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Review

20-HETE in neovascularization

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ABSTRACT

Cytochrome P450 4A/F (CYP4A/F) converts arachidonic acid (AA) to 20-HETE by ω-hydroxylation. The contribution of 20-HETE to the regulation of myogenic response, blood pressure, and mitogenic actions has been well summarized. This review focuses on the emerging role of 20-HETE in physiological and pathological vascularization. 20-HETE has been shown to regulate vascular smooth muscle cells (VSMC) and endothelial cells (EC) by affecting their proliferation, migration, survival, and tube formation. Furthermore, the proliferation, migration, secretion of proangiogenic molecules (such as HIF-1α, VEGF, SDF-1α), and tube formation of endothelial progenitor cells (EPC) are stimulated by 20-HETE. These effects are mediated through c-Src- and EGFR-mediated downstream signaling pathways, including MAPK and PI3K/Akt pathways, eNOS uncoupling, and NOX/ROS system activation. Therefore, the CYP4A/F-20-HETE system may be a therapeutic target for the treatment of abnormal angiogenic diseases.

Contents

1. Introduction

Neovascularization (including vasculogenesis and angiogenesis) is an important process that has been studied extensively over the last several decades. Vasculogenesis, the *de novo* formation of blood vessels, begins with islands of precursor angioblast cells that differentiate into endothelial cells (EC) which mature into a secondary vessel network [1]. On the other hand, angiogenesis is the sprouting of existing vascular structures into new capillary growth and occurs both as developmental and patho-physiological responses (e.g. cancer, atherosclerosis, ischemia, infectious diseases, diabetes, and retinopathy) to local hypoxia [2]. The angiogenic process involves a cascade of events that are initiated with the production, release, and binding of angiogenic factors to EC receptors, which leads to EC proliferation and directional migration toward gradients of pro-angiogenic factors. Consequently, there is lumen formation, branch development, anastomosis of the tip of one tube with another to form a loop (loop formation), and vessel stabilization to form mature blood vessels [3–7].

Angiogenesis requires many different cytokines and growth factors interacting with the endothelium and its microenvironment, such as hypoxia inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), stromal-derived factor-1 (SDF-1), placental growth factor (PIGF), and platelet-derived growth factor-BB (PDGF-BB) [8–10]. VEGF, the prominent angiogenic factor, promotes the proliferation, survival, migration, and tube formation of EC [11,12]. Furthermore, VEGF also mobilizes and recruits endothelial

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progenitor cells (EPC) from their bone marrow niche into blood circulation. EPC express C-X-C chemokine receptor-4 (CXCR4) that allows their migration to sites of neovascularization in response to its ligand, SDF-1. SDF-1 is typically released by target tissues, including ischemic tissues, primary tumors, and pre-metastatic sites of tumor [13–15]. Once EPC are recruited, they can promote neovascularization by secreting VEGF and other cytokines [16]. Thus, a positive feedback exists between VEGF secretion and EPC recruitment.

Mounting evidence in recent years has implicated that the CYP4A/F-20-HETE system plays an important role in regulation of the neovascularization process. 20-hydroxyeicosatetraenoic acid (20-HETE), a key bioactive lipid mediator, arises from arachidonic acid (AA) via metabolism by cytochrome P450 4A/F (CYP4A/F). 20-HETE is an important mitogen upstream of many growth factors [17]. In this review, we will focus on the role of the CYP4A/4F-20-HETE system in the regulation of neovascularization processes and the potential mechanisms involved, which may facilitate the development of therapeutic approaches to modulate vascular growth.

2. The CYP4A/F-20-HETE system: synthesis and distribution

Arachidonic acid is released from the cellular membrane by phospholipase A2 (PLA2) activation [18]. Free AA can then be metabolized via the cyclooxygenases, lipoxygenases, or CYP450 oxygenases to bioactive molecules such as prostaglandins, thromboxanes, leukotrienes, and CYP450-derived metabolites [19]. Specifically, the CYP450 family of enzymes catalyzes either epoxygenation (forms epoxy bonds across ω-5,6, ω-8,9, ω-11,12, or ω-14,15 via CYP2C and 2J) or hydroxylation (adds a hydroxyl group at the ω, ω-1, or ω-2 carbons via CYP4A and 4F) of AA yielding epoxyeicosatrienoic acids (EETs) or hydroxyeicosatetraenoic acids (HETEs). 20-HETE is the major metabolite of the CYP ω-hydroxylases [18,20,21]. Although both CYP4A11 and 4F2 are highly expressed in human kidney and liver [22,23], CYP4A11-catalyzed 20-HETE formation is quantitatively less important than the corresponding CYP4F2 component in human liver and kidney, while CYP4A22 has been identified as a highly homologous isoform of CYP4A11 [23–25]. The main sites of 20-HETE synthesis and action are kidneys, liver, vasculature, and lungs [26,27]. 20-HETE has also been shown to inhibit large conductance calcium-activated potassium channels in VSMC [21,28]. This action of 20-HETE, in turn, sensitizes the vasculature to myogenic and hormonal stimuli, which promotes vasoconstriction.

