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REVIEW

FAK Family Kinases: A Potential Therapeutic Target for Atherosclerosis

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Abstract: Atherosclerosis (AS) is a chronic progressive inflammatory disease of the vascular wall and the primary pathological basis of cardiovascular and cerebrovascular disease. Focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2), two highly homologous members of the FAK family kinases, play critical roles in integrin signaling. They also serve as scaffolding proteins that contribute to the assembly of cellular signaling complexes that regulate cell survival, cell cycle progression, and cell motility. Research indicates that the FAK family kinases is involved in the gene regulation of vascular cells and that aberrant expression of this family is associated with pathological changes in vascular disease. These findings establish the FAK family kinases as a critical signaling mediator in atherosclerotic lesions and inhibition of its activity has the potential to attenuate the pathological progression of AS. This review highlights the indispensable role of the FAK family kinases in abnormal vascular smooth muscle cell proliferation, endothelial cell dysfunction, inflammation, and lipid metabolism associated with AS. We also summarize therapeutic targets against the FAK family kinases, providing valuable insights into therapeutic strategies for AS.

Keywords: FAK family kinases, atherosclerosis, inhibitors, cellular signaling

Introduction

Cardiovascular disease (CVD) has a significant impact on mortality and morbidity worldwide, with atherosclerosis (AS) being the primary pathological basis of CVD.[1] Atherosclerosis involves the development of fibrofatty lesions within the arterial wall and is characterized by arterial wall thickening, intimal lipid deposition, and luminal narrowing.[2] The risk factors contributing to the development of AS include hypertension, smoking, diabetes mellitus, hyperlipidemia, inflammation, aging, and genetic predisposition.[3] Advances in cellular and molecular biology have greatly contributed to our understanding of AS and offer new perspectives for clinical management. Endothelial cells (EC), inflammatory cells, and vascular smooth muscle cells (VSMC) play pivotal roles in AS progression, with pathogenesis revolving around endothelial dysfunction, abnormal lipid metabolism, inflammatory response, oxidative stress, and macrophage polarization.[4] Recent research has indicated that the focal adhesion kinase (FAK) family kinases and its signaling pathways play critical roles in these pathogenic mechanisms, suggesting that the FAK family kinases is a potential target for AS therapy. This review presents the primary advances in the last 20 years, starting with the discovery of FAK family kinases and ending with the idea of using FAK family kinases as a potential therapeutic target for AS (Table 1).

FAK and proline-rich tyrosine kinase 2 (Pyk2) are members of the FAK family kinases and are key protein tyrosine kinases involved in integrin signaling. FAK was discovered in 1992 and named after its prominent localization in focal adhesions.[5,16,17] FAK has been implicated in various biological processes, including embryonic development, and several diseases, including CVD and cancer, through its regulation of cell migration. In 1992, Pyk2 was independently isolated as a focal adhesion protein similar to FAK, which is typically distributed throughout the cytoplasm but is enriched in the perinuclear region.[18] Pyk2 regulates downstream signaling through protein phosphorylation and plays a role in cell survival, migration, epithelial-mesenchymal transition, and carcinogenesis.[19] Currently, there is no comprehensive review explaining the specific roles of the FAK family kinases in AS progression. This review aims to address this gap.

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Table 1 Advances in Understanding the Mechanism of FAK Family Kinases-Mediated Atherosclerosis

