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Review article

Angiogenesis in the atherosclerotic plaque

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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, assymetric 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 palmityl 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 lipopoprotein; 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, lipoxygenase; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; NADPH, H^+, nicotinamide adenine dinucleotide phosphate; NRP-1, neuropilin-1; nNOS, neuronal nitric oxide synthase; NF-kB, 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

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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 constitutedby 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 II 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

Neovacularisation 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 O_2 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 filipodia 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 filipodia, 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 proteases 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