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Systematic Review

HIF-1α and VEGF Immunophenotypes as Potential Biomarkers in the Prognosis and Evaluation of Treatment Efficacy of Atherosclerosis: A Systematic Review of the Literature

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Abstract

Background: Hypoxia-inducible factor 1 alpha (HIF-1α) and its related vascular endothelial growth factor (VEGF) may play a significant role in atherosclerosis and their targeting is a strategic approach that may affect multiple pathways influencing disease progression. This study aimed to perform a systematic review to reveal current evidence on the role of HIF-1α and VEGF immunophenotypes with other prognostic markers as potential biomarkers of atherosclerosis prognosis and treatment efficacy. **Methods**: We performed a systematic review of the current literature to explore the role of HIF-1α and VEGF protein expression along with the relation to the prognosis and therapeutic strategies of atherosclerosis. We used the terms {"Atherosclerosis" [OR] "Atheroma" [OR] "atheromatous plaque" [OR] "plaque atherosclerotic"} [AND] {"HIF-1α"} [AND] {"VEGF"} from 2009 up to May 2024 and the Medline/Embase/PubMed database. We used methodological approaches to assess unbiased data [ROBIS (Risk of Bias in Systematic) tool]. We used study eligibility criteria, and data were collected and evaluated from original articles by two independent teams, judged by an independent reviewer, and reported by PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020. **Results**: We included 34 original studies investigating 650 human specimens, 21 different cell lines, and 9 animal models. Increased HIF-1α in vascular smooth muscle cells, macrophages, or endothelial cells, under hypoxia, chronic loss of nitric oxide (NO), or reduced micro ribonucleic acid (miRNA)-17 and miR-20, is associated with the upregulation of pro-inflammatory molecules, such as interleukin-1 beta (IL-1β) or tumor necrosis factor-alpha (TNF-α), increased migration inhibitory factor of macrophages, glycolytic flux, lipid accumulation, necroptosis via miR-383, and adverse effects in atherosclerosis and plaque vulnerability. However, increased HIF-1α in lymphocytes is associated with decreased interferon-gamma (IFN-γ) and a favorable prognosis. Increased VEGF in a coronary artery, activated macrophages, or chronic exposure to methamphetamine is associated with elevated levels of serum inflammatory cells (interleukin-18; IL18), p38 mitogen-activated protein kinase (MAPK) phosphorylation, lipopolysaccharide-induced tumor necrosis factor-alpha factor (LITAF), and signal transducer and activator of transcription 6 isoform B (STAT6B) overexpression, leading to atherosclerosis progression and plaque break. However, VEGF overexpression in serum is marginally associated with an elevated risk for atherosclerosis. In contrast, stable overexpression of VEGF in macrophages correlates with reduced hyperplasia after arterial injury, reduced foam cell formation, and attenuation of atherosclerosis progression. HIF-1α/VEGF immunophenotypes reflect atherosclerosis treatment efficacy using, among others, HIF-inhibitors, statins, polyphenols, miR-497-5p, methylation modification, adenosine receptor antagonists, natural products, or glycosides. **Conclusion**: We present an overview of HIF-1α/VEGF expression in chronic inflammatory-related atherosclerosis disease. Exploring pathogenetic mechanisms and therapeutic options, we included several studies using variable methods to evaluate HIF-1α/VEGF immunophenotypes with controversial and innovative results. Data limitations may include the use of different survival methods. Our data support HIF-1α/VEGF immunophenotypes as potential biomarkers of atherosclerosis prognosis and treatment efficacy.

Keywords: HIF-1α; VEGF; immunoassays; atherosclerosis; achromatic plaque; hypoxia; angiogenesis; atherosclerosis prognosis; atherosclerosis targeted therapy

1. Introduction

Atherosclerosis is an underlying pathophysiologic disease of the arterial wall and a leading cause of death worldwide [1,2]. Atherosclerosis is associated with chronic inflammation due to endothelial damage that contributes to the formation of atheromatous plaques in the arterial tunica intima. The developmental process of atheromatous plaques is slow and developed over several years through a complex series of cellular events occurring within the arterial wall and in response to various local vascular circulating factors. Atherosclerosis is the main cause of coronary artery disease (CAD) and stroke, which can happen when an atheromatic plaque ruptures and an atheroma is detached from it and blocks a blood vessel in the heart or the brain, respectively [1]. Moreover, atherosclerosis can lead to peripheral vascular disease [3] when the atheromatic plaque completely blocks the artery.

The relationship between hypoxia and endothelial dysfunction in atherosclerosis is complex and unclear [4]. Since many factors are involved in the signaling and action of hypoxia-inducible factor 1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF), they could be a target for atherosclerosis therapy. This systematic review article outlines the current evidence on the role of HIF-1α and VEGF immunophenotypes with other prognostic markers as potential biomarkers of atherosclerosis prognosis and treatment efficacy. Evidence of HIF-1α and VEGF protein expression using immunoassays in coronary artery cells, macrophages, or serum is presented, along with their potential involvement in atherosclerosis progression and atheromatic plaque vulnerability. Insights into the role of HIF-1α and VEGF immunophenotypes reflecting targeted therapy of atherosclerosis could suggest their use as potential biomarkers in the efficacy of atherosclerosis therapy.

Hypoxia, a condition in which the body's tissues have insufficient oxygen supply, can result from several factors, including respiratory issues, cardiovascular problems, and cancer [5,6], as discussed recently by our team [6]. Hypoxia triggers molecular mechanisms, such as stabilizing HIF-1α, a protein subunit of the HIF-1 transcription factor, which plays a key role in the cellular response to low oxygen levels. When oxygen levels drop, HIF-1α is stabilized and translocated to the cell nucleus, where it forms a complex with another subunit, HIF-1β. This complex activates the transcription of genes involved in variable processes, such as angiogenesis, erythropoiesis, and glycolysis [7]. The impact of hypoxia, particularly HIF-1α, is strongly associated with the pathogenesis of atherosclerosis [4]. Specifically, HIF-1α overexpression is associated with the formation, progression, and vulnerability of atherosclerotic plaques and inflammatory processes in atherosclerosis [4]. Therefore, HIF-1α is evolving as an attractive therapeutic target of atherosclerosis, with several promising research studies based on it in recent years.

The VEGFs represent a family of heparin-binding proteins that play a key role in multiple processes, including the pathways of angiogenesis, lymphopoiesis, and lymphangiogenesis, as well as in cells' responses to oxidative stress and inflammation and the management of lipid metabolism [8,9]. Its primary role is to stimulate the proliferation and growth of endothelial cells in all kinds of vessels. Moreover, VEGF can prevent the apoptosis of ischemic endothelial cells through the expression of various anti-apoptotic proteins. Hence, VEGF influences erythropoiesis, increases vascular permeability, and mediates inflammation with plural hemodynamic effects. Although VEGF has a key role in various physiological processes, the same properties make it play a role in the origin and maintenance of various pathological processes, including atherosclerosis [8].

There are five members of the VEGF family, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) [9], involved in the regulation and differentiation of the vascular system, particularly in the blood and lymph vessels, with the most significant VEGF-A for its mediating angiogenic effects [8]. There are three receptors targeted by VEGF, such as VEGF receptor 1 (also VEGFR-1 or Flt-1) and VEGF receptor 2 (also VEGFR-2, Flk-1, or KDR), mainly expressed in vascular endothelial cells (VECs) and VEGFR3, found in lymphatic endothelial cells (LECs) [9]. VEGF-A can be expressed in the cardiovascular system and other tissues under conditions of inflammation and hypoxia. Its secretion is upregulated by hypoxia-inducible factors (HIF), specifically by HIF-1α, and downregulated by low-density lipoprotein (LDL) and lipid-lowering drugs (rosuvastatin). It has been previously discussed that VEGF-A prevents the repair of an endothelial lesion and induces the expression of adhesion molecules in endothelial cells, stimulating the secretion of chemokines, which recruit monocytes to migrate into the blood vessel wall and promote endothelial permeability [9]. These monocytes accumulate oxidized LDL and transform into foam cells. VEGF-A additionally promotes the migration of vascular smooth muscle cells (VMSCs) into the plaque, which could lead to platelet activation, aggregation, and thrombus formation [9]. VEGF-A binds to and activates VEGFR-1 and VEGFR-2; the latter is mainly associated with pathological angiogenesis, including vessel formation in tumors [8]. VEGF-B can be found in many tissues, primarily the cardiovascular system, kidney, fat, lung, pancreas, and gallbladder. VEGF-B can only bind to VEGFR-1 and promote angiogenesis, with a substantial impact on neovascularization in the myocardium after a myocardial infarction (MI), and reduces oxidative stress to VECs. Additionally, it has a strong anti-apoptotic effect on myocardial cells after MI, inducing the uptake of fatty acids by endothelial cells, thus lowering the lipid levels in blood serum [10]. VEGF-C and VEGF-D share structural and functional homology to a greater degree than with the other VEGFs.

Specifically, VEGF-C is highly expressed in embryonic tissues but also expressed in an adult's organs. VEGF-C binds to VEGFR2 and VEGFR3, and its main function is regulating and shaping the lymphatic network. Moreover, VEGF-C has an angiogenetic, fibrogenetic, anti-apoptotic, and lipid-increasing effect [10]. VEGF-D is found mainly in the lung (embryonic and adult stages), heart, and intestine. VEGF-D also binds to VEGFR-2 and VEGFR-3 and induces lymphangiogenesis, angiogenesis, fibrogenesis, and a lipid-lowering effect [9,10]. In addition, the placental growth factor (PlGF) is another member of the VEGF family, which binds to VEGFR-1 [8].

HIF-1α and VEGF are strongly correlated through the same metabolic path, affecting angiogenesis and atherosclerosis [6,10]. Under hypoxia, HIF-1α activates the secretion of VEGF to promote angiogenesis, a higher blood and O_2 supply, and cell adaptation to hypoxia. HIF-1α-induced VEGF contributes to vascular remodeling, which can be classified as positive or negative. HIF, VEGF, and cytokines are primarily found in positively remodeled vascular plaques, which contain more macrophages, larger necrotic cores, and thin fibrous caps and, thus, are more prone to breakage [11]. On the other hand, negatively remodeled plaques are more stable [11].

Atherosclerosis results from various interactions between malfunctioning endothelial cells and smooth muscle cells, lipid aggregation, inflammation, calcification of the atheromatic plaques, degradation of the extracellular matrix, altered hemopoiesis, and genetic predispositions [12–14]. Specifically, the dysfunctional endothelium combined with the accumulation of lipids within the arteries and their effect on the tunica intima are the main initiators of atherosclerosis [12]. Various inflammatory cytokines are released, leading to the activation of inflammatory pathways responsible for the development of the fatty streak, the first evidence of atherosclerosis [12]. As more lipids are deposited in the plaque, more inflammatory cytokines are released, more monocytes are recruited, and the inflammation becomes chronic.

Several risk factors have been implicated in atherosclerosis (Table 1, Ref. [1–3,9,12–21]). In general, atherosclerosis is described as a preventable lifestyle disease. Hypercholesterolemia is the primary cause of atherosclerosis [14]. Mutations in the low-density lipoprotein receptor (*LDLR*) gene, as well as mutations in other genes, including the Apolipoprotein B (*APOB*) gene, proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, and low-density lipoprotein receptor adaptor protein 1 (*LDLRAP1*) gene have been found in individuals with familial hypercholesterolemia, a hereditary form of hypercholesterolemia [13,15,16]. Nutrition and obesity have been linked to the development of atherosclerosis [1,14]. Smoking, recurrent exposure to environmental and psychological stressors, as well as inadequate or poor-quality sleep, are also risk factors for the development of atherosclerosis [12,14]. The mechanism underlying the aforementioned factors has been linked to the activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. This activation leads to low-grade inflammation, which in turn affects the functioning of the endocrine and vascular systems [14].