Study	Results	Ref.
Hanks et al, 1992	Chicken and mouse FAK(PTK2) cDNA is cloned.	[5]
Whitney et al, 1993	Human FAK cDNA is cloned.	[6]
Schaller et al, 1994	Y397 autophosphorylation site of FAK binds to SH2 domain of Src.	[7]
Rocic et al, 2001	PYK2 regulates VSMC protein synthesis.	[8]
Nowakowski et al, 2002	The structures of kinase domains for FAK are the first determined.	[9]
Ceccarelli et al, 2006	Two crystal structures of an NH2-terminal fragment of FAK containing the FERM domain.	[10]
Sayers et al, 2008	FRNK expression promotes SMCs maturation during vascular development and following vascular injury.	[11]
Son et al, 2014	Petunidin directly suppresses FAK activity attenuate aortic SMCs migration.	[12]
Yurdagul et al, 2016	Macrophage recruitment and VCAM-1 expression were decreased in FAK-KD (Cre+) mice.	[13]
Jeong et al, 2019	VS-4718 induced loss of FAK activity and increased nuclear FAK blocks neointimal hyperplasia.	[14]
Velatooru et al, 2021	FAK K152 SUMOylation plays a key role in endothelial activation and senescence.	[15]

This review summarizes the existing literature on the important role of the FAK family kinases in AS. Here, we review the structure, function, and activation pathways of the FAK family kinases. We then discuss the pathological involvement of the FAK family kinases in AS, focusing on four interrelated processes: VSMC proliferation, EC dysfunction, foam cell formation, and the development of inflammatory responses. In addition, we provide an overview of drugs that inhibit the FAK family of kinases.

Overview of the FAK Family Kinases

Structure of the FAK Family Kinases

FAK (gene symbol: PTK2) and Pyk2 (gene symbol: PTK2B) exhibit a similar structural composition, comprising three primary elements: a central kinase domain, an N-terminal FERM domain, and a C-terminal fragment domain that contains an adhesion-targeting sequence (Figure 1). The FERM domain comprises three distinct substructures (F1, F2, and F3).[19] The nuclear localization sequence within the F2 lobe of the FERM structural domain and the nuclear export sequence in the kinase structural domain play crucial roles in facilitating the shuttling of FAK and Pyk2 between the nucleus and cytoplasm of the cell.[20] The proline-rich FAT structural domain targets FAK to the adhesion complex, where both FAK and Pyk2 localize to integrin-containing sites by binding paxillin and talin, resulting in focal adhesion formation.[21] However, Pyk2 exhibits reduced adhesion localization because of its inability to bind talin. Further, the FAT region contains the tyrosine phosphorylation sites Y861 and Y925 that are phosphorylated by Src kinase. FAK and Pyk2 possess three proline-rich regions: one situated between the FERM and central kinase structural domains, and the other two located between the central kinase and FAT structural domains.

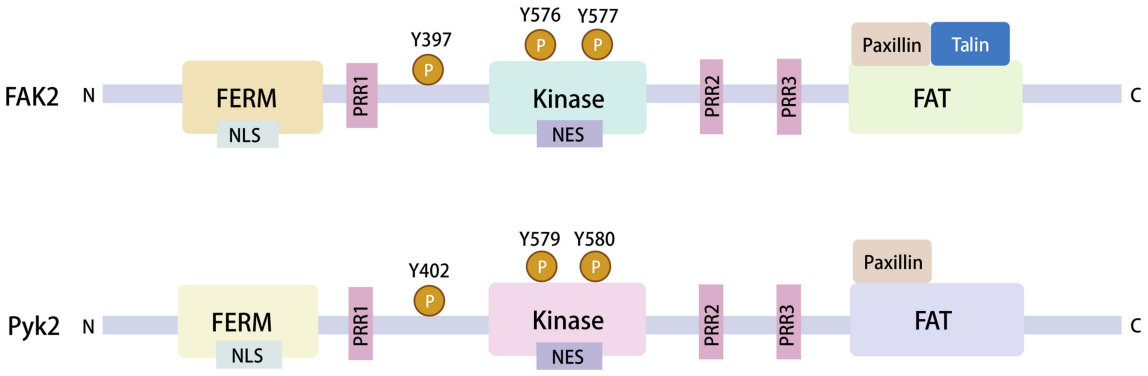

Figure 1 The structure of the FAK Kinase Family. TFAK and Pyk2 consist of three structural domains: the central kinase structural domain, the N-terminal FERM structural domain, and a C-terminal structural domain containing an adhesion targeting sequence. The central kinase structural domain is flanked by three proline-rich regions. The nuclear localization sequence (NLS) of the FERM structural domain and the nuclear export sequence (NES) of the kinase structural domain facilitate the shuttling of FAK and Pyk2 between the nucleus and the cytoplasm. Key sites for tyrosine phosphorylation (P) are marked in yellow.

