.
- [248] J. Igarashi, M. Miyoshi, T. Hashimoto, Y. Kubota, H. Kosaka, Hydrogen peroxide induces S1P1 receptors and sensitizes vascular endothelial cells to sphingosine 1-phosphate, a platelet-derived lipid mediator, *Am. J. Physiol. Cell Physiol.* 292 (2) (2007) C740–C748.
- [249] C. Cinq-Frais, C. Coatrieux, M.H. Grazide, Y.A. Hannun, A. Negre-Salvayre, R. Salvayre, N. Auge, A signaling cascade mediated by ceramide, src and PDGFRbeta coordinates the activation of the redox-sensitive neutral sphingomyelinase-2 and sphingosine kinase-1, *Biochim Biophys Acta*, 1831(8), 2013, pp.1344–1356.
- [250] G. Murugesan, G.M. Chisolm, P.L. Fox, Oxidized low density lipoprotein inhibits the migration of aortic endothelial cells in vitro, *J. Cell Biol.* 120 (4) (1993) 1011–1019.
- [251] C.H. Chen, W. Jiang, D.P. Via, S. Luo, T.R. Li, Y.T. Lee, P.D. Henry, Oxidized low-density lipoproteins inhibit endothelial cell proliferation by suppressing basic fibroblast growth factor expression, *Circulation* 101 (2) (2000) 171–177.
- [252] J. Amaral, J.W. Lee, J. Chou, M.M. Campos, I.R. Rodriguez, 7-Ketocholesterol induces inflammation and angiogenesis in vivo: a novel rat model, *PLoS One* 8 (2) (2013) e56099.
- [253] S. Schoors, U. Bruning, R. Missiaen, K.C. Queiroz, G. Borgers, I. Elia, A. Zecchin, A.R. Cantelmo, S. Christen, J. Goevia, W. Heggermont, L. Godde, S. Vinckier, P.P. Van Veldhoven, G. Eelen, L. Schoonjans, H. Gerhardt, M. Dewerchin, M. Baes, K. De Bock, B. Ghesquiere, S.Y. Lunt, S.M. Fendt, P. Carmeliet, Fatty acid carbon is essential for dNTP synthesis in endothelial cells, *Nature* 520 (7546) (2015) 192–197.
- [254] A. Negre-Salvayre, C. Coatrieux, C. Ingueneau, R. Salvayre, Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors, *Br. J. Pharm.* 153 (1) (2008) 6–20.
- [255] I. Fleming, The pharmacology of the cytochrome P450 epoxygenase/soluble epoxide hydrolase axis in the vasculature and cardiovascular disease, *Pharm. Rev.* 66 (4) (2014) 1106–1140.
- [256] A.D. Watson, N. Leitinger, M. Navab, K.F. Faull, S. Horkko, J.L. Witztum, W. Palinski, D. Schwenke, R.G. Salomon, W. Sha, G. Subbanagounder, A.M. Fogelman, J.A. Berliner, Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo, *J. Biol. Chem.* 272 (21) (1997) 13597–13607.
- [257] K. Uchida, Role of reactive aldehyde in cardiovascular diseases, *Free Radic. Biol. Med.* 28 (12) (2000) 1685–1696.
- [258] A. Negre-Salvayre, N. Auge, V. Ayala, H. Basaga, J. Boada, R. Brenke, S. Chapple, G. Cohen, J. Feher, T. Grune, G. Lengyel, G.E. Mann, R. Pamplona, G. Poli, M. Portero-Otin, Y. Riahi, R. Salvayre, S. Sasson, J. Serrano, O. Shamni, W. Siems, R.C. Siow, I. Wiswedel, K. Zarkovic, N. Zarkovic, Pathological aspects of lipid peroxidation, *Free Radic. Res.* 44 (10) (2010) 1125–1171.