Atherosclerosis is a chronic inflammatory condition defined by the buildup of lipids, fibrous tissue, and calcification inside large and medium-sized arteries [17] (Table 1). Accumulating evidence suggests that the inflammation surrounding the atheromatic plaque links the various risk factors and the development of atherosclerosis [1,3,12,18,19]. A growing body of research has proposed that pro-inflammatory substances, such as cytokines, are secreted and accumulated within the arteries due to the oxidation of the lipoproteins that are present in the blood, and this, in turn, leads to the recruitment of white blood cells (mainly monocytes and macrophages, but also T-cells and B-cells in the area) and consequently inflammation [1,2,12,14]. Other researchers do not embrace this hypothesis because using antioxidant medicines has not managed to decrease atherosclerosis. Therefore, they have proposed different mechanisms regarding the presence of inflammation, including accumulated LDL [1], while various experimental studies have provided evidence in favor of the lipid oxidation hypothesis and have managed to do this by manipulating different variables, including transgenic expres-

Table 1. Factors contributing to atherosclerosis, pathogenesis, and histopathological findings.

Factors Contributing to Atherosclerosis	Pathogenesis of Atherosclerosis	Histopathology of Atherosclerosis
Nutrition and obesity [1,14]	Genetics and Lifestyle [3,21]	Calcification in the tunica media [21]
Sedentary lifestyle and poor cardiovascular fitness [14]	Dyslipidemia [1,20]	The core of lipids is surrounded by a fibrous layer [12,21]
Smoking [12,14]	Accumulation of lipids in the arteries – Damaged endothelium [17]	Foam cells [21]
Environmental and psychological stressors	Oxidative stress + Inflammation [1,9,12,18,19]	Ulceration [21]
Inadequate/poor quality sleep [14]	Recruitment of macrophages + Phagocytosis [1,12]	Thrombosis [21]
Genetics [13,15,16]	Foam cells – Cytokines [2]	Intimal thickening [12,21]
	Formation of atheroma [1,2,12]	Narrowing of the lumen and stenosis [21]
	Calcification [17]	

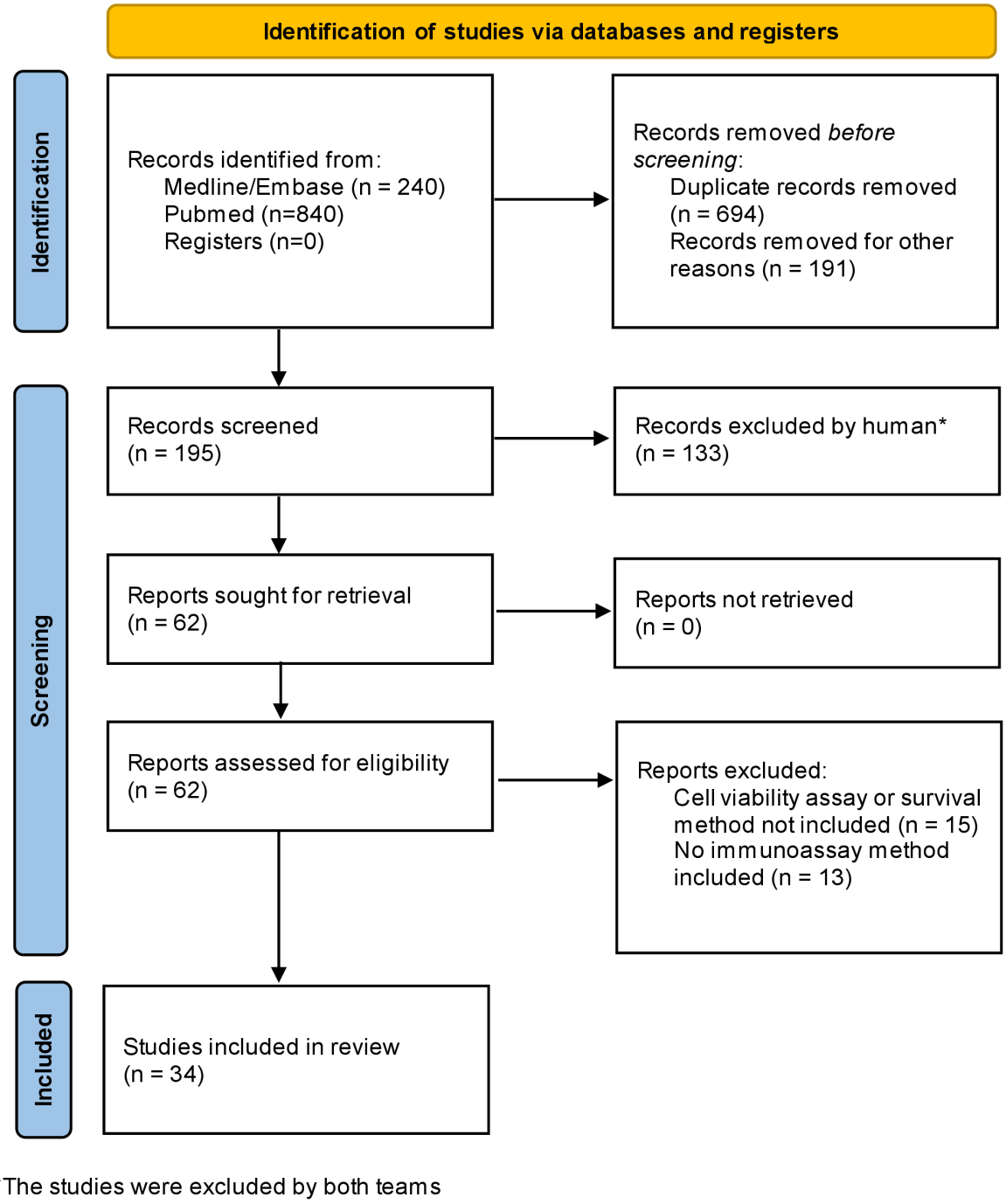

Fig. 1. The diagram shows the articles included in this study (by PRISMA 2020).

sion of antibodies in animals or by targeting specific receptors implicated in the processing of lipids [1]. In addition, several parameters have been parallelized to plaque pathogenesis, such as LDL/(high-density lipoproteins) HDL levels, (apolipoprotein-AI) Apo-AI levels, triglycerides, oxidized low-density lipoprotein (ox-LDL), and inflammation mediators, which are also used as biomarkers, such as C-reactive protein (CRP) [20].

Regarding the histopathological findings of atherosclerosis, it has been reported that coronary arteries show signs of calcification in the tunica media, a "heart" full of lipids and surrounded by a fibrous layer and numerous foam cells [12,21]. Features of ulceration and thrombosis can be found, as well as intimal thickening, narrowing of the lumen, and stenosis of various degrees (Table 1). Severe stenosis in large arteries can be identified

macroscopically, but a smaller degree requires a light microscope.

Atheromatic lesions are either fibrotic or abundant in foam cells by using hematoxylin-eosin staining or monoclonal antibodies against various antigens (immunohistochemical procedures) [21] (Table 1). Specifically, histologically, the atheromatous plaque is characterized by the accumulation of monocytes, macrophages/large foam cells with high lipid content, and smooth muscle proliferation in response to cytokines secreted by damaged endothelial cells (Table 1).

2. Methods

We used PRISMA to report our systematic review and ROBIS (Risk of Bias in Systematic) as a quality assessment tool for the risk of bias, as follows:

2.1 Review Scope and Assessment of Relevant Literature

We explored the literature for HIF-1α and VEGF expression in atherosclerosis by performing a review using the terms {"Atherosclerosis" [OR] "Atheroma" [OR] "atheromatous plaque" [OR] "plaque atherosclerotic"} [AND] {"HIF-1α"} [AND] {"VEGF"} from 2009 up to May 2024 (PubMed; Medline/Embase), following the PRISMA 2020 principles [22]. We assessed the relevant literature using Yale Medical Library and University of Thessaly Library tools for screening and filtering data.

2.2 Study Eligibility Criteria

We used inclusion criteria such as (i) evaluation of atherosclerosis prognosis based on HIF-1α/VEGF expression and other biomarkers related to HIF-1α/VEGF, using the application of immunohistochemistry or other immunoassays for protein expressions, such as immunofluorescence, western blot, and ELISA (enzyme-linked immunosorbent assay), (ii) evaluation of HIF-1α/VEGF protein expression in association with the effectiveness of atherosclerosis therapeutic strategies based on cell viability, or cell proliferation, microscopic or imaging evaluation, or animal or patient survival methods.

We used exclusion criteria, such as (i) data not evaluating protein levels of HIF-1α or VEGF; (ii) data not reporting HIF-1α or VEGF and prognosis; (ii) review articles; (iii) original articles not in English; and (iv) studies before 2009.

2.3 Data Collection and Study Appraisal

We extracted data regarding (i) atherosclerosis prognosis and immunophenotypes of HIF-1α and VEGF, as well as (ii) HIF-1α/VEGF immunophenotypes and atherosclerosis therapeutic strategies. Methodological assays for HIF-1α/VEGF evaluation and atherosclerosis cell viability/proliferation, microscopic examination or imaging evaluation, or animal or human survival assessment were examined for the extracted data. Increased or decreased HIF-1α or VEGF protein expression levels, cell proliferation or cell viability, animal or patient survival, or changes of atheromatous plaque were measured. We selected articles that included methodological assays for HIF-1α/VEGF and atherosclerosis development or progression, as well as for the efficacy of atherosclerosis-targeted therapy.

2.4 Synthesis and Findings

Our search revealed 34 full-text articles (Fig. 1) by PRISMA 2020 [22], including 650 human specimens, 21 different cell lines, and 9 different animal models. Most of these articles (53%), particularly those that discussed HIF-1α and VEGF-related immunophenotypes in atherosclerosis-targeted therapy (76%), were published between 2019 and 2024. The main details of the studies about the HIF-1α/VEGF immunophenotypes in atherosclerosis are shown in Tables 2,3,4 and summarized in Figs. 2,3,4,5.

It is worth mentioning that based on our search, a great volume of atherosclerosis-related articles was published from 2009 to May 2024 (PubMed: 112,414; Embase: 99,856; Medline: 178,200), showing a health emergency due to the size of the disease.

2.5 Assessment of Bias

Two separate teams worked on this search and reviewed all titles and abstracts. Full articles were retrieved from any article deemed relevant by either reviewer. Data were extracted from relevant methodological articles. Each team discussed it face-to-face before sending it to an independent reviewer, who made the final judgment. We used ROBIS to assess the risk of bias [23]. Assessing the overall risk of bias in the interpretation of review findings and considering limitations, such as the use of different survival methods, no concerns were found, and the review study is rated as "low risk of bias".

3. Results

3.1 HIF-1α Immunophenotypes and Atherosclerosis Prognosis

We found 9 studies investigating HIF-1α protein expression through immunoassays and atherosclerosis prognosis, using 10 different cell cultures and 1 animal model (Table 2, Ref. [24–32]). Among the 9 studies, 8 presented the adverse effect of HIF-1α immunoreactivity on atherosclerotic plaques, while only 1 study showed a favorable prognosis.

Lv *et al.* [24] showed that hypoxia stimulation at 2% oxygen leads to the activation of HIF-1α in neonatal rat aorta smooth muscle cells (NRSMCs) and promotes cell proliferation (Table 2; Fig. 2). Another important study by Maier *et al.* [25] revealed that hypoxia-inducible protein 2 (*HIG2*)/hypoxia-inducible lipid droplet-associated (*HILPDA*), is a target of HIF-1, highly expressed in atherosclerotic plaques, mediating neutral lipid accumulation in macrophages, and is crucial to foam-cell formation

Table 2. HIF-1α immunophenotype and atherosclerosis prognosis.

