Review

CXCL16 in Vascular Pathology Research: from Macro Effects to microRNAs

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Chemokines and their receptors have become significant factors in atherosclerosis research. CXCL16 is a multifunctional agent located on a separate locus to all other known chemokines and binds only to its "unique" receptor named CXCR6. As a scavenger receptor, adhesion molecule, and chemokine, it quickly became an interesting target in atherosclerosis research as all its functions have a role in vascular pathology. The investigation of the role of CXCL16 in atherosclerosis, although shown in *in vitro* studies, animal knockout models, and CXCL16 gene polymorphisms, haplotypes, and circulating levels, still shows puzzling results. Genetic and epigenetic studies have just scratched the surface of research necessary for a better assessment of the significance and perspective of this marker in plaque development and progression. In this review, we will summarize current knowledge about CXCL16 in atherosclerosis. Additionally, we will point out the importance of bioinformatics tools for the detection of potentially new CXCL16 regulatory networks through microRNA activity. This review aims to provide a better understanding of the underlying mechanisms, define more specific biomarkers, and discover new therapeutic targets.

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1. Introduction

Research in the field of atherosclerosis identified chemokines and their receptors as important mediators of atherosclerotic plaque formation. Some of them are markers of disease progression, so there is a tendency to make them candidates for therapeutic intervention in both the prevention of atherogenesis and treatment of atherosclerosis[1, 2]. Because CXC chemokine ligand 16 (CXCL16) is described as a scavenger receptor, adhesion molecule, and chemokine, it quickly became interesting target in atherosclerosis research as all its functions have a role in atherogenesis[3]. However, its role in atherosclerosis is not straightforward as assumed. Taking all the described functions of CXCL16 into consideration, we see a dual function of this molecule as it may be both proatherogenic and antiatherogenic depending on its role in different blood vessel compartments and the stage of atherosclerosis[4, 5].

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2. CXCL16

2-1. CXCL16: Unique Chemokine on Separate gene Locus

There are four subfamilies of chemokines described so far based on the arrangement of N-terminal cysteines. They include C, CC, CXC, and CX3C, where X represents an amino acid residue other than cysteine[6, 7]. CXCL16 is a multi-domain chemokine that exists in the transmembrane and in a soluble

form. The transmembrane form is a 30-kDa glycoprotein of 254 amino acids. It consists of an N-terminal signal sequence and a C-X-C motif chemokine domain, followed by a mucin stalk, hydrophobic transmembrane domain for binding and suspension, and cytoplasmic tail. The tail has a YXPV motif, which is a potential substrate for tyrosine kinase phosphorylation (**Fig. 1**). The stalk is highly glycosylated at the serine and threonine residues, which probably provides protection against proteolytic cleavage and folding[8-10]. This domain also participates in CXCL16-mediated cell adhesion[11]. The described type of organization is a novelty in the C-X-C chemokine subfamily as it is very similar to C-X3-C fractalkine/neuroactin[12, 13]. CXCL16 also contains six cysteine residues in the chemokine domain[9], which was a characteristic previously observed only in the CC chemokine family (**Fig. 1**)[14]. This chemokine is also unique as it is located on chromosome 17p13, a separate locus to all other known chemokines[9].

Transmembrane chemokines are synthesized as transmembrane molecules and are transported to the cell surface. The conversion of the transmembrane into its soluble form is performed by proteolytic cleavage[15, 16]. It was found that the disintegrin-like metalloproteinase ADAM-10 and TNFα-converting enzyme ADAM-17 cleave CXCL16 from the cell surface and release the soluble form[17-20]. While ADAM-10 mediates both constitutive and inducible cleavage[17, 18, 20], ADAM-17 seems to be involved only in the inducible shedding of CXCL16[21]. The position of the protease cleavage site in CXCL16 is not clearly determined, but it is probably located in the mucin stalk domain, above the cell membrane (**Fig. 2**)[9]. Signaling pathways that facilitate the increased cleavage of CXCL16 by ADAMs are still unknown[3].

Recent studies showed that cleavage is not the only way of forming the chemokine soluble form, as was previously thought. An alternatively spliced isoform of CXCL16 found to be expressed by murine dendritic cells and transfected HEK293 cells was termed the CXCL16v isoform. This isoform was not expressed on the cell membrane but was secreted as a 10 kDa protein, which had all proinflammatory abilities as the transmembrane form of CXCL16 (**Fig. 2**)[22].