- [259] I. Escargueil-Blanc, R. Salvayre, N. Vacaresse, G. Jurgens, B. Darblade, J.F. Arnal, S. Parthasarathy, A. Negre-Salvayre, Mildly oxidized LDL induces activation of platelet-derived growth factor beta-receptor pathway, *Circulation* 104 (15) (2001) 1814–1821.
- [260] F. Robbesyn, N. Auge, C. Vindis, A.V. Cantero, R. Barbaras, A. Negre-Salvayre, R. Salvayre, High-density lipoproteins prevent the oxidized low-density lipoprotein-induced epidermal [corrected] growth factor receptor activation and subsequent matrix metalloproteinase-2 upregulation, *Arterioscler Thromb. Vasc. Biol.* 25 (6) (2005) 1206–1212.
- [261] A. Negre-Salvayre, O. Vieira, I. Escargueil-Blanc, R. Salvayre, Oxidized LDL and 4-hydroxynonenol modulate tyrosine kinase receptor activity, *Mol. Asp. Med.* 24 (4–5) (2003) 251–261.
- [262] G. Robino, M. Parola, F. Marra, A. Caligiuri, R.M. De Franco, E. Zamara, G. Bellomo, P. Gentilini, M. Pinzani, M.U. Dianzani, Interaction between 4-hydroxy-2,3-alkenals and the platelet-derived growth factor-beta receptor. Reduced tyrosine phosphorylation and downstream signaling in hepatic stellate cells, *J. Biol. Chem.* 275 (51) (2000) 40561–40567.
- [263] C. Vindis, I. Escargueil-Blanc, M. Elbaz, B. Marcheix, M.H. Grazide, K. Uchida, R. Salvayre, A. Negre-Salvayre, Desensitization of platelet-derived growth factor receptor-beta by oxidized lipids in vascular cells and atherosclerotic lesions: prevention by aldehyde scavengers, *Circ. Res.* 98 (6) (2006) 785–792.
- [264] I. Edirisinghe, I. Rahman, Cigarette smoke-mediated oxidative stress, shear stress,

- and endothelial dysfunction: role of VEGFR2, *Ann. NY Acad. Sci.* 1203 (2010) 66–72.
- [265] L. Fang, C. Liu, Y.I. Miller, Zebrafish models of dyslipidemia: relevance to atherosclerosis and angiogenesis, *Transl. Res.* 163 (2) (2014) 99–108.
- [266] S. Tatematsu, S.A. Francis, P. Natarajan, D.J. Rader, A. Saghatelian, J.D. Brown, T. Michel, J. Plutzky, Endothelial lipase is a critical determinant of high-density lipoprotein-stimulated sphingosine 1-phosphate-dependent signaling in vascular endothelium, *Arterioscler Thromb. Vasc. Biol.* 33 (8) (2013) 1788–1794.
- [267] L. Zhu, L. Fang, AIBP, A. Novel, Molecule at the Interface of Cholesterol Transport, Angiogenesis, and Atherosclerosis, *Methodist Debakey Cardiovasc. J.* 11 (3) (2015) 160–165.
- [268] K.A. Parham, J.R. Zebol, K.L. Tooley, W.Y. Sun, L.M. Moldenhauer, M.P. Cockshell, B.L. Gliddon, P.A. Moretti, G. Tigyi, S.M. Pitson, C.S. Bonder, Sphingosine 1-phosphate is a ligand for peroxisome proliferator-activated receptor-gamma that regulates neovascularization, *FASEB J.* 29 (9) (2015) 3638–3653.
- [269] B. Ho-Tin-Noe, J. Le Dall, D. Gomez, L. Louedec, R. Vranckx, M. El-Bouchtaoui, L. Legres, O. Meilhac, J.B. Michel, Early atheroma-derived agonists of peroxisome proliferator-activated receptor-gamma trigger intramedial angiogenesis in a smooth muscle cell-dependent manner, *Circ. Res.* 109 (9) (2011) 1003–1014.
- [270] D. Harrison, K.K. Griendling, U. Landmesser, B. Hornig, H. Drexler, Role of oxidative stress in atherosclerosis, *Am. J. Cardiol.* 91 (3A) (2003) 7A–11A.