A/a	Article (year)	Materials	Methods	HIF-1α-related remarks	Significant association with adverse outcomes
(1)	Poitz *et al.* (2013) [31]	Monocytes culture	WB	HIF-1α was activated during monocyte-to-macrophage differentiation during hypoxia, which was partially mediated by a micro ribonucleic acid (miRNA)-dependent mechanism (negative regulators, such as miR-17 and miR-20a).	Yes
(2)	Karshovska *et al.* (2020) [32]	M-HIF1$^{-/-}$ mice on a C57Bl/6J background; BMDMs cell culture	IHC; *In situ* PCR (polymerase chain reaction) hybridization	HIF-1α activation in inflammatory macrophages promoted necrotic core formation and lesion progression through miR-383-mediated ATP depletion.	Yes
(3)	Maier *et al.* (2017) [25]	ApoE- HILPDA tie2-Cre Cko cell culture	Immunoblotting; IHC	HIF-1α expression induced transcriptional activation of *HIG2/HILPDA* which was crucial for foam-cell formation and regulated PGE2 production, affecting early lesion formation and progression of atherosclerosis.	Yes
(4)	Lv *et al.* (2015) [24]	Sprague-Dawley Rat VSMCs cell culture	WB	HIF-1α was overexpressed under hypoxia stimulation at 2% oxygen in NRSMCs, promoting cell proliferation.	Yes
(5)	Zhang *et al.* (2016) [26]	Sprague-Dawley Rat HUVECs cell culture	Immunoassays	HIF-1α and the NF-κB-related proinflammatory pathway were upregulated, leading under hypoxia to early-stage atherosclerosis mainly by the expression of pro-inflammatory cytokines.	Yes
(6)	Tawakol *et al.* (2015) [28]	HUASMCs; Human & murine macrophages cell culture	IHC	The pro-inflammatory activity of HIF-1α and PFKFB3 increased under hypoxia, potentiating macrophage glycolytic flux.	Yes
(7)	Cattaneo *et al.* (2011) [30]	HUVECs cell culture	IHC, ELISA	Nucleus accumulation of HIF-1α in the nucleus caused by NO deficiency in HUVECs induced pseudohypoxia, resulting in mitochondrial dysfunction and reduced energy production.	Yes
(8)	Fu *et al.* (2010) [29]	HUASMCs cell culture	Western blot; ELISA	HIF-1α increased and rapidly induced MIF expression in human VSMCs, influencing the progression of atherosclerosis.	Yes
(9)	Ben-Shoshan *et al.* (2009) [27]	ApoE mice cell culture	IHC	Increased expression of HIF-1α in mouse lymphocytes reduced IFN-γ expression and attenuated atherosclerotic lesions.	No

HIF, hypoxia-inducible factor; ApoE, atherosclerosis-prone apolipoprotein E-deficient; WB, Western blot; IHC, immunohistochemical; IFN-γ, interferon-γ; HUASMCs, human umbilical artery smooth muscle cells; MIF, migration inhibitory factor; VSMCs, vascular smooth muscle cells; HUVECs, human umbilical vein endothelial cells; NO, nitric oxide; NRSMCs, neonatal rat smooth muscle cells; PFKFB3, 6-phosphofructo-2-kinase; *HIG2/HILPDA*, hypoxia-inducible protein 2/hypoxia-inducible lipid droplet-associated protein; PGE2, prostaglandin E2; M-HIF1$^{-/-}$, myeloid cell-specific deletion of HIF-1α; BMDMs, bone marrow-derived macrophages; ELISA, enzyme-linked immunosorbent assay; NF-κB, nuclear factor-kappaB.

Table 3. VEGF and HIF-1α-induced and VEGF immunophenotypes and atherosclerosis prognosis.

A/a	Article (year)	Materials	Methods	VEGF-related remarks	Significant association with adverse outcomes
(1)	Bialecka *et al.* (2024) [38]	70 plasma samples from patients with early-onset CAD	ELISA	VEGF levels were higher in CAD compared to healthy individuals; however, there were no significant correlations between VEGF plasma concentrations and atherosclerosis progression.	No
(2)	Cui *et al.* (2024) [34]	ApoE$^{-/-}$ mice; aortic endothelial cells	IHC; IF	VEGF significantly increased under chronic exposure to methamphetamine (METH), promoting angiogenesis and vessel rupture in atherosclerotic plaques.	Yes
(3)	Pauli *et al.* (2020) [37]	100 human specimens (CAD patients)	ELISA	There was a weak correlation between plasma VEGF and the risk of atherosclerosis.	No
(4)	Yan *et al.* (2019) [40]	RAW 264.7 cell culture; ApoE$^{-/-}$ mice	IHC, IF; WB	Stable VEGF overexpression in macrophages attenuated the intimal hyperplasia after arterial injury.	No
(5)	Guo *et al.* (2018) [36]	60 human carotid specimens and 346 human coronary artery samples	IHC; IF	The CD163/HIF-1α/VEGF-A pathway produced alternative macrophages, which promoted plaque angiogenesis, leakiness, and inflammation.	Yes
(6)	Yan *et al.* (2017) [39]	RAW 264.7 cell culture	ELISA	Stable overexpression of VEGF down-regulated CD36 in macrophages and reduced foam cell formation, attenuating the progression of atherosclerosis.	No
(7)	Vm *et al.* (2016) [33]	74 human specimens (38 HRD patients; 36 CAD patients without HRD)	ELISA	Increased VEGF positively correlated with serum IL-18 levels and was identified as a factor involved in coronary artery disease pathophysiology.	Yes
(8)	Tang *et al.* (2013) [35]	macLITAF$^{-/-}$ cells, U2OS cell, RAW 264.7 cell, endothelial ell culture; TamLITAF$^{-/-}$ and wild-type mice	ELISA	The overexpression VEGF in macrophages and endothelial cells induced p38α phosphorylation, which activated transcriptional factors LITAF and STAT6B. These factors upregulated VEGF, angiogenesis, and atherosclerosis.	Yes

macLITAF$^{-/-}$, macrophage-specific LITAF-deficient; ELISA, enzyme-linked immunosorbent assay; IF, Immunofluorescence; WB, Western blot; HRD, heart rhythm disorders; CAD, coronary artery disease.

Table 4. HIF-1α and VEGF-related immunophenotypes in atherosclerosis targeted therapy.

A/a	Article (year)	Materials	Methods	HIF-1α/VEGF-related remarks	Significant association with therapeutic outcomes
(1)	Bao *et al.* (2024) [44]	RAW264.7 cells; ApoE$^{-/-}$ mice	WB; IF	LCZ696, a CNP, enhanced HIF-1α downregulation via PHD2 and reduced the inflammatory phenotype, foam cell formation, and necroptosis in macrophages. LCZ696 amplified the bioactivity of CNP and ameliorated atherosclerotic plaque formation.	Yes
(2)	Ji *et al.* (2024) [47]	ApoE$^{-/-}$ mice	WB; ELISA	HIF-1α levels were reduced in aortic tissues treated with BHD to modulate M1/M2 macrophage polarization, decrease inflammatory factors, increase anti-inflammatory factors, and reduce plaque area.	Yes
(3)	Ma *et al.* (2024) [53]	Sprague-Dawley rats	WB	VEGF levels were reduced by NR1 in arterial endothelium, accompanied by reduced levels of serum lipid profiles, inflammatory factors, enhanced levels of NO, and a noticeable reduction in plaque pathology.	Yes
(4)	Li *et al.* (2024) [54]	C57BL/6J; (ApoE$^{-/-}$); Pericytes-ECs	IHC; IF; WB	VEGF-A significantly decreased by NR1 treatment. NR1 ameliorated atherosclerosis development and reversed atherosclerotic plaque vulnerability.	Yes
(5)	Bai *et al.* (2024) [56]	HUVEC-12; ApoE$^{-/-}$ mice	IHC; WB	VEGF was suppressed by DFMG, inducing an anti-atherosclerotic effect. This effect is mediated by miR-140 through the negative regulation of TLR4/NF-κB.	Yes
(6)	Lu *et al.* (2024) [58]	HUVECs	WB	VEGF downregulation was alleviated by inhibiting miR-497-5p, attenuating ox-LDL dysfunction in endothelial cells through activating the p38/MAPK pathway, and preventing angiogenic capacity.	Yes
(7)	Yan *et al.* (2023) [55]	Rat thoracic aortic endothelial cells	WB; IF	VEGF and HIF-1α expression was suppressed via STAT3 (signal transducer and activator of transcription 3) by glycosides, significantly improving atherosclerosis.	Yes
(8)	Villa-Roel *et al.* (2022) [43]	Human aortic endothelial cells; C57BL/6 & ApoE$^{-/-}$ mice	WB	HIF-1α protein expression was decreased by PX-478 in a concentration-dependent manner. PX-478 reduced plasma cholesterol and atherosclerosis.	Yes
(9)	Makaritsis *et al.* (2022) [48]	ApoE$^{-/-}$ mice	IHC	HIF-1α was reduced and eNOS was increased in macrophages of the atheromatous plaque under the effect of crocin, mediating atherosclerosis reduction via eNOS and HIF-1α.	Yes
(10)	Wang *et al.* (2022) [42]	Hif1a$^{fl/fl}$, Hif1a^{DAdipo}, ApoE$^{-/-}$ mice; 3T3-L1 & HEK293T	WB	Direct inhibition of HIF-1α by PX-478 significantly reduced HIF-1α, cholesterol levels, inflammatory responses, and atherogenesis.	Yes
(11)	Kai *et al.* (2021) [57]	HUVECs	WB	Inhibition of long non-coding RNA (lncRNA) *NORAD* (non-coding RNA Activated by DNA Damage) enhanced VEGF expression, alleviating vascular endothelial cell injury and atherosclerosis.	Yes
(12)	Dong *et al.* (2021) [51]	HUVECs	ELISA	VEGF expression/secretion, cell proliferation migration, and tube formation were inhibited by METTL3 knockdown in ox-LDL-treated HUVECs, preventing the progress of atherosclerosis.	Yes
(13)	Marino *et al.* (2020) [50]	HUVECs	ELISA	VEGF was reduced by anthocyanins (peonidin and petunidin-3-glucoside and their metabolites), restricting atherosclerosis.	Yes

Table 4. Continued.

A/a	Article (year)	Materials	Methods	HIF-1α/VEGF-related remarks	Significant association with therapeutic outcomes
(14)	Yuan *et al.* (2018) [52]	HUVECs	ELISA; WB	The combination of TMP and PF reduced VEGF immunophenotype, in addition to VEGFR2, JAG1, and Notch1, contributing to plaque stability in atherosclerosis.	Yes
(15)	Rahtu-Korpela *et al.* (2016) [46]	HIF-P4H-2- hypomorphic/C699Y-LDL receptor–mutant mice; LDL receptor–deficient mice	WB	HIF-P4H-2 inhibition (FG-4497) stabilized HIF-1α and HIF-2α, resulting in a 50% reduction in atherosclerotic plaque areas, reduced WAT weights, macrophage aggregated numbers, and increased autoantibodies against oxidized LDL.	Yes
(16)	Hisada *et al.* (2012) [45]	Human coronary artery smooth muscle cells	WB; Other Immunoassays	HIF-1α protein expression was inhibited by fluvastatin, attenuating HIF-1-dependent ET-1 gene expression and inducing a potentially beneficial effect on atherosclerosis.	Yes
(17)	Gessi *et al.* (2010) [49]	Human myelomonocytic U937 cell culture	WB; ELISA	VEGF was reduced by adenosine receptor antagonists, preventing foam cell formation and atherosclerosis.	Yes

HUVECs, human umbilical vein endothelial cells; ECs, endothelial cells; LDL, low-density lipoprotein; Hif1a$^{\Delta Adipo}$, Adipocyte-specific HIF-1α knockout; Apoe$^{-/-}$ mice, Atherosclerosis-prone apolipoprotein E-deficiency mice; Hif1a$^{fl/fl}$ mice, flanking exon 2 of the hypoxia-inducible factor 1, alpha subunit gene mice; nSMase2 (also called SMPD3), type 2-neutral SMase; PHD2, prolyl hydroxylase domain-containing protein 2; BHD, Buyang Huanwu decoction; NR1, Notoginsenoside R1; CNP, C-type natriuretic peptide; DFMG, 7-difluoromethoxy-5,4′-dimethoxygenistein; TMP, tetramethylpyrazine; PF, paeoniflorin; WAT, white adipose tissue; MAPK, mitogen-activated protein kinase; TLR4, Toll-like receptor 4; ox-LDL, oxidized low-density lipoprotein; METTL3, methylated regulation; JAG1, Jagged1.

and controlled prostaglandin E2 (PGE2) production (Table 2; Fig. 2). Thus, it promotes early lesion formation and the progression of atherosclerosis. In addition, based on Zhang *et al*. [26], hypoxia through the HIF-1α-nuclear factor-kappaB (NF-κB) proinflammatory pathway produces an increased expression of proinflammatory cytokines, leading to the formation of early-stage atherosclerosis (Table 2; Fig. 2). These interactions result in promoted proinflammatory cytokines production, such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), which in turn upregulate CRP and interleukin-6 (IL-6) production, the key genes in the progression of atherosclerosis. In parallel, proinflammatory cytokines can further enhance NF-κB transcriptional activity and transcriptional activation of HIF-1α, thus atherosclerosis [26]. However, Ben-Shoshan *et al*. [27] used an *in vivo* model to show the potential anti-inflammatory properties of HIF-1α to reduce atherosclerotic plaque. Specifically, they showed the direct effects of HIF-1α expression on the lymphocytic cytokine profile and a marked decrease in the transcription of interferon-gamma (IFN-γ) and attenuation of experimental atherosclerosis, accompanied by a parallel increase in interleukin-10 (IL-10) expression [27] (Table 2; Fig. 2). This model provided a potential immunomodulatory approach to atherosclerosis.