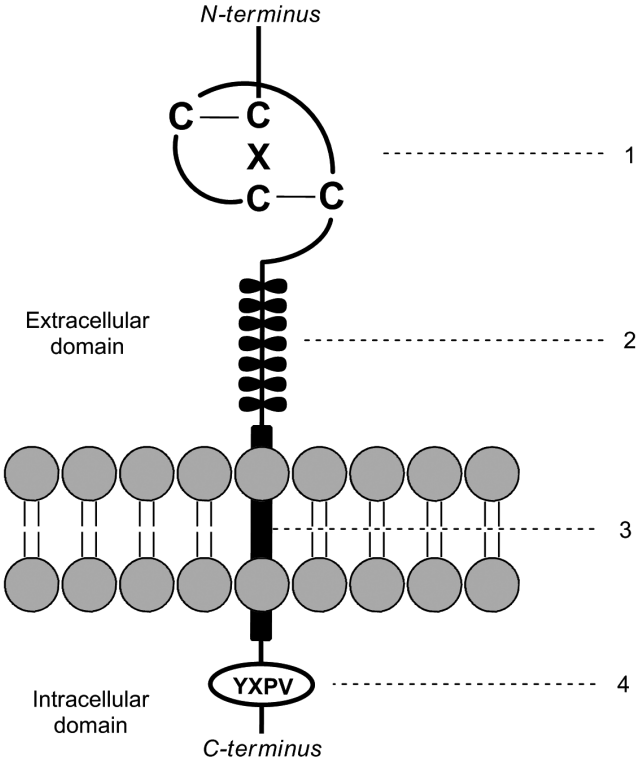

Fig. 1. Four domains of CXCL16 chemokine.

1) C-X-C motif chemokine domain with N-terminal signal sequence, 2) Mucin stalk, 3) Hydrophobic transmembrane domain, 4) Cytoplasmic tail with YXPV motif

2-2. CXCL16: Direct and Indirect Regulation

The expression of the CXCL16 chemokine was observed in various types of cells. Human monocyte-derived macrophages express CXCL16 on activation and in atherosclerotic lesions[10, 23-25]. CXCL16 expression at the mRNA level was also confirmed in B-cells[8], dendritic cells[11, 25], smooth muscle cells[18, 26, 27], T-cells[28], and endothelial cells[3, 18, 26].

Studies that investigated the regulation of CXCL16 gene expression by proinflammatory stimuli in endothelial smooth muscle cells, human mononuclear cells, and atherosclerotic lesions of ApoE$^{-/-}$ mice have shown that the expression of CXCL16 mRNA is induced by INFγ and TNFα, either separately or synergistically[8, 18, 24, 27, 29]. In ApoE$^{-/-}$ mice treated only with IL-18, which is known to be an inducer of INFγ, a similar effect occurred as when they were subjected to direct INFγ treatment. In this way, it was demonstrated that CXCL16 mRNA expression could be also indirectly triggered[30]. All these findings reflect the complexity of the CXCL16 regulation pathway, which could be modified on different levels of the CXCL16 gene regulatory network.

2-3. CXCL16: a Unique Molecule with "private" Receptor and Multiple Functions

The receptor for CXCL16 is a chemokine orphan, G protein-coupled receptor, first discovered as an HIV/SIV coreceptor, termed Bonzo/TYMSTR/