Biochemical Mechanisms for FAK Family Kinases Activation

FAK activation involves key steps such as autophosphorylation of Tyr397 and site-specific dimerization. The recruitment of FAK to the adhesion foci is facilitated by either the FAT or FERM structural domains. This recruitment leads to the dissociation of the latter from the catalytic structural domain, relieving autoinhibition and initiating initial kinase activation. Subsequently, dimer formation occurs via FERM-FERM or FERM-FAT interactions,[22] allowing FAK autophosphorylation on Tyr397.[23] Tyr397, which is adjacent to the first proline-rich sequence, plays a significant role in mediating phosphorylation and triggering maximal activity of the FAK catalytic region. In addition, it serves as a docking site for highly active Src family kinases (SFKs).[24]

Further, FAK is activated through growth factor signaling-mediated cell adhesion. It directly interacts with the cytoplasmic portion of various growth factor receptors, such as Met proto-oncogene receptor tyrosine kinase (rearranged during transfection), epidermal growth factor receptor, and vascular endothelial growth factor (VEGF). Integrin signaling, on the other hand, can activate FAK through the plasma membrane lipid phosphatidylinositol 4.5-bisphosphate. The binding of phosphatidylinositol 4,5-bisphosphate to a crucial region of the regulatory FERM structural domain triggers FAK aggregation in the lipid bilayer that induces FAK autophosphorylation. Phosphorylation of Src in the FAK activation loop results in release of the FERM/kinase chain and full catalytic activation of FAK.[25]

Pyk2 activation does not rely solely on the recruitment of focal adhesions. The activation of Pyk2 is regulated by cytokines, growth factors, and, critically, by an elevation in cytoplasmic Ca^{2+} levels induced by various ligands, such as antidiuretic hormone and platelet-derived growth factor (PDGF).[26] Experimental evidence for the inhibitory interaction between the FERM domain and the structural domain of Pyk2 kinase remains unavailable,[27,28] although key tyrosine residues within Pyk2 may play a crucial role in SFK-mediated phosphorylation.

Biological Functions of FAK Family Kinases

FAK and Pyk2, members of the FAK family kinases, serve as downstream substrates for kinase phosphorylation, transmit cellular signals, and act as scaffolding proteins in the assembly of signaling complexes. Activation of the FAK family kinases is modulated by various stimuli and plays a crucial role in regulating processes such as cell survival, proliferation, and motility.[29] FAK modulates the actin cytoskeleton to regulate cell adhesion and directional motility. Similarly, Pyk2 contributes to the regulation of cell phenotype and modulates the cytoskeleton during cell attachment and spreading.[30] FAK is actively involved in the growth and development of the heart, kidneys, and nervous system. Pyk2 does not affect embryonic growth or development. However, it plays a vital role in the developmental regulation of the immune system in mice, with Pyk2 deficiency leading to defective humoral immune responses in marginal B-zone cells.[31] FAT of the Pyk2 structural domain is involved in regulating bone resorption, and its deficiency in mice contributes to AS development.[32]

The FAK Family Kinases are Involved in Atherosclerosis

FAK family kinases are involved in atherosclerotic disease progression through multiple signaling cascades (Figure 2) that we will address in detail in subsequent sections to provide a theoretical basis for the use of the FAK family of kinases for treating AS.