Macrophages, endothelial, and smooth muscle cells are the most relatable cells of atherosclerosis. Tawakol *et al*. [28], using human and murine macrophage cultures, showed that elevated HIF-1α, hexokinase II, and ubiquitous 6-phosphofructo-2-kinase (PFKFB3) expression is associated with an increase in pro-inflammatory activation and glycolysis (Table 2; Fig. 2). Specifically, they showed that under hypoxic conditions, increased levels of HIF-1α are associated with the upregulation of proinflammatory cytokines after transcriptional induction of PFKFB3, while the activated macrophages produce further cytokines to perpetuate the inflammatory cycle. The authors also showed that glycolysis after PFKFB3 expression has a profound effect on macrophage viability [28].

In vascular smooth muscle cells (VSMCs), HIF-1α is a potent and rapid inducer of macrophage migration inhibitory factor (MIF) expression, a proinflammatory factor influencing the pathogenesis of atherosclerosis. Fu *et al*. [29] showed that hypoxia plays a key role in vascular remodeling and directly affects VSMCs' functions through migration inhibitory factor (MIF)'s expression (Table 2; Fig. 3). They showed that up-regulation of MIF expression appears to be dependent on HIF-1α, mediating the hypoxia response of VSMCs, including cell migration and proliferation. MIF regulates VSMC proliferation and migration within the vessel wall and the migration of VSMCs from the media into the neointima, contributing to vessel narrowing during the atherosclerotic process.

Chronic loss of nitric oxide (NO)-induced profound modifications in endothelial cell physiology, with important consequences for endothelial cell dysfunction associated with atherosclerosis progression [30]. Specifically, Cattaneo *et al*. [30], using an *in vitro* model, showed that accumulation of HIF-1α in normoxia is related to a lack of NO in human endothelial cells, inducing pseudohypoxia and mitochondrial dysfunction with consequent decreased energy production and enhanced chemotactic migration (Table 2; Fig. 3).

Regarding genetics, Poitz *et al*. [31] showed that during monocyte-to-macrophage differentiation in the progression of atherosclerosis, the expression of HIF-1α subunits is regulated by micro ribonucleic acid (miRNAs) (Table 2; Fig. 3). They showed that miR-17 and miR-20a negatively influence the hypoxic HIF-activity and gene expression in primary human macrophages by directly binding to their 3′UTR [31]. Also, Karshovska *et al*. [32], using a mouse model, showed that elevated levels of HIF-1α in inflammatory macrophages increase necroptosis through miR-383-mediated ATP depletion, thus increasing atherosclerosis by necrotic core formation (Table 2; Fig. 3).

3.2 VEGF and HIF-1α-Induced VEGF Immunophenotypes and Atherosclerosis Prognosis

The association of elevated levels of VEGF in coronary artery cells, macrophages, or plasma with a higher or lower risk of atherosclerosis is unclear yet. We found 8 studies exploring VEGF and HIF-1α immunophenotypes and their association with atherosclerosis prognosis, using 650 human specimens, 5 different cell cultures, and 3 different animal models (Table 3, Ref. [33–40]). Four of these studies presented the adverse effect of VEGF immunoreactivity on atherosclerotic plaques, while the rest 4 of the 8 studies proved a favorable prognosis (Table 3).

Specifically, based on Vm *et al*. [33], HIF-1α-induced VEGF promotes angiogenesis, which mediates the growth of plaques and activates the influx of inflammatory cells and erythrocytes, resulting in the plaque break (Table 3; Fig. 3). They showed that elevated VEGF levels are associated with serum IL-18 levels and higher coronary artery disease (CAD) severity [33]. Furthermore, Cui *et al*. [34] provided recent preclinical evidence that VEGF is significantly increased in aortic endothelial cells after chronic exposure to methamphetamine (METH), supporting VEGF-mediated angiogenesis and vessel rupture in atherosclerotic plaques (Table 3; Fig. 3). In addition, Tang *et al*. [35] (Table 3; Fig. 3), using *in vitro* and *in vivo* models, showed that increased VEGF levels in macrophages or endothelial cells induce p38α phosphorylation, which consequently activates both transcriptional factors lipopolysaccharide-induced tumor necrosis factor-alpha factor (LITAF) and signal transducers and activators of transcription 6 isoform B (STAT6B) to upregulate VEGF, increasing angiogenesis and atherosclerosis.

The way that HIF-1α and VEGF are correlated under hypoxic conditions has been reported in inflamma-

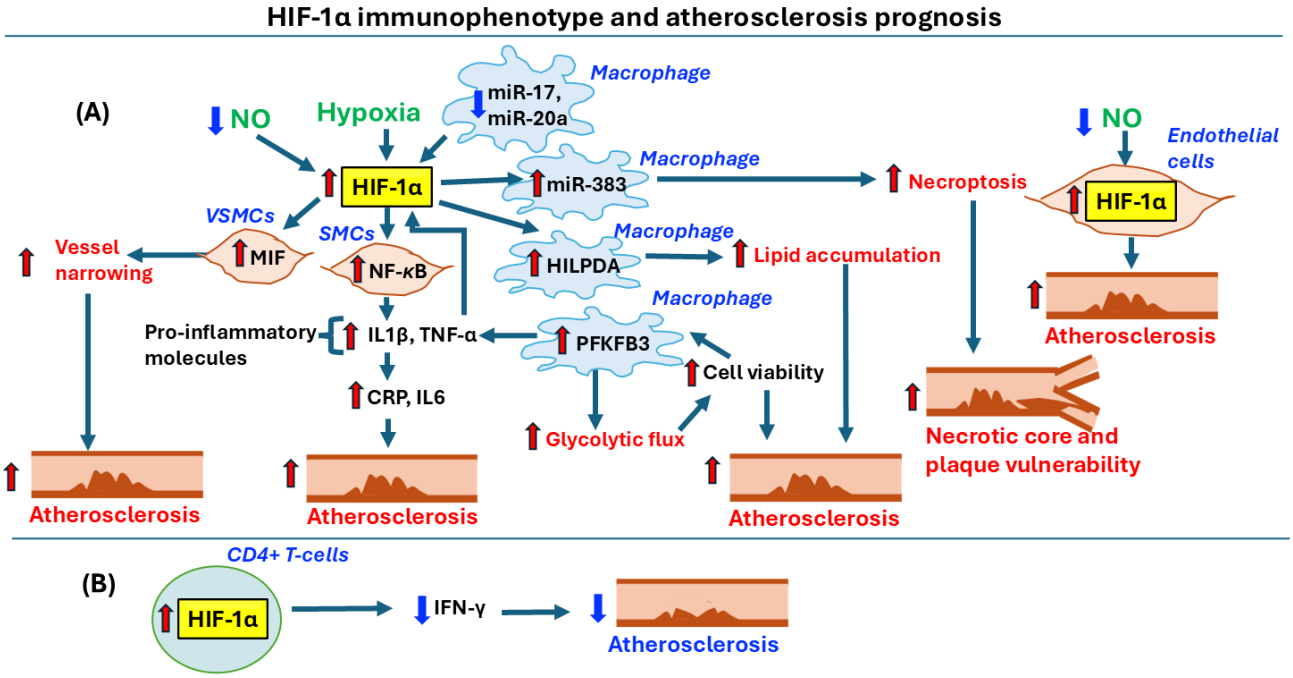

Fig. 2. Hypoxia-inducible factor 1alpha immunophenotype and atherosclerosis prognosis. (A) Increased HIF-1α immunophenotypes can be found in vascular smooth muscle cells (VSMCs), macrophages, or endothelial cells under hypoxic conditions, chronic loss of nitric oxide (NO), or reduced levels of miRNAs, such as miRNA-17 and miR-20a. These increased HIF-1α immunophenotypes are associated with the upregulation of pro-inflammatory molecules (IL-1β, TNF-α) via NF-κB, increased MIF, and vessel narrowing, glycolytic flux, and increased macrophage cell viability via PFKFB3, lipid accumulation via HILPDA, neckfbroptosis via miR-383, and lead to atherosclerosis progression and atheromatic plaque vulnerability. (B) On the contrary, the increased levels of HIF-1α found in lymphocytes (CD4+ spleen-derived T-cells) are associated with decreased IFN-γ and attenuated atherosclerotic lesions. CRP, C-reactive protein; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha.

tion and tumor angiogenesis [9]. Induced by HIF-1α, VEGF-A primarily has either harmful or beneficial effects on atherosclerosis [9]. Guo *et al.* [36] used human atherosclerotic samples and showed elevated levels of HIF-1α and VEGF-A expression in CD163+ macrophages are associated with plaque progression (Table 3; Fig. 3). Specifically, they showed that HIF-1α promoted VEGF-mediated increase in intraplaque angiogenesis. These findings highlighted a nonlipid-driven mechanism. Based on this mechanism, macrophages promote plaque angiogenesis, leakiness, inflammation, and progression via the CD163/HIF1α/VEGF-A pathway.

On the contrary, Pauli *et al.* [37], in a study group of 100 Caucasian patients, concluded that the protein levels of circulating VEGF are only marginally associated with an elevated risk of atherosclerosis (Table 3; Fig. 3). Similarly, a recent clinical study by Bialecka *et al.* [38] showed that although CAD patients have higher levels of VEGF in their plasma compared to healthy individuals, plasma VEGF levels may not be a reliable marker for the vascular condition (Table 3; Fig. 3). Notably, Yan *et al.* [39] revealed that stable overexpression of VEGF in macrophages downregulates expression of CD36 and reduces foam cell formation, attenuating the progression of atherosclerosis (Table 3; Fig. 3). In another study, Yan *et al.* [40] also highlighted that stable overexpression of VEGF in macrophage cell cultures produces high NO levels, contributing to the attenuation of intimal hyperplasia after arterial injury (Table 3; Fig. 3).

3.3 HIF-1α and VEGF Immunophenotypes in Atherosclerosis Targeted Therapy

A wide investigation field is correlated with atherosclerosis therapy since its pathogenic characteristics and genetics seem to complicate medical research [21,41]. Since HIF-1α and VEGF expression is detected in atherosclerotic plaques and the HIF and VEGF pathway affects the progression of atherosclerosis, disruption of this pathway might be effective in the treatment of atherosclerosis, while altered HIF and VEGF immunophenotypes may reflect the effectiveness of the treatment. Our research revealed 17 studies that explored the association of HIF-1α and VEGF immunophenotypes with targeted therapy of atherosclerosis, using 7 cell cultures and 7 different animal models (mice, rats) (Table 4, Ref. [42–58]; Fig. 4).