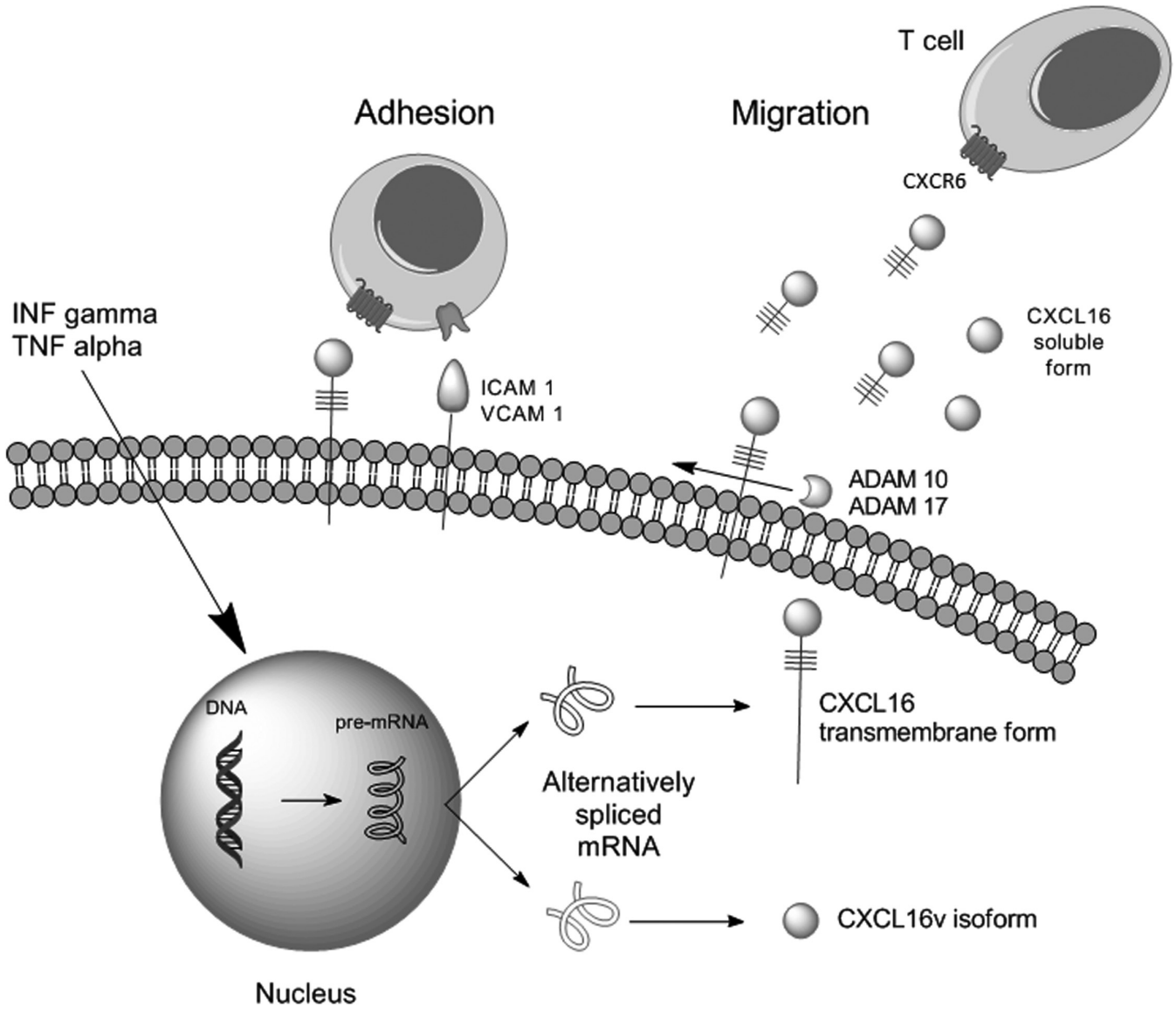

Fig. 2. The role of CXCL16 in T-cell migration and adhesion.

CXCL16 gene expression can be up-regulated by a number of inflammatory stimuli (e.g., IFN-γ, TNF-α). Two alternatively spliced mRNA variants of the CXCL16 gene code for different functional CXCL16 protein isoforms: the transmembrane CXCL16 and soluble CXCL16v isoform. The metalloproteolytic cleavage of the transmembrane form of CXCL16 with ADAM10 and ADAM17 produces the soluble form of this chemokine. The two soluble isoforms induce the migration of T cells through an interaction with CXCR6. The subsequent firm adhesion of migrating T cells to the vascular wall is achieved with the synergistic activity of the CXCL16 transmembrane form and ICAM1 and VCAM1.

STRL33.[31-33]. It was named as CXCR6 according to chemokine and receptor nomenclature[34]. The interaction between CXCL16 and CXCR6 is unique as it is shown that this chemokine does not bind to any other known chemokine receptor[8]. CXCR6 is expressed in smooth muscle cells[35], dendritic cells[36], B cells[29, 37], macrophages[38], natural killer T cells, bone marrow cells, CD4+ and CD8+ T cells[8, 9, 25, 37, 39-42], and platelets[43].

The soluble form of CXCL16 acts as a chemoattractant for nearly all cells that express its "private" receptor, at least *in vitro*[8, 9, 29, 44]. As this type of chemotactic guidance of inflammatory cells into the blood vessel intima depicts another critical step of the plaque evolution process[45], there was a presumption that the impaired levels of circulating CXCL16 could