- [271] R.S. Frey, M. Ushio-Fukai, A.B. Malik, NADPH oxidase-dependent signaling in endothelial cells: role in physiology and pathophysiology, *Antioxid. Redox Signal* 11 (4) (2009) 791–810.
- [272] D.I. Brown, K.K. Griendling, Regulation of signal transduction by reactive oxygen species in the cardiovascular system, *Circ. Res.* 116 (3) (2015) 531–549.
- [273] R. Salvayre, A. Negre-Salvayre, C. Camare, Oxidative theory of atherosclerosis and antioxidants, *Biochimie* 125 (2016) 281–296.
- [274] J. Ruef, Z.Y. Hu, L.Y. Yin, Y. Wu, S.R. Hanson, A.B. Kelly, L.A. Harker, G.N. Rao, M.S. Runge, C. Patterson, Induction of vascular endothelial growth factor in balloon-injured baboon arteries. A novel role for reactive oxygen species in atherosclerosis, *Circ. Res.* 81 (1) (1997) 24–33.
- [275] S.C. Bir, X. Shen, T.J. Kavanagh, C.G. Kevil, C.B. Pattillo, Control of angiogenesis dictated by picomolar superoxide levels, *Free Radic. Biol. Med.* 63 (2013) 135–142.
- [276] Y.W. Kim, T.V. Byzova, Oxidative stress in angiogenesis and vascular disease, *Blood* 123 (5) (2014) 625–631.
- [277] R. Ross, Atherosclerosis—an inflammatory disease, *N. Engl. J. Med.* 340 (2) (1999) 115–126.
- [278] A. Leclercq, X. Houard, M. Philippe, V. Ollivier, U. Sebbag, O. Meilhac, J.B. Michel, Involvement of intraplaque hemorrhage in atherothrombosis evolution via neutrophil protease enrichment, *J. Leukoc. Biol.* 82 (6) (2007) 1420–1429.
- [279] A.G. Arroyo, M.L. Iruela-Arispe, Extracellular matrix, inflammation, and the angiogenic response, *Cardiovasc. Res.* 86 (2) (2010) 226–235.
- [280] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, *Nature* 473 (7347) (2011) 317–325.
- [281] E. Pardali, M.J. Goumans, P. ten Dijke, Signaling by members of the TGF-beta family in vascular morphogenesis and disease, *Trends Cell Biol.* 20 (9) (2010) 556–567.
- [282] C.M. Ghajar, S.C. George, A.J. Putnam, Matrix metalloproteinase control of capillary morphogenesis, *Crit. Rev. Eukaryot. Gene Expr.* 18 (3) (2008) 251–278.
- [283] F. De Smet, I. Segura, K. De Bock, P.J. Hohensinner, P. Carmeliet, Mechanisms of vessel branching: filopodia on endothelial tip cells lead the way, *Arterioscler Thromb. Vasc. Biol.* 29 (5) (2009) 639–649.
- [284] E.D. Karagiannis, A.S. Popel, Distinct modes of collagen type I proteolysis by matrix metalloproteinase (MMP) 2 and membrane type 1 MMP during the migration of a tip endothelial cell: insights from a computational model, *J. Theor. Biol.* 238 (1) (2006) 124–145.
- [285] N. Auge, F. Maupas-Schwalm, M. Elbaz, J.C. Thiers, A. Waysbort, S. Itohara, H.W. Krell, R. Salvayre, A. Negre-Salvayre, Role for matrix metalloproteinase-2 in oxidized low-density lipoprotein-induced activation of the sphingomyelin/ceramide pathway and smooth muscle cell proliferation, *Circulation* 110 (5) (2004) 571–578.
- [286] S. Langlois, D. Gingras, R. Beliveau, Membrane type 1-matrix metalloproteinase (MT1-MMP) cooperates with sphingosine 1-phosphate to induce endothelial cell migration and morphogenic differentiation, *Blood* 103 (8) (2004) 3020–3028.