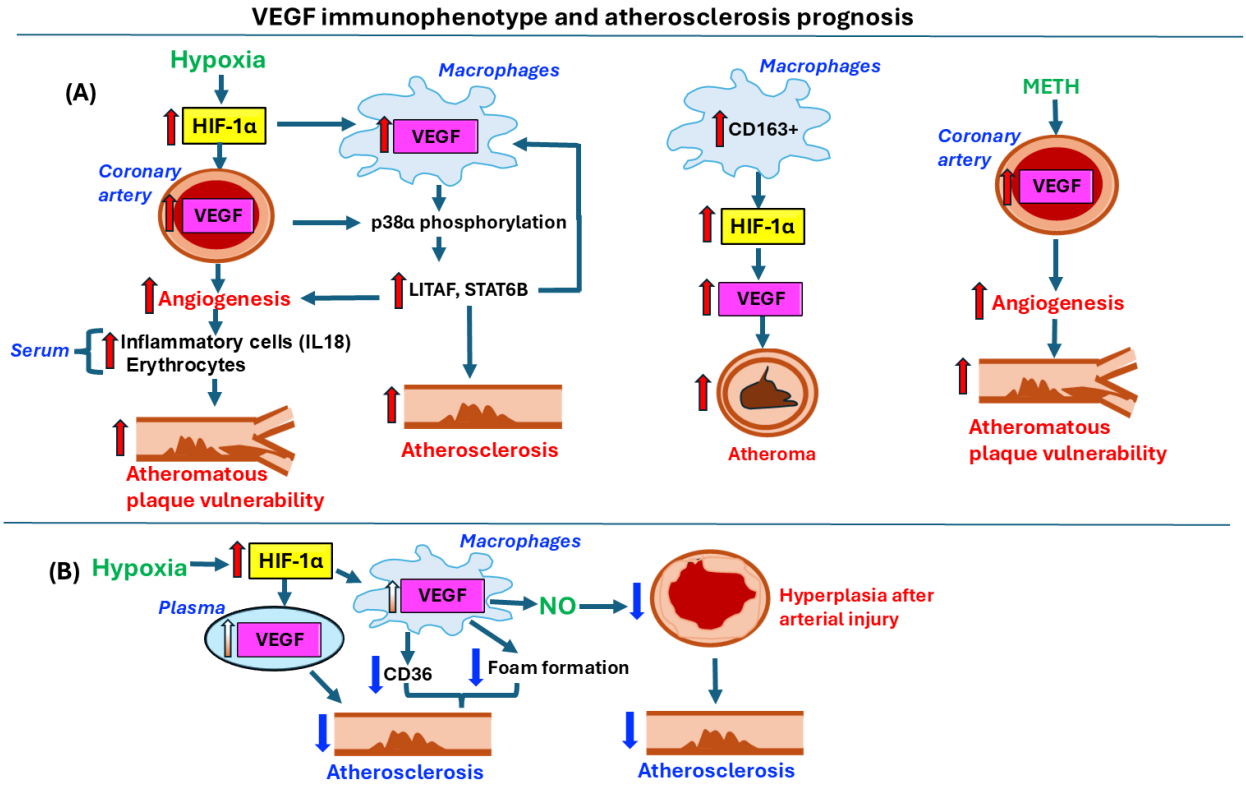

Fig. 3. Vascular endothelial growth factor immunophenotypes and atherosclerosis prognosis. (A) HIF-1α-induced increased VEGF immunophenotype can be found in coronary artery cells under hypoxia, increasing inflammatory cells (IL18) in serum and angiogenesis and leading to atheromatic plaque break. Similarly, HIF-1α-induced increased VEGF immunophenotype can be promoted in activated macrophages or endothelial cells, inducing p38α phosphorylation and LITAF and STAT6B overexpression and leading to increased angiogenesis and atherosclerosis progression. Finally, increased HIF-1α and VEGF immunophenotypes can be shown in activated macrophages (CD163+), leading to atheromatic plaque progression. VEGF is significantly increased in aortic endothelial cells after chronic exposure to METH (methamphetamine), contributing to angiogenesis and vessel rupture in atherosclerotic plaques. (B) Stable VEGF overexpression under hypoxic conditions can be found in serum but is marginally associated with an elevated risk for atherosclerosis. Also, stable VEGF overexpression can be found in macrophages under hypoxia and is associated with reduced CD36, foam formation, and attenuation of atherosclerosis progression. Similarly, the stable VEGF overexpression found in macrophages is associated with elevated NO, contributing to the attenuation of intimal hyperplasia after arterial injury. VEGF, vascular endothelial growth factor; LITAF, lipopolysaccharide-induced tumor necrosis factor-alpha factor; STAT6B, signal transducers and activators of transcription 6 isoform B.

3.3.1 HIF-1α Inhibitors

Wang *et al.* [42] showed an effective reduction of HIF-1α immunophenotypes and activity using a specific HIF-1α inhibitor known as PX-478 (MedChemExpress, Monmouth Junction, NJ, USA), with a direct impact on the development of atherosclerosis (Table 4). Specifically, this inhibitor works by reducing the production of ceramide in fatty tissues, which in turn leads to a decrease in cholesterol levels and a reduction in inflammatory reactions. Indeed, Villa-Roel *et al.* [43], using a mouse model, proved the efficacy of PX-478 in the treatment of atherosclerosis by decreasing plasma cholesterol levels and reducing atheroma (Table 4; Fig. 4).

3.3.2 LCZ696 and C-type Natriuretic Peptide (CNP)

Bao *et al.* [44] experimental data showed that CNP (R&D System, Minneapolis, MN, USA), an endogenous peptide that is a crucial regulator of vascular homeostasis, can enhance plaque stability and alleviate macrophage inflammatory responses by promoting HIF-1α degradation via PHD (prolyl hydroxylase domain-containing protein) 2, suggesting a novel atheroprotective role of CNP. They also showed that administration of LCZ696 (Novartis Pharma Schweiz AG; Basel, Switzerland), an orally bioavailable drug that combines an angiotensin receptor blocker (valsartan) with sacubitril, a specific inhibitor of the neutral endopeptidase (NEP), recently approved for treating chronic heart failure with reduced ejection fraction, can amplify the bioactivity of CNP and improve atherosclerotic plaque formation [44] (Table 4; Fig. 4).

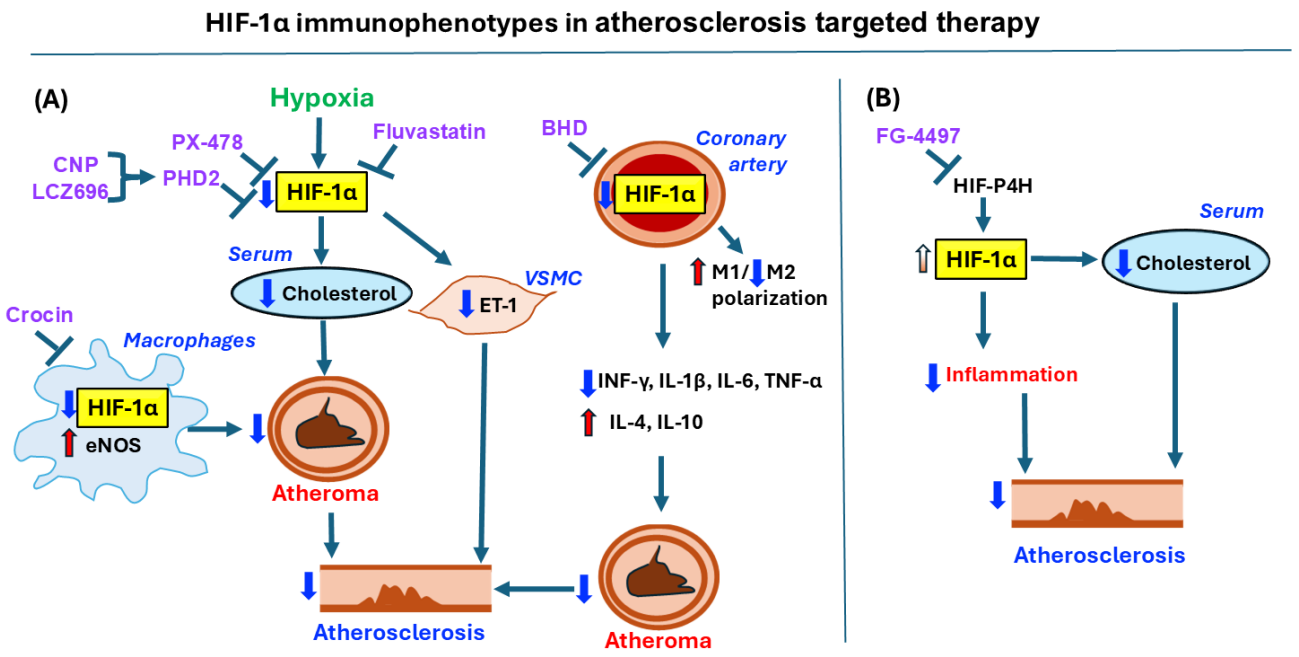

Fig. 4. HIF-1α immunophenotypes in atherosclerosis targeted therapy. (A) HIF-1α inhibitors (PX-478) or statins (Fluvastatin) can suppress hypoxia-induced HIF-1α immunophenotype, leading to reduced levels of cholesterol in serum and endothelin-1 (ET-1) in vascular smooth muscle cells (VSMC), respectively, and preventing atherosclerosis. CNP, a C-type natriuretic peptide, enhanced plaque stability by promoting HIF-1α degradation via a prolyl hydroxylase domain-containing protein 2 (PHD2), while the LCZ696 drug amplifies CNP bioactivity. Crocin, an active ingredient of saffron, reduces atheromatous plaque by reducing HIF-1α and increasing endothelial nitric oxide synthase (eNOS) in macrophages of atheromatous plaque, Buyang Huanwu decoction (BHD), a famous herbal prescription that has been used to treat stroke, can modulate M1/M2 macrophage polarization by inhibiting HIF-1α levels in coronary arteries, preventing atherosclerosis. (B) The HIF-prolyl 4-hydroxylase (HIF-P4Hs) inhibitor, FG-4497, can stabilize HIF-1α expression and reduce serum cholesterol levels, protecting against the development of atherosclerosis.

3.3.3 Statins

Another association has been presented between HIF-1α and statins. Fluvastatin (Novartis Pharma Schweiz AG; Basel, Switzerland) inhibits HIF-1α binding to the hypoxia response element (HRE) of the plasminogen activator inhibitor-1 gene in VSMCs and endothelial cells [45] (Table 4). Specifically, Hisada *et al.* [45] showed that hypoxia (1% O_2), compared with the normoxic condition (21% O_2), can significantly induce the expression of endothelin-1 (ET-1) secretion in VSMCs, contributing to atherosclerosis. On the other hand, they showed that fluvastatin can inhibit HIF-1α protein expression, attenuating HIF-1-dependent ET-1 gene expression in conjunction with the stimulation of ubiquitin/proteasome-dependent degradation of HIF-1α, suggesting fluvastatin's potential therapeutic application for atherosclerotic diseases [45] (Table 4; Fig. 4).

3.3.4 HIF-prolyl 4-hydroxylases (HIF-P4H) Inhibitors

HIF-P4Hs play a significant role in the hypoxia response, regulating the stability of HIF-1α [59]. A recent study by Rahtu-Korpela *et al.* [46] revealed that the HIF-P4H inhibitor FG-4497 (FibroGen, San Francisco, USA) stabilizes HIF-1α expression and reduces serum cholesterol levels, protecting against the development of atherosclerosis (Table 4; Fig. 5). Specifically, they showed that HIF-P4H-2 inhibition could be beneficial in preventing chronic inflammation in atherosclerosis through the increased autoantibody levels against ox-LDL.