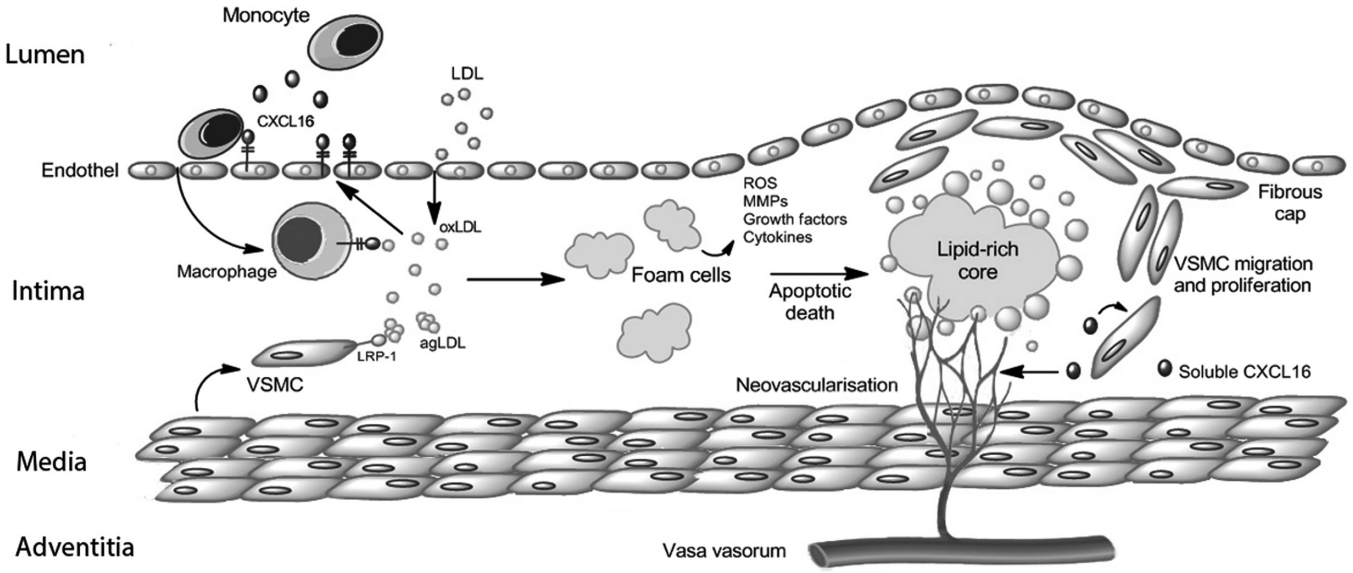

Fig. 3. Potential roles of CXCL16 in the development of atherosclerosis.

The transmembrane form of CXCL16 acts as a scavenger receptor in addition to its role in adhesion. The scavenging of oxLDL particles in the blood vessel intima is the main cause of macrophage to foam cell transformation and formation of an atherosclerotic lipid-rich core. Foam cells also produce different factors that could further destabilize atherosclerotic plaques, such as ROS, MMPs, growth factors, and cytokines. Soluble CXCL16 may direct monocyte migration, smooth muscle cell proliferation, and plaque angiogenesis, which all contribute toward plaque evolution and stability.

cause the development of atherosclerosis.

This chemokine also has functions that are beyond leukocyte recruitment.[10, 46]. The dysfunction of blood vessel endothelium leads to its impaired permeability and the infiltration of circulating LDL to vessel intima. Infiltrated LDL binds to extracellular matrix within the arterial wall and becomes modified in different ways, e.g., oxidized[47, 48]. Oxidized LDL (oxLDL) is the target of different scavenger receptors expressed on the macrophages in the arterial intima. The ingestion of oxLDL by macrophages appears to play a key role in foam cell transformation and subsequent atherosclerotic progression[5]. It was described that CXCL16 also has scavenging properties besides chemotaxis and adhesion and therefore, could promote foam cell formation[10]. These lipid-rich cells release cytokines, growth factors, matrix metalloproteinases (MMPs), reactive oxygen species (ROS), and tissue factors, which lead to inflammation, vascular remodeling, and plaque vulnerability[49].

It was shown that CXCL16 also has proangiogenic activity by inducing the proliferation of human umbilical vein endothelial cells (HUVECs)[50]. It is known that angiogenesis in the early stages of plaque development promotes stabilization as it feeds intimal cells[51, 52]. However, in the later stages of plaque development, these small, fragile blood vessels are susceptible to burst, making the environment of advanced plaques prone to destabilization (**Fig. 3**).

3. Role of CXCL16 in Atherosclerosis

3-1. CXCL16 and Atherosclerotic Plaque Formation: Atherogenesis vs. Atheroprotection

It has been shown that CXCL16 mRNA and protein expression are significantly upregulated in coronary and carotid atherosclerotic plaques than in healthy blood vessels[24]. The immunohistochemical staining of carotid plaques confirmed a higher expression of CXCL16 and CXCR6 in plaque regions rich in macrophages and T cells as was expected for a scavenger receptor[3, 23, 24]. It was reported that human and mice smooth muscle cells express CXCL16[27, 38], but this was not the case in rabbit arteries[53], which potentiates need and caution regarding a species-specific approach. The study conducted on rabbit lesion-prone sites of endothelium implies that CXCL16 is involved even in the initial stages of atherosclerosis[53] and not only in advanced plaques. This abundance of CXCL16 in atherosclerotic lesions observed in different cell types and in different stages of plaque development was a good reason to investigate the role of CXCL16 in the pathogenesis of atherosclerotic plaques. Although a lot of studies focused on its detrimental effects, not

all described CXCL16 as strictly proatherogenic factor.

In animal models for atherosclerosis, it was shown that the deletion of CXCL16 and CXCR6 demonstrates different effects. CXCL16 knockout mice developed larger plaques in both the aortic arc and root compared with atherosclerotic mice without CXCL16 disruption. The authors proposed that the reason for increased lesion size could be the affected clearance of apoptotic cells by CXCL16, which is in line with its possible atheroprotective role[54]. Earlier, the general opinion was that scavenger receptors are highly atherogenic by producing massive cholesterol depositions in the blood vessel intima[23, 55]. Today, it is known that scavenger function also regulates apoptotic cell clearance, thus maintaining vascular wall homeostasis[56]. However, further *in vitro* experiments showed that macrophages from CXCL16-deficient mice internalize apoptotic thymocytes equally well compared with those in control mice, which brought into question the proposed atheroprotective mechanism. The authors suggested the role of CXCL16 in oxLDL scavenging as an additional mechanism for the observed atheroprotective role. They revealed that macrophages originating from CXCL16-deficient mice exhibit a significant decrease in the internalization of oxLDL[54]. In contrast, another *in vitro* study demonstrated that oxLDL uptake mediated by CXCL16 plays a critical role in foam cell formation, which makes it proatherogenic[57]. It is clear that these different findings impose the need for more functional and *in vivo* studies to elucidate if there is a dual role of CXCL16 in atherosclerosis.