- [287] B. Annabi, S. Thibeault, Y.T. Lee, N. Bousquet-Gagnon, N. Eliopoulos, S. Barrette, J. Galipeau, R. Beliveau, Matrix metalloproteinase regulation of sphingosine 1-phosphate-induced angiogenic properties of bone marrow stromal cells, *Exp. Hematol.* 31 (7) (2003) 640–649.
- [288] S. Raab, K.H. Plate, Different networks, common growth factors: shared growth factors and receptors of the vascular and the nervous system, *Acta Neuropathol.* 113 (6) (2007) 607–626.
- [289] I. Yana, H. Sagara, S. Takaki, K. Takatsu, K. Nakamura, K. Nakao, M. Katsuki, S. Taniguchi, T. Aoki, H. Sato, S.J. Weiss, M. Seiki, Crosstalk between neovessels and mural cells directs the site-specific expression of MT1-MMP to endothelial tip cells, *J. Cell Sci.* 120 (Pt 9) (2007) 1607–1614.
- [290] P. Libby, E. Folco, Tension in the plaque: hypoxia modulates metabolism in atheroma, *Circ. Res.* 109 (10) (2011) 1100–1102.
- [291] J.B. Michel, R. Virmani, E. Arbustini, G. Pasterkamp, Intraplaque hemorrhages as the trigger of plaque vulnerability, *Eur. Heart J.* 32 (16) (2011) 1977–1985 (1985a, 1985b, 1985c).
- [292] R. Khurana, M. Simons, J.F. Martin, I.C. Zachary, Role of angiogenesis in cardiovascular disease: a critical appraisal, *Circulation* 112 (12) (2005) 1813–1824.
- [293] M.J. McCarthy, I.M. Loftus, M.M. Thompson, L. Jones, N.J. London, P.R. Bell, A.R. Naylor, N.P. Brindle, Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology, *J. Vasc. Surg.* 30 (2) (1999) 261–268.
- [294] P. Gao, Z.Q. Chen, Y.H. Bao, L.Q. Jiao, F. Ling, Correlation between carotid intraplaque hemorrhage and clinical symptoms: systematic review of observational studies, *Stroke* 38 (8) (2007) 2382–2390.
- [295] F.D. Kolodgie, A.P. Burke, G. Nakazawa, Q. Cheng, X. Xu, R. Virmani, Free cholesterol in atherosclerotic plaques: where does it come from, *Curr. Opin. Lipidol.* 18 (5) (2007) 500–507.
- [296] G.S. Abela, K. Aziz, A. Vedre, D.R. Pathak, J.D. Talbott, J. Dejong, Effect of cholesterol crystals on plaques and intima in arteries of patients with acute coronary and cerebrovascular syndromes, *Am. J. Cardiol.* 103 (7) (2009) 959–968.
- [297] P. Duester, H. Kono, K.J. Rayner, C.M. Sirois, G. Vladimer, F.G. Bauernfeind, G.S. Abela, L. Franchi, G. Nunez, M. Schnurr, T. Espevik, E. Lien, K.A. Fitzgerald, K.L. Rock, K.J. Moore, S.D. Wright, V. Hornung, E. Latz, NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals, *Nature* 464 (7293) (2010) 1357–1361.
- [298] K. Rajamaki, J. Lappalainen, K. Oorni, E. Valimaki, S. Matikainen, P.T. Kovanen, K.K. Eklund, Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation, *PLoS One* 5 (7) (2010) e11765.
- [299] D.N. Tziakas, J.C. Kaski, G.K. Chalikias, C. Romero, S. Fredericks, I.K. Tentes, A.X. Kortsaris, D.I. Hatseras, D.W. Holt, Total cholesterol content of erythrocyte membranes is increased in patients with acute coronary syndrome: a new marker of clinical instability?, *J. Am. Coll. Cardiol.* 49 (21) (2007) 2081–2089.