3.3.5 Buyang Huanwu Decoction (BHD)

Ji *et al.* [47], using an *in vivo* model, showed that BHD, a famous herbal prescription composed of seven kinds of Chinese medicine (Chinese Herbs Direct, Torrance, CA, USA) that has been used to treat stroke, can inhibit HIF-1α levels in aortic tissue to prevent atherosclerosis (Table 4; Fig. 4). Specifically, they showed that BHD regulates the polarization of M1/M2 macrophages by reducing the level of blood lipids, inhibiting the expression of inducible nitric oxide synthase (*iNOS*), arginase (*Arg-1*), pyruvate kinase M1/2 (*PKM2*)/*HIF-1A*, as well as glycolytic enzymes, decreasing the level of inflammatory factors while increasing the level of anti-inflammatory factors to inhibit inflammatory reactions and reduce plaque area [47].

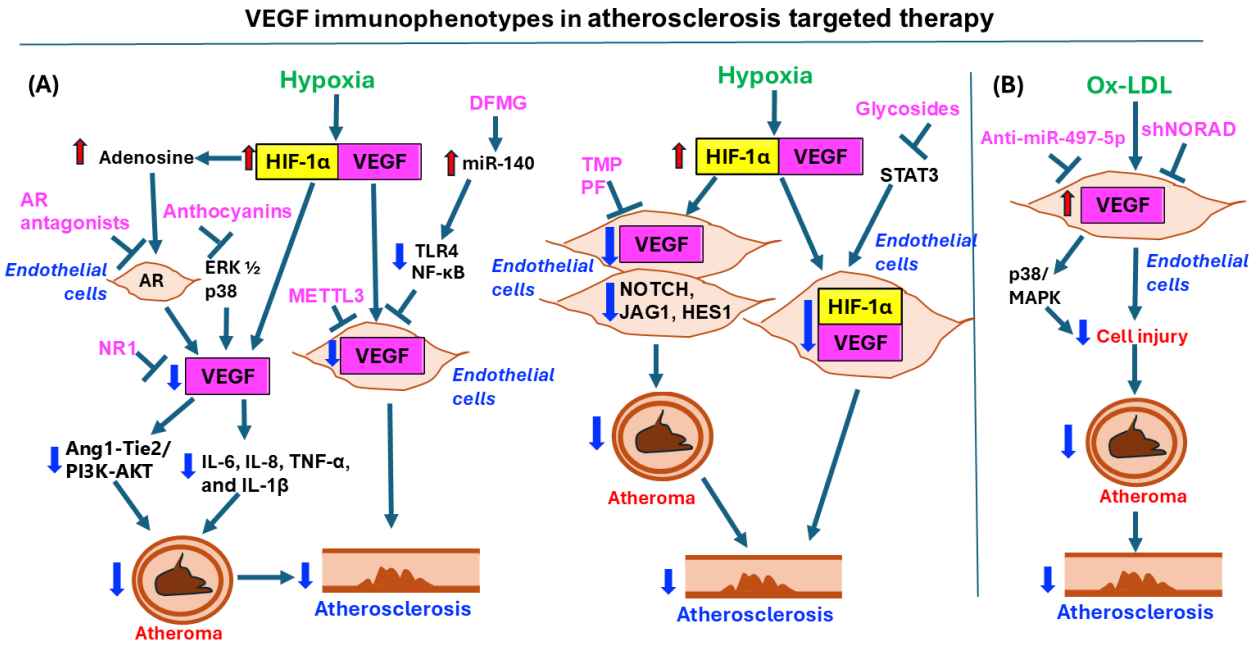

Fig. 5. VEGF immunophenotypes in atherosclerosis targeted therapy. (A) Reduced VEGF immunophenotype is associated with the effect of antagonists of adenosine receptors (AR) in endothelial cells, preventing hypoxia-related adenosine action and atheromatous growth. Similarly, reduced VEGF immunophenotype is associated with the effect of polyphenols, such as anthocyanins, suppressing angiogenesis and atherosclerotic progression. Similarly, NR1 (notoginsenoside R1) can inhibit VEGF with a therapeutic effect on the vulnerable plaque by suppressing the Ang1-Tie2 (angiopoietin 1/angiopoietin receptor)/PI3K-AKT (phosphoinositide-3 kinase/serine/threonine-protein kinase) pathway. Inhibition of VEGF by DFMG (7-difluoromethoxy-5,4′-dimethoxygenistein) provides anti-atherosclerotic effects through negative regulation by miR-140, inhibiting TLR4/NF-κB/VEGF signaling pathway. NR1 can also reduce the expression of VEGF levels in arterial endothelium and decrease the levels of inflammatory factors (such as IL-6, TNF-α, and IL-1β), inducing a noticeable reduction in plaque pathology. Inhibiting VEGF by methylated regulation (METTL3) can also reduce VEGF immunophenotypes, preventing atherosclerosis. Downregulation of VEGF and its related factors Notch1 (NOTCH1), Jagged1 (JAG1), and Hes1 (HES1) in endothelial cells treated by the combination of natural products, such as tetramethylpyrazine (TMP) and paeoniflorin (PF) contributes to the plaque stability in atherosclerosis. Finally, reduced HIF-1α and VEGF immunophenotypes are associated with the effect of the glycoside combination via the inhibition of STAT3, to improve atherosclerosis. (B) Increased VEGF immunophenotypes can be found in endothelial cells after the inhibition of lncRNA *NORAD* (non-coding RNA Activated by DNA Damage), alleviating vascular endothelial cell injury and atherosclerosis development. Inhibition of miR-497-5p can alleviate ox-LDL-induced downregulation of VEGFA and dysfunction of endothelial cells through the activation of the p38/MAPK pathway. shNORAD, short-hairpin RNA (shRNA) specific for *NORAD*.

3.3.6 Crocin (Saffron)

Saffron (Krokos, Kozani, Greece) is the commercial name of the dried stigmata of *Crocus sativus L*. flowers (*C. sativus*). Crocin is the main biologically active carotenoid of saffron. Makaritsis *et al*. [48], using an *in vivo* model, showed that Crocin reduces atherosclerosis by modulating endothelial nitric oxide synthase (eNOS) and HIF-1α expression without affecting plasma cholesterol (Table 4; Fig. 5). Specifically, they showed that crocin can significantly decrease HIF-1α and increase eNOS in atheromatous plaque macrophages of treated mice but did not affect plasma LDL or HDL.

3.3.7 Adenosine Receptor (AR) Antagonists

Hypoxia stabilizes HIF and leads to the accumulation of adenosine. Adenosine modulates HIF-1α, VEGF, interleukin-8 (IL-8), and foam cell formation by activating A_{2B} and A_3 adenosine receptors (AR). Based on Gessi *et al*. [49] (Table 4; Fig. 5), VEGF expression is reduced by AR antagonists, preventing atheromatous growth. In particular, A_3 and A_{2B} or mixed A_3/A_{2B} blockers may be useful to block important steps in atherosclerotic plaque development mediated by adenosine [49] (Table 4).

3.3.8 Polyphenols

Marino *et al*. [50] (Table 4) showed that VEGF is decreased in endothelial cells treated with polyphenols, such as anthocyanins (peonidin and petunidin-3-glucoside and their metabolites), which are mainly found in berries and grapes, perhaps via the inhibition of extracellular signal-regulated kinase (ERK) 1/2 and p38 pathways (Fig. 5). This results in the restriction of angiogenesis and atherosclerotic progression.

3.3.9 Methylation Modification

The most common modification in eukaryotic RNA transcripts is N6-methyl adenosine (m6A), which is dysregulated in atherosclerosis. m6A-mediated regulation of gene expression is catalyzed by m6A methyltransferases (m6A writer). Methylated regulation 3 (METTL3), an m6A writer, has been related to the dysfunction of vascular endothelium. Dong *et al*. [51] showed that VEGF secretion, cell proliferation, migration, and tube formation could be inhibited in ox-LDL-treated Human Umbilical Vein Endothelial Cells (HUVEC) after the METTL3 knockdown (Table 4; Fig. 5). This condition prevents the progress of atherosclerosis and creates new prospects in METTL3-targeted therapies.

3.3.10 Natural Products—Tetramethylpyrazine (TMP) and Paeoniflorin (PF)

Apart from chemical compounds that can be used as therapeutic agents, some natural products are associated with VEGF-mediated anti-atherosclerosis effects. As reported by Yuan *et al*. [52], downregulation of VEGF and angiogenesis-related factors Notch1 (NOTCH1), Jagged1 (JAG1), and Hes1 (HES1) in endothelial cells treated with the combination of tetramethylpyrazine (TMP) or ligustrazine and paeoniflorin (PF) (Merck KGaA, Darmstadt, Germany) contributes to the increase in plaque stability (Table 4; Fig. 5).

3.3.11 Nature products: Notoginsenoside R1 (NR1)

Ma *et al*. [53], using an *in vivo* model, showed that NR1 (Millipore Sigma, Burlington, MA, USA), due to its unique anti-inflammatory properties, may potentially prevent the progression of atherosclerosis (Table 4; Fig. 5). Specifically, they showed that NR1 can reduce the expression of VEGF levels in arterial endothelium, as well as improve serum lipid profiles, decrease the levels of inflammatory factors (IL-6, IL-33, TNF-α, and IL-1β), and enhance the levels of plasma NO. They also showed that atherosclerosis lesions in NR1-treated rats showed a marked reduction in plaque pathology, reducing lipid deposition and decreasing the amount of calcium salt. Li *et al*. [54] also showed the therapeutic effect of NR1 on the vulnerable plaque by suppressing the effect induced by VEGF-A. Specifically, they showed that NR1 treatment can inhibit VEGF-A-stimulated intraplaque neovascularization by suppressing angiopoietin-1-tyrosine kinase receptor 2 (Ang1-Tie2)/phosphoinositide 3-kinase-protein kinase B (PI3K-AKT) paracrine signaling pathway (Table 4; Fig. 5).

3.3.12 Glycosides

Glycosides are effective extracts of Buyang Huanwu decoction (BYHWD) (Chinese Herbs Direct, Torrance, CA, USA), a traditional Chinese medicine derived from Yi Li Gai Cuo, which consists of seven crude herbs and has been proven to protect blood vessels and prevent atherosclerosis. Yan *et al*. [55] examined the effect of glycoside combinations of BYHWD on the expression of STAT-3, HIF-1α, HIF-1β, and VEGF and its pathway proteins for treating atherosclerosis (Table 4; Fig. 5). The researchers showed that the expression of HIF-1α and VEGF is abolished in cells treated with a combination of glycosides through the inhibition of STAT3, which is involved in the secretion pathway of VEGF.

3.3.13 DFMG (7-difluoromethoxy-5,4′-dimethoxygenistein) Synthesized with a Natural Phytoestrogen

DFMG (Pharmacy Department of Hunan Normal University, Changsha, People's Republic of China) is a new chemical entity synthesized with genistein, a natural phytoestrogen. Based on experimental data by Bai *et al*. [56], DFMG can inhibit VEGF expression and angiogenesis in atherosclerosis plaques. Specifically, they showed that the anti-atherosclerotic effects of DFMG are mediated through the negative regulation by miR-140, inhibiting the Toll-like receptor 4 (TLR4)/NF-κB/VEGF signaling pathway [56] (Table 4; Fig. 5).

3.3.14 Inhibition of Long Non-coding RNA (lncRNA)-non-coding RNA Activated by DNA Damage (NORAD)

Kai *et al*. [57] showed that the VEGF immunophenotype is suppressed in endothelial cells treated with ox-LDL via lncRNA *NORAD* (Hechuang Biotechnology Co., Ltd., Shijingshan District Zhenjiang, Jiangsu, China), while inhibition of lncRNA *NORAD* can reverse the VEGF immunophenotype, alleviating vascular endothelial cell injury and growth of atherosclerosis. This data supports lncRNA *NORAD* as a new therapeutic target for atherosclerosis (Table 4; Fig. 5).

3.3.15 Inhibition by miRNAs

Lu *et al*. [58] recently showed that inhibition of miR-497-5p can attenuate ox-LDL-induced downregulation of VEGF-A and dysfunction of endothelial cells through the activation of the p38/mitogen-activated protein kinase (MAPK) pathway (Table 4; Fig. 5). They showed that ox-LDL exposure can reduce cell viability and angiogenic capacity, coupled with increased apoptosis, inflammation,

and oxidative stress in endothelial cells, partially mediated by miR-497-5p upregulation.