Data regarding the role of CXCR6 in atherosclerosis mainly propose its proatherogenic effect, but again, the mechanism of its action is not clearly defined. ApoE$^{-/-}$ CXCR6$^{-/+}$ mice did not express any significant pathological difference in the aortic arch compared with control animals[38], where CXCR6-deficient homozygotes showed less accumulation of certain lymphocytes and macrophages into the aorta's wall[38]. These findings suggest that CXCR6 has a proatherogenic influence, but the paradox remains, as the earlier mentioned CXCL16-deficient mice also had less CXCR6 positive cells in lesions, but larger plaques[54].

The reason for the contradiction of the results could be explained in different ways. The multiple functions of CXCL16, such as adhesion, chemotaxis and scavenging, participate in both proatherogenic and atheroprotective processes and have different relative contribution to atherogenesis. Therefore, in different time courses of disease development and under different stimuli, certain functions could predominate and override others. On the other hand, species-specific differences in CXCL16 functioning have already been observed in different animal models. This is the reason why these data have to be evaluated and verified in further functional studies, and in different animal models and human clinical studies.

3-2. Potential Role of CXCL16 in Atherothrombosis

Under physiological conditions, platelet adhesion to the vascular wall followed by platelet activation is crucial for primary hemostasis. On the other hand, they could also be the processes underlying acute arterial thrombotic occlusion at the regions of atherosclerotic plaque rupture, which is a pathophysiological mechanism causing myocardial infarction and ischemic stroke. It is known that endothelial cells play a major role in vascular physiology, so abnormalities in their structure and function may contribute to thrombosis[58]. Therefore, the culture of HUVECs became one of the best *in vitro* models in vascular pathology research[50, 53, 59, 60]. A recent study has shown for the first time that CXCL16 increases platelet adhesion to the HUVEC monolayer under conditions of high arterial shear stress in *in vitro* flow chamber experiments[43]. Moreover, the authors verified this process *in vivo* after carotis ligation by intravital microscopy. They also found that CXCL16 triggers platelet activation and aggregation in response to low-dose ADP activation. Platelet adhesion, activation, and aggregation are major mechanisms underlying thrombotic artery occlusions. Therefore, the authors concluded that CXCL16 expression in atherosclerotic lesions and release from inflammatory cells could be an additional local proadhesive stimulus for circulating platelets that could lead to increased platelet adhesion at the sites of vascular injury. Thus, CXCL16 could play a decisive role in linking inflammatory vascular diseases and thrombosis[43].

4. CXCL16 gene Polymorphisms and microRNA

4-1. CXCL16 gene Polymorphisms and Circulating Levels as Potential Biomarkers in Atherosclerosis

Research of the CXCL16 gene variants in atherosclerotic vascular pathology (**Table 1**) has also provided ambiguous results. The most investigated single-nucleotide polymorphism (SNP) A181V (rs2277680) is located in the fourth exon of the CXCL16 gene. In this non-synonymous polymorphism, alanine is substituted with valine at codon 181 located in the mucin stalk. An earlier study showed that although there was no significant difference in allele frequency between

Table 1. CXCL16 SNPs associated with vascular pathology

SNP	Observed effects	Ref.
rs2277680 (A181V)	The V allele was independently associated with an increased mean percentage of stenosis in patients with MI and with a smaller minimal luminal diameter of coronary arteries before PTCA.	[61]
rs2277680 (A181V)	The V allele inhibits monocyte adhesion by CXCR6.	[65]
rs2277680 (A181V) and rs1050998 (I123T)	The T123V181 haplotype was significantly and independently associated with carotid atherosclerosis plaque occurrence than the more frequent I123A181. The T123V181 haplotype has the ability to significantly change the CXCL16−CXCR6 interaction compared with the wild type I123A181.	[66]
rs3744700 (T/G)	Homozygous individuals for the G allele had a significantly increased susceptibility to CAD than the T allele carriers. The GG genotype was associated with significantly higher CXCL16 plasma levels in both patients and controls.	[68]