- [300] D.N. Tziakas, G.K. Chalikias, D. Stakos, I.K. Tentes, S.V. Chatzikyriakou, K. Mitrousi, A.X. Kortsaris, H. Boudoulas, J.C. Kaski, Cholesterol composition of erythrocyte membranes and its association with clinical presentation of coronary artery disease, *Coron. Artery Dis.* 19 (8) (2008) 583–590.
- [301] D. Steinberg, S. Parthasarathy, T.E. Carew, J.C. Khoo, J.L. Witztum, Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity, *N. Engl. J. Med.* 320 (14) (1989) 915–924.
- [302] H. Esterbauer, M. Dieber-Rotheneder, G. Waeg, G. Striegl, J. Gurgens, Biochemical, structural, and functional properties of oxidized low-density lipoprotein, *Chem. Res. Toxicol.* 3 (2) (1990) 77–92.
- [303] R. Stocker, J.F. Kearney Jr., Role of oxidative modifications in atherosclerosis, *Physiol. Rev.* 84 (4) (2004) 1381–1478.
- [304] K. Yunoki, T. Naruko, R. Komatsu, S. Ehara, N. Shirai, K. Sugioka, M. Nakagawa, C. Kitabayashi, Y. Ikura, A. Itoh, K. Kusano, T. Ohe, K. Haze, A.E. Becker, M. Ueda, Enhanced expression of haemoglobin scavenger receptor in accumulated macrophages of culprit lesions in acute coronary syndromes, *Eur. Heart J.* 30 (15) (2009) 1844–1852.
- [305] E. Nagy, J.W. Eaton, V. Jeney, G.P. Soares, Z. Varga, Z. Galajda, J. Szentmiklosi, G. Mehes, T. Csonka, A. Smith, G.M. Vercellotti, G. Balla, J. Balla, Red cells, hemoglobin, heme, iron, and atherogenesis, *Arterioscler Thromb. Vasc. Biol.* 30 (7) (2010) 1347–1353.
- [306] Y.C. Chen, A.V. Bui, J. Diesch, R. Manasseh, C. Hausding, J. Rivera, I. Haviv, A. Agrotis, N.M. Htun, J. Jowett, C.E. Hagemeyer, R.D. Hannan, A. Bobik, K. Peter, A novel mouse model of atherosclerotic plaque instability for drug testing and mechanistic/therapeutic discoveries using gene and microRNA expression profiling, *Circ. Res.* 113 (3) (2013) 252–265.
- [307] S. Kolb, R. Vranckx, M.G. Huisse, J.B. Michel, O. Meilhac, The phosphatidylserine receptor mediates phagocytosis by vascular smooth muscle cells, *J. Pathol.* 212 (3) (2007) 249–259.
- [308] S. Kalet-Litman, P.R. Moreno, A.P. Levy, The haptoglobin 2-2 genotype is associated with increased redox active hemoglobin derived iron in the atherosclerotic plaque, *Atherosclerosis* 209 (1) (2010) 28–31.
- [309] A.P. Levy, R. Asleh, S. Blum, N.S. Levy, R. Miller-Lotan, S. Kalet-Litman, Y. Anbinder, O. Lache, F.M. Nakhoul, R. Asaf, D. Farbstain, M. Pollak, Y.Z. Soloveichik, M. Strauss, J. Alshiek, A. Livshits, A. Schwartz, H. Awad, K. Jad, H. Goldenstein, Haptoglobin: basic and clinical aspects, *Antioxid. Redox Signal* 12 (2) (2010) 293–304.
- [310] S. Sugiyama, Y. Okada, G.K. Sukhova, R. Virmani, J.W. Heinecke, P. Libby, Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes, *Am. J. Pathol.* 158 (3) (2001) 879–891.
- [311] B.S. van der Veen, M.P. de Winther, P. Heeringa, Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease, *Antioxid. Redox Signal* 11 (11) (2009) 2899–2937.