4. Discussion

Atherosclerosis is associated with chronic inflammation due to endothelial damage leading to the formation of atheromatous plaques. HIF-1α regulates inflammation, angiogenesis, response to oxidative stress, and development of foam cells [60]. Also, HIF-1α adjusts the pathophysiology of macrophage foam cells [61,62]. Here, we performed a systematic review to show the association of HIF-1α and VEGF immunophenotypes in atherosclerosis prognosis and targeted therapy. Despite the increasing interest in hypoxia and atherosclerosis, especially because of its therapeutic perspective, the relevant literature was not extensive. We found 34 preclinical and clinical studies proving the association of HIF-1α and VEGF immunophenotypes with atherosclerosis prognosis or the effectiveness of therapy. Data limitations of this study may include the use of different survival methods. Our data are summarized in Fig. 6. In general, elevated HIF-1α and VEGF levels in coronary artery cells and macrophages are strongly associated with poor prognosis of atherosclerosis. Since data of elevated HIF-1α and VEGF have also been reported to correlate with a favorable prognosis, therapeutic approaches that either reduce or stabilize HIF-1α and VEGF immunophenotypes are applied for the attenuation of atherosclerosis.

We present that hypoxia-induced elevated HIF-1α and VEGF expression in smooth muscle cells can be found in early-stage atherosclerosis development [24], accompanied by increased levels of a proinflammatory pathway of NF-κB, CRP, and IL-6 [26]. Kimura *et al*. [63] previously discussed that serum VEGF may be an arteriosclerosis-promoting cytokine in humans and proven that elevated CRP and VEGF can be detected earlier than the development of arteriosclerosis in smokers.

We also show that HIF-1α overexpression induces increased MIF expression, which mediates smooth muscle cell migration and proliferation and, thus, is linked to vessel narrowing and atherosclerosis progression [29]. Similarly, Kastora *et al*. [64], in a review article, discussed data from animal studies, presenting that upregulation of VEGF decreases lumen stenosis and neointimal hyperplasia, both protective factors against atherosclerosis.

Moreover, we present that elevated HIF-1α expression induces transcriptional activation of HILPDA in macrophages, which mediates lipid accumulation and plaque formation [25]. In addition, we show that elevated HIF-1α expression in human endothelial cells due to its accumulation resulting from chronic loss of NO can lead to modifications to endothelial cell physiology and dysfunction and the progression of atherosclerosis [30]. Under hypoxic conditions, elevated levels of HIF-1α, hexokinase II, and PFKFB3 are also associated with enhanced proinflammatory activity and macrophage glycolytic flux, which affect macrophage viability [28]. However, increased HIF-1α levels in mouse lymphocytes have also been associated with a favorable prognosis due to the direct effect of HIF-1α on the lymphocytic cytokine profile, causing a significant reduction in IFN-γ, the transcription of, and attenuation of atherosclerosis [27].

Regarding genetics, we show that increased levels and activity of HIF-1α in hypoxic-induced macrophages can be negatively regulated by miR-17 and miR-20a. This can influence the differentiation from monocytes to macrophages in the progression of atherosclerosis [31]. Furthermore, we show that elevated levels of HIF-1α in inflammatory macrophages can increase necroptosis via microRNA-mediated ATP depletion, leading to atherosclerosis and plaque vulnerability [32].

As mentioned above, HIF-1α and VEGF, primarily induced by HIF-1α, strongly correlate through the same metabolic path, affecting angiogenesis and atherosclerosis [65]. Induced by HIF-1α, VEGF-A primarily has either harmful or beneficial effects on atherosclerosis. Earlier studies have shown that hypoxia and inflammation trigger VEGFA's secretion in VMSCs and macrophages [9,65–67]. In addition, it has been previously shown that VEGF contributes to vascular remodeling [11]. HIF, VEGF, and cytokines are primarily found in positively remodeled vascular plaques, which contain more macrophages, larger necrotic cores, and thin fibrous caps and, thus, are more prone to breakage, while negatively remodeled plaques are more stable [11].

Based on our data, HIF-1α-induced VEGF correlates with a higher CAD severity by mediating the growth and breaking of plaques, the latter due to the activation of the influx of inflammatory cells and erythrocytes [33]. Specifically, we present that elevated VEGF levels in coronary artery cells are associated with serum IL-18 levels and a higher CAD severity, and thus, VEGF may be involved in CAD pathophysiology [33]. We show that chronic exposure to METH can induce elevated levels of VEGF in the coronary artery that mediate angiogenesis and vessel rupture in atherosclerotic plaque [34]. Moreover, we show that elevated VEGF in macrophages or endothelial cells can activate, via p38α phosphorylation, both LITAF and STAT6B to further upregulate VEGF, angiogenesis, and atherosclerosis [35]. Hypoxia and inflammation trigger VEGFA's secretion in VMSCs and macrophages [9]. Here, we present that increased HIF1α and VEGF-A expression in CD163+ macrophages is associated with plaque progression [36], highlighting a nonlipid-driven mechanism of angiogenesis via the CD163/HIF1α/VEGF-A pathway.

On the contrary, we present preclinical and clinical data that support a correlation of reduced risk of atherosclerosis when VEGF levels are stable. Specifically, we present clinical data [37,38] showing that circulating VEGF (VEGF-A) levels are only marginally associated with a considerable risk of atherosclerosis. In addition, we present *in*

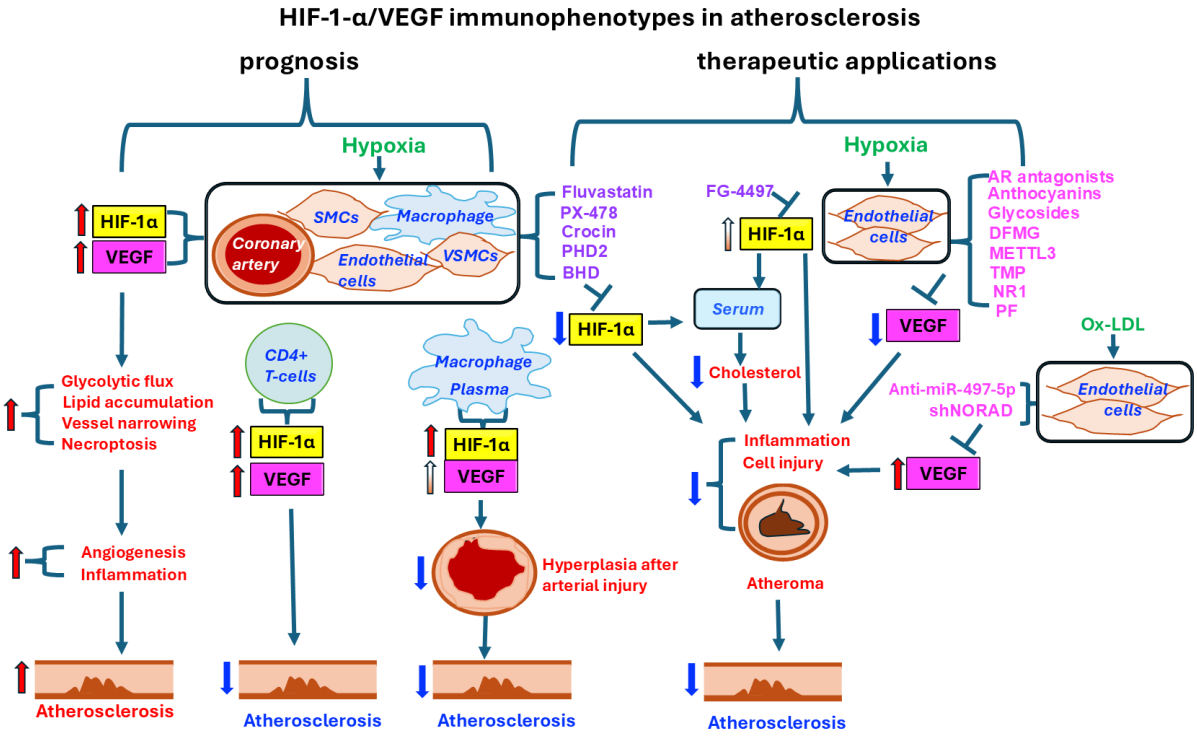

Fig. 6. The Role of HIF-1α and VEGF as Biomarkers in the Prognosis and Evaluation of Treatment Efficacy of Atherosclerosis. Prognosis: Hypoxic-induced increased HIF-1α and VEGF immunophenotypes in vascular smooth muscle cells (VSMCs), macrophages, or endothelial cells are associated with glycolytic flux, lipid accumulation, vessel narrowing, and necroptosis, inducing increased inflammation and angiogenesis, leading to atherosclerosis progression. However, increased levels of HIF-1α and VEGF found in lymphocytes (CD4+ spleen-derived T-cells) are associated with attenuated atherosclerotic lesions. Similarly, increased levels of HIF-1α with stable VEGF overexpression in macrophages or plasma contribute to the attenuation of intimal hyperplasia after arterial injury and a favorable prognosis for atherosclerosis. Targeted therapy: Targeting HIF-1α using PX-478, fluvastatin, a prolyl hydroxylase domain-containing protein 2 (PHD2), Buyang Huanwu decoction (BHD), or Crocin can suppress hypoxia-induced HIF-1α immunophenotype in coronary artery cells, endothelial cells, or macrophages or reduce cholesterol levels in serum, preventing atherosclerosis. Similarly, the application of HIF-prolyl 4-hydroxylases (HIF-P4Hs) inhibitor, FG-4497, can stabilize HIF-1α expression and reduce cholesterol in serum, protecting against the development of atherosclerosis. In parallel, targeting VEGF using adenosine receptors (AR), polyphenols (anthocyanins), notoginsenoside R1 (NR1), 7-difluoromethoxy-5,4′-dimethoxygenistein (DFMG), glycosides, tetramethylpyrazine (TMP), and paeoniflorin (PF) or methylated regulation (METTL3) in endothelial cells can regulate hypoxic-related atherosclerotic progression by decreasing the levels of inflammation, reducing atheromatique plaque pathology, or alleviating vascular endothelial cell injury. However, the inhibition of lncRNA *NORAD* can increase VEGF immunophenotypes in endothelial cells, alleviating ox-LDL-induced vascular endothelial cell injury and atherosclerosis development. Similarly, inhibition of miR-497-5p can alleviate ox-LDL-induced downregulation of VEGFA and dysfunction of endothelial cells, contributing to atherosclerosis prevention.

vitro data showing that stable overexpression of VEGF correlates with reduced macrophage foam cell formation and down-regulation of CD36 expression in macrophages, attenuating the progression of atherosclerosis [39]. Similarly, we present *in vitro* and *in vivo* data that stable overexpression of VEGF can promote the expression of anti-apoptotic proteins and produce high NO levels, contributing to the attenuation of intimal hyperplasia after arterial injury [40]. However, the association of stable levels of VEGF in blood with a higher or lower risk of atherosclerosis needs further clarification. Moreover, based on data from animal models and humans treated for cancer, anti-angiogenic factors, including blocking VEGF-A and the signaling downstream from their receptors, may reduce the formation of atheromatic plaques [68,69].