Besides the human isoforms, different isoforms of 20-HETE synthases are also present in animals. Similar to the human isoforms, expression of these enzymes is organ- and gender-specific in mice [29]. *Cyp4a12a* has been identified as the major 20-HETE synthase that is prominent in males, and *Cyp4a14* has been demonstrated to be the predominant 20-HETE synthase in females [29–31]. However, *Cyp4a10* was found in the kidneys of all mice [29]. The main isoforms in rats [32] were CYP4A1 [32–36] and CYP4A2 [37–40], which also vary by gender. CYP4A protein has been found in the brain, prostate, intestine, and lungs of rat and rabbits [32,41,42]. 20-HETE synthase expression and function can be altered by the states of diseases [28] and in response to drugs. For example, Cyp4a10 and Cyp4a14 were shown to be increased in the liver and *ob*/*ob* in *db*/*db* diabetic animals [43,44].

3. 20-HETE and vascular cell functions associated with angiogenesis

The production of 20-HETE has long been shown in EC from systemic circulations, pulmonary small arteries, as well as VSMC [28,32]. Recent studies have expanded our knowledge of the actions of 20-HETE on vascular cell functions associated with angiogenesis. EC proliferation is one of the early steps in angiogenesis. 20-HETE was shown to induce the proliferation of human EC *in vitro* via stimulating superoxide formation and the production of both VEGF and HIF-1α, both essential regulators of angiogenic responses in EC [45,46]. In addition, 20-HETE increases EC migration, another important step in the angiogenic cascade [25,45–48]. Furthermore, 20-HETE induces neovascularization in the rat cornea *in vivo* [49]. Thus, 20-HETE activates both release of angiogenic factors and the growth responses of vascular cells both *in vitro* and *in vivo*. Ishizuka et al. have also shown that both CYP4A overexpression in human umbilical vein endothelial cell (HUVEC) [48] and addition of exogenous 20-HETE to non-transfected EC induced their activation [48]. Activation of EC results in the production of cytokines [50], such as VEGF. In turn, elevated levels of VEGF increase EC proliferation.

Similar to EC proliferation, the regulation of vascular cell survival and apoptosis is another cellular mechanism for neonatal vascular remodeling [51]. 20-HETE and its stable analog, 20-hydroxy-eicosa-5(Z),14(Z)-dienoic acid (WIT003), were found to have a protective effect on bovine pulmonary artery ECs (BPAEC) and pulmonary arterial smooth muscle cells (PASMC) by promoting survival and preventing apoptosis by acting on, at least in part, the intrinsic apoptotic pathway [47,52]. By preserving the integrity of the endothelium, 20-HETE increases the chance of EC exposure to pro-angiogenic molecules and plays a role in the angiotensin II-induced neo-intimal formation [53].

The growth of new blood vessels depends on the formation of both capillary-like tubes of EC and the subsequent infiltration of VSMC [54]. The directional migration of EC toward gradients of pro-angiogenic factors is a prerequisite of lumen formation. 20-HETE treatment not only induced EC migration [46], but also promoted VSMC migration via PDGF [55], which is another crucial step in angiogenic responses. 20-HETE treatment induced cytoskeletal changes in EC, promoting a spindle shape [46], which would be easier for forming sprouts out of existing vessels. Therefore, 20-HETE plays a critical role in angiogenic responses via regulating the proliferation, migration, tube formation, and survival of both EC and VSMC.

4. 20-HETE and endothelial progenitor cell functions associated with angiogenesis

Accumulating evidence suggests that the regulatory influence of 20-HETE on angiogenesis involves actions on the EPC function. Recent studies in stem cell biology suggest that bone-marrow-derived EPC also play a pivotal role in postnatal vasculogenesis, the *de novo* formation of new blood vessels essential for organ and tissue growth, wound healing, and tumor neovascularization [56–65]. EPC activate the "angiogenesis switch", a crucial step in the transition of an avascular, dormant area to a vascularized, rapidly growing tissue [66–69].

Under normal physiological conditions, EPC are in a quiescent state within the bone marrow niche, with a low frequency of EPC in circulating blood. Conversely, when the endothelium is perturbed, as occurs in tumor neovascularization, wounds, or ischemia, bone marrow EPC are mobilized and their numbers in the blood greatly increase. The most prominent factors promoting mobilization and differentiation of EPC include VEGF, angiopoietin-1, PlGF [70–73], and SDF-1α. EPC and other $CXCR4^+$ bone marrow derived cells can be recruited to ischemic tissues, primary tumor sites, and pre-metastatic tissues partially through increases in HIF and its target genes SDF-1α and VEGF [56,58,74–77]. After incorporation into the vasculature, EPC gradually differentiate toward EC and form neo-vessels.