Abbreviations: MI, myocardial infarction; CAD, coronary artery disease; PTCA, percutaneous transluminal coronary angioplasty

post MI patients and control group, the less frequent V allele is independently associated with an increased mean percentage of stenosis in the same group of patients. In an additional group of patients undergoing percutaneous transluminal coronary angioplasty (PTCA) with stent implantation, the same allele is associated with a smaller minimal luminal diameter of coronary arteries before PTCA[61]. It was suggested that this amino acid substitution changes the cleavage site and thus increases the amount of soluble CXCL16, which could stimulate the chemotaxis of T cells and the proliferation of smooth muscle cells, and contribute to atherosclerosis[61]. Later studies showed that the circulating levels of CXCL16 are better predictive markers for atherosclerosis and its end points (myocardial infarction, stroke, and mortality)[62, 63] than the A181V polymorphism[64]. Further, it was shown that the A181V polymorphism influences the functionality of CXCL16 in a way that the V allele inhibits monocyte adhesion; however, it has not been associated with susceptibility to human coronary heart disease[65]. Recently, we performed a preliminary study investigating the I123T (rs1050998) polymorphism (also located in the mucin stalk domain) and A181V polymorphism haplotypes in carotid atherosclerosis. Although CXCL16 mRNA expression in carotid plaques showed no significant difference according to haplotypes, the T123V181 haplotype was significantly and independently associated with carotid atherosclerosis plaque occurrence compared with the more frequent I123A181[66]. We also analyzed the soluble plasma levels of CXCL16 according to haplotypes, but only in controls. We found no significant difference in plasma CXCL16 levels between the T123V181 and I123A181 haplotypes. In addition, we performed bioinformatics analysis by the information-spectrum method[67] to investigate the potential effects of these haplotypes on CXCL16−CXCR6 interactions. We found that the same rare haplotype, T123V181, has the ability to significantly change the CXCL16−CXCR6 interaction than the wild-type I123A181[66]. This result should be functionally evaluated and confirmed, while the effects of these haplotypes on mRNA levels should be confirmed in a representative number of tissue specimens. The largest angiography-based case-control study comprising 1175 patients with coronary artery disease (CAD) and 850 controls was conducted in the Chinese Han population. Five SNPs were evaluated for their association with increased risk of CAD and CXCL16 plasma levels, including the previously mentioned rs2277680 (A181V)[68]. The only SNP that showed a significant association with CAD was the rs3744700 (T/G) located in a fourth intron of CXCL16, where the T allele, marked as ancestral, is substituted with the G allele. The G allele frequency was significantly higher in the patients with CAD and individuals homozygous for this allele had significantly increased susceptibility to CAD than the T allele carriers. The detailed *in silico* analysis of intron 4 led to a finding that this polymorphism affects the GATA-binding site and therefore, probably influences CXCL16 gene transcription[68]. The GG genotype was also associated with significantly higher plasma levels of CXCL16 than other genotypes[64, 68]. It is still debatable whether this SNP affects CXCL16 expression or splicing as it was shown that intronic

variations can influence gene splicing and could represent the functional markers of human diseases[68, 69]. At the end, the SNP rs3744700 could be a neutral marker that resides in linkage disequilibrium with another, rare, functional polymorphism in the CXCL16 gene or with a mutation of another neighboring gene or even locus located in a distant region of the genome[68]. As the discovery of the rs3744700 polymorphism effects are a novelty, it is necessary to validate these results in other populations due to environmental and ethnical differences.

Several studies that evaluated CXCL16 circulating levels as a potential biomarker of vascular pathology also provided inconsistent results. While two studies showed that the lower concentrations of CXCL16 could correlate with CAD and acute coronary syndrome[70, 71], one study claimed that patients with CAD had higher CXCL16 levels[72] and another one did not find any association of serum CXCL16 with CAD in patients with rheumatoid arthritis[73]. These conflicting findings necessitate additional clinical studies on larger study groups and in different populations. Although CXCL16 circulating levels were shown to be an independent predictor of long-term mortality and heart failure development in patients with acute coronary syndrome, the combination with additional independent biomarkers in a multimarker panel makes it even more informative[74]. CXCL16 may also be a promising biomarker for idiopathic pulmonary arterial hypertension as its plasma levels are significantly increased in patients than in healthy controls[75]. This could be due to right ventricular remodeling and inflammation in pulmonary circulation. It was described that myocardial CXCL16 expression is enhanced in experimental and clinical heart failure and that CXCL16 promotes the matrix remodeling of the ventricles by stimulating MMP activity in cardiomyocytes and fibroblasts *in vitro*[76]. The increased serum levels of CXCL16 also have the potential to become an independent biomarker for atherosclerotic ischemic stroke, particularly in large arteries[77].

4-2. CXCL16 and microRNA

MicroRNAs (miRs) are approximately 22 nucleotides long RNAs constituting a dominant class of small non-coding RNAs in most somatic tissues[78]. During the RNA silencing process, the base pairing of miRs with target mRNA guides the RNA-induced silencing complex (RISC), which subsequently recruits different factors promoting translational repression, mRNA deadenylation, and mRNA degradation[79]. The target sites of miRs are most often located in the 3′ untranslated region (UTR) of mRNA[80]. The most important domain of miRs for mRNA targeting is the miR seed region that spans from nucleotide 2 to 7 in its 5′ end, but it is also known that the rest of the mature miR sequence helps target recognition[78]. It was predicted that more than 60% of human protein-coding genes contain at least one conserved miR target site. Therefore, it is not surprising that the dysregulation of certain miRs often leads to the development of various human diseases. Cardiovascular diseases are among them. Because there are already thorough reviews that summarize current knowledge about miRs implicated in atherosclerosis susceptibility, development, and progression[81-88], we will focus on the part of atherosclerosis pathology where different miRs could potentially alter vascular physiology by the direct or indirect regulation of CXCL16 gene expression.