A mechanism by which HIF-1α and VEGF interact under hypoxia and their association with angiogenesis have been previously proposed [64]. This mechanism may include oxidative stress through heat shock protein 27 (HSP27), tissue trauma, and inflammation through activator protein 1 (AP-1), caveolin-1 (Cav-1), or nitric oxide synthase (NOS). VEGF-A bindings to VEGFR2 may lead to downstream proangiogenic cascades [52]. In addition, cross-activation of the VEGF receptors by multiple

VEGF family members may lead to variable effects [70]. Another mechanism is the promotion of the production of VEGF by HIF-1 connection to the HRE in the VEGF promoter region. VEGFR-1 and VEGFR-2 are the two main receptors expressed on endothelial cells, mediated by HIF-1. Under hypoxia, HIF upregulates VEGFR-1 directly by binding to the enhancer element in the *VEGFR-1* promoter, while *VEGFR-2* is post-transcriptional upregulated [41,71]. Based on a molecular mechanism proposed by Chen *et al.* [72], HIF-1α and VEGF interaction may lead to negative regulation of atherosclerosis. This mechanism shows silent information regulator 1 (SIRT1) to negatively regulate atherosclerotic angiogenesis via mammalian target of rapamycin 1 (mTORC1) and HIF-1α signaling that may result in a decreasing VEGF secretion in atherosclerotic plaques [72]. Also, a computational mechanistic model created by Zhao & Popel [73] may shed some light on the molecular control of the HIF-1/VEGF pathway. Based on this model, under hypoxia, members of the let-7 miR family are identified as hypoxia-responsive miRNAs (HRMs) whose levels are robustly upregulated by HIF-1. Mature miR-7 targets argonaute family 1 (*AGO1*) mRNA, essential for miRNA function. This leads to the downregulation of *AGO1* and other miRNAs that target *VEGF*, thus freeing *VEGF* from translational repression to promote angiogenesis [73].

The above observations may explain our data summarized in Fig. 6, showing that increased HIF-1α and VEGF immunophenotypes may have a different prognosis in atherosclerosis depending on the cellular environment and conditions, as well as on the VEGF subtype analyzed due to complex interactions between VEGFs and VEGFRs. Based on our data, VEGF inhibition can prevent hypoxia-associated atherosclerosis through the negative regulation of the Ang1-Tie2/PI3K-AKT or TLR4/NF-κB/VEGF signaling pathway, the latter using small regulatory molecules such as miR-140 (Fig. 5).

How HIF-1α and VEGF are correlated under different cellular environments and pathological conditions, such as inflammation and tumor angiogenesis, and how they correlate with factors that may influence their different pathological effects on atherosclerosis have been previously discussed [9,64,68,74]. Under hypoxic conditions, HIF-1α activates the secretion of VEGF and other transcriptional factors [66], which promotes angiogenesis, a higher blood and O_2 supply, and cell adaptation to hypoxia [9,11]. HIF-1α and VEGF-A are both increased in the early phase of ischemia or infarction; however, persistent oxygen deficiency may hinder the production of angiogenic factors due to widespread cell deaths [75]. Moreover, dysregulation of vascular homeostasis in atherogenesis during aging may include VEGF downregulation, which is strongly correlated with the decreased levels of P-STAT3 (phospho-STAT3) and P-CREB (phospho-cAMP response element-binding protein) in association with HIF-1α [65,66]. In addition, the upregulation of HIF-1α and VEGF has been associated with cytokine networks and apoptosis [76]. Based on a proposed mechanism, caspase 1/interleukin 1-β may interact with HIF-1α and VEGF during retinopathy damage to the blood vessels [76]. Specifically, hypoxia-induced activation of HIF-1α stimulates VEGF production, which leads to angiogenesis, and vasculogenesis may, in parallel, lead to caspase-1 and an acute inflammatory response. Caspase-1 is a cysteine protease that responds to apoptotic stimuli and can cleave precursors to inflammatory cytokines into active forms. This results in activating and releasing pro-inflammatory molecules, such as IL-1β, and activation of programmed cell death. Blocking the caspase-1/IL-1β signaling cascade can inverse this process. Although it is not clear yet how the presence of VEGF-A may affect caspase 1, IL-1β, and HIF-1α signaling within retinal capillaries, it is suggested that HIF-1α and VEGF may affect retinal capillaries.

In addition, HIF-1α and VEGF have also been reported to play a significant role in tumor angiogenesis [77]. More specifically, when cancer cells proliferate, forming a solid tumor, there is an imbalance between oxygen supply and oxygen demand. Therefore, newly activated HIF-1α is not ubiquitinated and is targeted to proteasomal degradation. Activated HIF-1α upregulates the expression of various proangiogenic genes, such as *VEGF*, and their receptors, such as *VEGFR1*, also known as *Flt-1* or *Flk-1*, as well as *Ang1*, *Ang2*, and *Tie2*. VEGF is considered a primary effector of tumor angiogenesis via "angiogenic switching, in which tumor cells stimulate tumor progression through angiogenesis induction by supplying oxygen and nutrients through the newly created capillaries. A hypoxia-independent stimulation of HIF-1α in solid tumors through antioncogenes' alterations has also been described [77]. Similar findings are also seen in studies testing anti-HIF agents in anti-angiogenesis therapy in carcinoma [78–80].

The potential therapeutic implications of targeting HIF-1α for atherosclerosis have been previously discussed [41]. Here, we show that HIF-1α immunophenotypes are reduced after PX-478 application, which is an inhibitor of HIF-1α, resulting in reduced cholesterol levels in plasma and reduced atheroma, directly affecting the development of atherosclerosis [43]. Furthermore, we present that CNP endogenous peptide promotes HIF-1α degradation, enhancing plaque stability, while LCZ696, a drug, can amplify the bioactivity of CNP and ameliorate atherosclerotic plaque formation [44]. In addition, we show that HIF-1α expression is reduced in the coronary artery wall by statins [45]. Specifically, we found that HIF-1α expression and its dependent ET-1 expression are reduced in VSMCs by fluvastatin application, resulting in the attenuation of atherosclerosis [45]. On the other hand, we present that HIF-P4As inhibition can stabilize HIF-1α expression, reducing serum cholesterol and inflammation and protecting against the development of atherosclerosis [46]. Fi-

nally, we show that natural products, such as BHD, a classic herbal formula of traditional Chinese medicine, or crocin, can inhibit HIF-1α expression in aortic tissue to prevent atherosclerosis [47,48].

Due to the direct relation of HIF-1α with VEGF, it has been previously discussed that the application of angiogenesis inhibitors may improve plaque stability and reduce plaque progression. Thus, several drugs targeting VEGF are being processed in the preclinical and clinical stages; however, anti-angiogenic therapy is still debatable because of the side effects on vasculature [8,68]. About VEGF immunophenotypes, we show that VEGF is reduced in endothelial cells through the application of polyphenols, methylation modification, antagonists of adenosine receptors, or natural products [49–55]. Specifically, we show that VEGF and IL-8 expression is reduced by adenosine receptor antagonists blocking important steps in adenosine-mediated atherosclerotic plaque development [49]. In addition, we show that reduced VEGF levels are induced by polyphenols, such as anthocyanins, resulting in restriction of angiogenesis, glycosides, and atherosclerotic progression [50]. Similarly, we present that VEGF is reduced in endothelial cells after *METTL3* knockdown, preventing atherosclerosis [51]. Moreover, we present natural products with anti-atherosclerotic effects, such as the combination of TMP and PF, and NR1, an herbal monomer isolated from *Panax notoginseng (Burk.) F. H. Chen*, can reduce the expression of VEGF levels in arterial endothelium [52–54]. Specifically, we show that the downregulation of protein expression of VEGF and angiogenesis-related factors, including Notch1, in TMP and PF-treated cells contributes to the increase of plaque stability [52]. T*riptolide* (*TPL*) has also previously been discussed by others as another natural compound that mediates the downregulation of eNOS, VEGF, and VEGFR2 and could limit atherosclerotic plaque enlargement [9].

Here, we present that NR1 can induce a reduced VEGF immunophenotype, decrease the levels of inflammatory factors [53], and induce a noticeable reduction in plaque pathology by suppressing the Ang1-Tie2/PI3K-AKT paracrine signaling pathway [54]. We also show that DFMG, a new chemical entity synthesized with genistein, a natural phytoestrogen, can inhibit VEGF protein levels, mediated by miR-140 negative regulation of the TLR4/NF-κB signaling pathway, showing anti-atherosclerotic effects [56]. Furthermore, we present that the expression of HIF-1α and VEGF can be abolished in cells treated with a combination of glycosides through STAT3 inhibition, preventing atherosclerosis [55]. On the other hand, we show that the inhibition of lncRNA *NORAD* can reverse VEGF-reduced immunophenotype, alleviating vascular endothelial cell injury and atherosclerosis development [57]. Finally, we show that miRNAs may play a role in therapeutic approaches for atherosclerosis. Specifically, we present that inhibition of miR-497-5p can attenuate ox-LDL-induced downregulation of VEGFA and dysfunction of endothelial cells through the p38/MAPK pathway [58].

In addition to the data presented here, earlier studies also discussed the effect of VEGF on specific anti-VEGF drugs, such as monoclonal antibodies against the VEGF signaling pathway (VSP), VEGF-modified macrophages, or soluble forms of VEGF receptors, to treat atherosclerosis. VEGF-modified macrophages seem to decrease lipid accumulation, which reduces cell foam formation [9,39]. Also, soluble forms of VEGF receptors have been previously discussed to improve the effects on atherosclerotic plaques by binding to VEGF and regulating its concentration in the plaque area [64]. Furthermore, VSP monoclonal antibodies can inhibit VEGF actions, influencing the survival and proliferation of endothelial cells and VSMCs [9]. However, they are also connected with cardiotoxic side effects [9]. Finally, melatonin has been previously discussed as a therapeutic target in atherosclerosis through VEGF [63]. Specifically, a recent study showed that melatonin can significantly and dose-dependently inhibit VEGF-induced angiogenesis in endothelial cells as well as attenuate smoking-induced atherosclerosis by activating the nuclear factor erythroid 2-related factor 2 (*NRF2*) pathway in endothelial cells [68].

5. Conclusion

In conclusion, hypoxia is involved in atherosclerosis, and the role of HIF-1α and its induced VEGF as potential biomarkers in the prognosis and evaluation of treatment efficacy of atherosclerosis has been thoroughly studied with experimental and clinical data. Increased levels of HIF-1α and VEGF in coronary artery cells or macrophages are associated with proinflammatory cell production, leading to atherosclerosis via increased glycolytic flux, lipid accumulation, vessel narrowing, necroptosis, and atheromatic plaque break. Chronic loss of NO or specific miRNAs can also regulate this process. However, overexpression of HIF-1α in lymphocytes or overexpression of VEGF in macrophages is associated with reduced foam formation and hyperplasia after arterial injury, attenuating atherosclerosis development or progression. In parallel, HIF-1α or VEGF immunophenotypes are altered in coronary artery endothelial cells or macrophages after the application of atherosclerosis-targeted therapy by using HIF-1α inhibitors, HIF-P4H inhibitors, statins, polyphenols, lnRNAs, methylation modification, adenosine receptor antagonists, natural products, or miRNA blockers, supporting the disruption of the HIF-1α/VEGF pathway as an effective approach in atherosclerosis treatment.

Although the development of proper methods to accurately evaluate hypoxia in atherosclerotic lesions is still a challenge for the future, meta-analyses, including similar enough research studies, may reveal the clinical or methodological heterogeneity from various sources exploring the role of HIF-1α/VEGF as a biomarker for the risk of

atherosclerosis and treatment efficacy. However, a growing body of data supports the involvement of HIF-1α and its related VEGF in various atherosclerosis aspects. Further investigation in large clinicopathological experimental models and validation of the predictive value of HIF-1α expression in atheromatous plaque regulation is warranted, which may contribute to novel personalized treatment strategies in clinical practice. It is expected that such large-scale clinical studies, including anti-HIF or anti-VEGF therapeutic strategies and similar methodological approaches for the qualification and quantification of HIF-1α and VEGF immunophenotypes, will provide evidence of HIF-1α and VEGF profiles as a reliable and valuable clinical tool for detecting, prognosis, and monitoring patients with atherosclerosis.

Abbreviations

HIF-1α, Hypoxia-inducible factor 1 alpha; VEGF, vascular endothelial growth factor.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and are available from the corresponding author upon reasonable request.

Author Contributions

Study conception and design: DPV, MI, KPM; literature search: DPV, PGD, AP, DG, MP, NL; data analysis: DPV; interpretation of data and results: DPV, PGD, AP, DG, MP, NL, KZ, SGD, KPM, MI; draft manuscript preparation: DPV, PGD, AP. All authors contributed to editorial changes in the manuscript. All authors reviewed the results and approved the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL27004.

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