- [312] A. Leclercq, X. Houard, S. Loyau, M. Philippe, U. Sebbag, O. Meilhac, J.B. Michel, Topology of protease activities reflects atherothrombotic plaque complexity, *Atherosclerosis* 191 (1) (2007) 1–10.
- [313] S. Mansilla, Y. Boulaftali, L. Venisse, V. Arocas, O. Meilhac, J.B. Michel, M. Jandrot-Perrus, M.C. Bouton, Macrophages and platelets are the major source of protease nexin-1 in human atherosclerotic plaque, *Arterioscler Thromb. Vasc. Biol.* 28 (10) (2008) 1844–1850.
- [314] M.A. Boulhel, B. Derudas, E. Rigamonti, R. Dievert, J. Brozek, S. Haulon, C. Zawadzki, B. Jude, G. Torpier, N. Marx, B. Staels, G. Chinetti-Gbaguidi, PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties, *Cell Metab.* 6 (2) (2007) 137–143.
- [315] J.J. Boyle, H.A. Harrington, E. Piper, K. Elderfield, J. Stark, R.C. Landis, D.O. Haskard, Coronary intraplaque hemorrhage evokes a novel atheroprotective macrophage phenotype, *Am. J. Pathol.* 174 (3) (2009) 1097–1108.
- [316] A. Simonini, M. Moscucci, D.W. Muller, E.R. Bates, F.D. Pagani, M.D. Burdick,

- R.M. Strieter, IL-8 is an angiogenic factor in human coronary atherectomy tissue, *Circulation* 101 (13) (2000) 1519–1526.
- [317] E.C. Griffith, Z. Su, S. Niwayama, C.A. Ramsay, Y.H. Chang, J.O. Liu, Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2, *Proc. Natl. Acad. Sci. USA* 95 (26) (1998) 15183–15188.
- [318] P.M. Winter, A.M. Neubauer, S.D. Caruthers, T.D. Harris, J.D. Robertson, T.A. Williams, A.H. Schmieder, G. Hu, J.S. Allen, E.K. Lacy, H. Zhang, S.A. Wickline, G.M. Lanza, Endothelial alpha(v)beta3 integrin-targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis, *Arterioscler Thromb. Vasc. Biol.* 26 (9) (2006) 2103–2109.
- [319] A. Ahmed, T. Fujisawa, X.L. Niu, S. Ahmad, B. Al-Ani, K. Chudasama, A. Abbas, R. Potluri, V. Bhandari, C.M. Findley, G.K. Lam, J. Huang, P.W. Hewett, M. Cudmore, C.D. Kontos, Angiopoietin-2 confers Atheroprotection in apoE^{-/-} mice by inhibiting LDL oxidation via nitric oxide, *Circ. Res.* 104 (12) (2009) 1333–1336.
- [320] S.H. Wilson, J. Herrmann, L.O. Lerman, D.R. Holmes Jr., C. Napoli, E.L. Ritman, A. Lerman, Simvastatin preserves the structure of coronary adventitial vasa vasorum in experimental hypercholesterolemia independent of lipid lowering, *Circulation* 105 (4) (2002) 415–418.
- [321] W. Cheungpasitporn, F.T. Chebib, L.D. Cornell, M.L. Brodin, S.H. Nasr, C.A. Schinstock, M.D. Stegall, H. Amer, Intravitreal Antivascular Endothelial Growth Factor Therapy May Induce Proteinuria and Antibody Mediated Injury in Renal Allografts, *Transplantation* 99 (11) (2015) 2382–2386.
- [322] N. Maurea, C. Coppola, G. Piscopo, F. Galletta, G. Riccio, E. Esposito, C. De Lorenzo, M. De Laurentis, P. Spallarossa, G. Mercurio, Pathophysiology of cardiotoxicity from target therapy and angiogenesis inhibitors, *J. Cardiovasc Med. (Hagerstown)* 17 (Suppl 1) (2016) S19–S26.