There is a lot of evidence that miRs play a very important role in atherosclerosis and lipoprotein metabolism through mechanisms that affect endothelial integrity, macrophage inflammatory response to atherogenic lipids, vascular smooth muscle cell proliferation, and cholesterol synthesis[89]. Recent findings describe a linear pathway induction of miR-221 by Staphylococcal nuclease domain-containing protein 1 and NFkB and the subsequent activation of CXCL16 as an angiogenic factor in hepatocellular carcinoma[90]. The authors assumed that there is a negative regulator of CXCL16 gene expression, which is a target of miR-221. They also suggested that the described downregulation of the tissue inhibitor of metalloproteinase-3 mediated by miR-221 increases the expression of ADAM10 and ADAM17[91] and thus, enhances the production of the angiogenic form of CXCL16, the soluble form[90]. If these results could be extrapolated to blood vessel pathology, the induction of CXCL16 by miR-221 could contribute, as previously described, to plaque stability or thrombotic prognosis via the formation of the vasa vasorum, for example. Although angiogenesis in the early stages of plaque formation has atheroprotective effects because it prevents the apoptosis of vascular smooth muscle cells and formation of lipid rich core, in advanced atherosclerosis, it could have a completely different effect. In advanced atherosclerosis, angiogenesis results in blood vessels becoming more fragile and susceptible to burst[92]. However, the simple prediction of miR-221 role in vascular pathology is not possible due to cell-specific functions. It was shown that although highly expressed in both vascular smooth muscle cells and endothelial cells, the function of miR-221 differs in these cells. In vascular smooth muscle cells, it has proliferative and migratory effects. On the other hand, it has antimigratory and antiproliferative effects in endothelial cells[93].

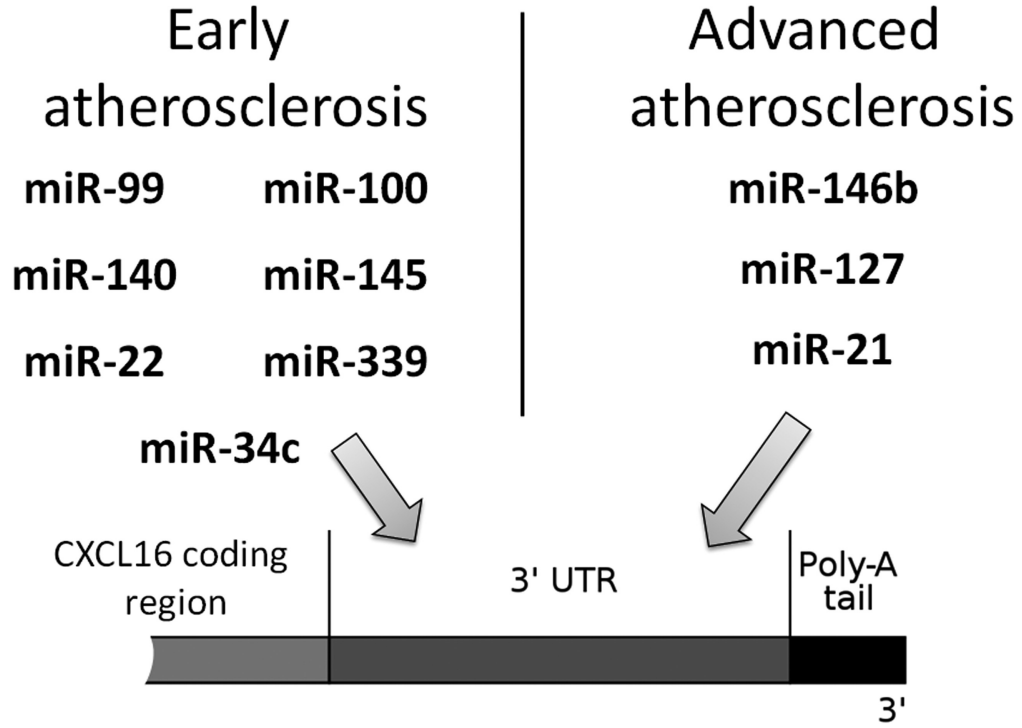

Fig. 4. miRNAs from co-inertia analysis with potential target sites in the 3′ UTR of CXCL16, according to ComiRNet.

miRs associated with early and advanced atherosclerotic plaque in the co-inertia analysis were filtered using the ComiRNet database of miR target predictions to extract those with potential target sites in the 3′ UTR of CXCL16.

Other findings of angiogenesis inhibition by miR-221 in HUVECs[60, 94, 95] could demonstrate how it is difficult to extrapolate data about miR-221 in different tissues. Recently, by an *in silico* analysis, we found miR-221 to have a role in the advanced stages of atherosclerosis[96].

Our recent study, which was based on the computational analysis of miRs associated with the early and advanced stages of atherosclerosis, revealed potentially characteristic miRs for these two stages. Some of them were already described in terms of atheroprotective or atherogenic function, but some were a novelty in atherosclerosis research[96]. Generally, knowledge about interactions between different miRs and CXCL16 in atherosclerosis or any other pathology is very scarce. Therefore, for the purpose of this review, we wanted to test if the most significant miRs from our study have potential target sites in the CXCL16 mRNA 3′ UTR. The impaired function of these post-transcriptional regulators could highly affect the temporal expression of CXCL16, and it was reported that the altered levels of this chemokine show different actions depending on the stage of the disease[53, 54, 97, 98].

Nowadays, multiple miR target prediction algorithms are developed based on different criteria such as target conservation, seed-target complementarity, seed pairing stability, free energy of duplex, etc.[99-106]. We decided to use the ComiRNet database of predicted miR regulatory network[107] to search for the potential targets of our miRs in the CXCL16 3′ UTR sequence. Using ComiRNet, we found that certain miRs that we previously associated with early atherosclerotic plaque (miR-99, miR-100, miR-140, miR-145, miR-22, miR-339, and miR-34c) and advanced plaque (miR-146b, miR-127, and miR-21)[96] have their target sites in the CXCL16 mRNA 3′ UTR sequence (**Fig. 4**). Although some of these miRs have already been described as atherosclerosis contributors in a positive or negative context,[96] none of them have undergone functional study for the CXCL16 interaction. Therefore, further research on the role of CXCL16 in atherosclerosis should take into account different miRs and their expression in the different stages of atherosclerosis.

According to crosslinking, ligation, and sequencing of hybrids (CLASH), it was discovered that two

miRs noncanonicaly target CXCL16 mRNA (miR-744 and miR-92a)[108]. miR-92a and miR-744 were already investigated in atherosclerosis, although miR-92a has far more evidence for its role in vascular pathology. It was discovered that miR-744 modulates the expression of TGF-β1, the factor for which aberrant expression is implicated in numerous pathological processes including atherosclerosis[109]. Its effect on CXCL16 gene expression in atherosclerosis is still not evaluated. miR-92a was described in atherosclerosis as a circulating biomarker for chronic cardiovascular diseases[110, 111] and coronary endothelial dysfunction[112]. During a screening study[113] in pursuit of miRs that have an impact on atherosclerosis, the authors searched for the so-called "atheromirs" *in vitro* using HUVECs. The search for "atheromirs" was based on two criteria that are crucial for the pathogenesis of atherosclerosis: (1) miRs that exhibit a change in expression by exposure to oxLDL under low shear stress and (2) miRs that are not affected by oxLDL exposure under high shear stress[113]. The most dysregulated miR was miR-92a, which has been confirmed to noncanonicaly bind CXCL16 mRNA[108, 113]. The *in vivo* validation in $LDLR^{-/-}$ mice confirmed that miR-92a was highly expressed in atherosclerosis prone regions in normocholesterolemic mice than in atherosclerosis resistant regions. This difference in miR-92a expression was further emphasized when the $LDLR^{-/-}$ mice were on a high-fat diet. The inhibition of miR-92a expression prevents endothelial activation and dysfunction *in vivo* and promotes the anti-inflammatory phenotype[113]. Another study showed that miR-92a inhibition improves re-endothelialization, which enhances functional recovery following vascular injury *in vivo*[114]. The described findings about miR-92a in atherosclerosis make it a potential therapeutic target in vascular pathology. The functional validation of the interaction between this miR and CXCL16 imply the existence of an interplay among these factors in atherogenesis. This conclusion opens the door for an additional aproach in the research of CXCL16 in atherosclerosis by taking into account the context of different miRs that are already solely described, besides the temporal and spatial context, as important factors in this chronic inflammatory disease.

5. Conclusions

Atherosclerosis is the main underlying process that could lead to endpoint events such as myocardial infarction and stroke. It is still the most common killer in the Western world as well as in low-income countries. Nowadays, we are already assured of the importance of chemokines in atherogenesis and the progression and destabilization of atherosclerotic plaques. The CXCL16 chemokine with its unique receptor expresses a high degree of complexity through its multiple functions, different forms, and not clearly defined nature during the course of the chronic inflammatory milieu of atherosclerosis. All heterogeneous data from association, knockout, and gene expression studies conducted on different blood vessel wall compartments, in different atherosclerotic phenotypes, and in different animal models require additional research. Further research should be carefully designed to take into account the temporal and spatial approach, species-specific differences in CXCL16 functions, cell specificity, and complex genetic and epigenetic networks. Thorough analysis of the CXCL16 gene, haplotype, and miRs could have the potential to reveal new disease pathways and new targets for therapeutic intervention. This kind of thorough research is a path toward identification of novel biomarkers as well as novel therapeutic targets in the future.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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