Regulation of FAK Family Kinases in VSMCs

VSMCs are located in the medial layer of the arterial wall and play a crucial role in regulating vascular tone, blood pressure, and blood flow.[33] While VSMCs residing in mature vessels have low proliferative and migratory activity, VSMCs may migrate from the medial to the intimal layer of the arterial wall in response to physical and inflammatory stimuli that cause damage to the vascular endothelium and undergo a phenotypic switch from a "contractile" to a highly "synthetic" phenotype. This phenotypic transition involves the proliferation and migration of VSMCs and production of extracellular matrix components.[34] Targeted regulation of VSMC proliferation and migration is instrumental in effectively mitigating AS. FAK plays a critical role in regulating VSMC proliferation and migration. Inhibiting FAK

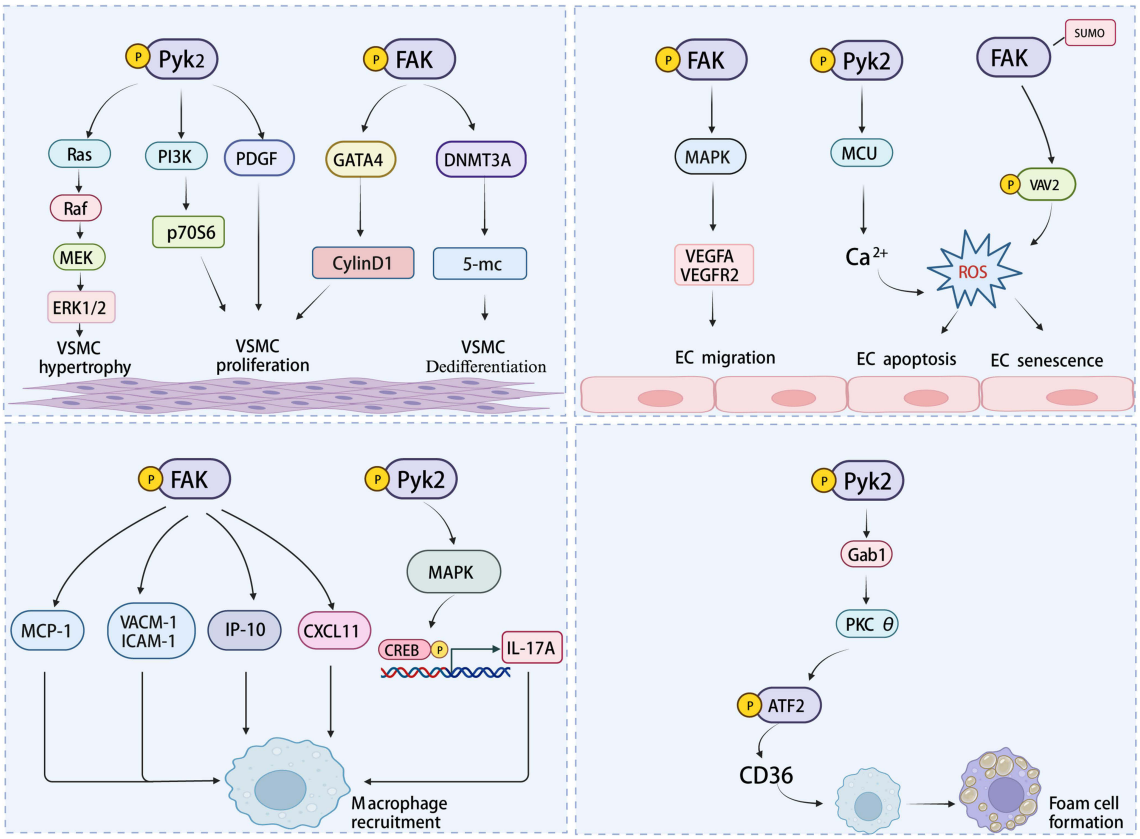

Figure 2 Potential role of FAK family kinases in atherosclerosis. FAK family kinases are involved in the pathogenesis of atherosclerosis by regulating VSMC phenotypic switching, EC dysfunction, foam cell formation, and inflammatory response mechanisms.

Abbreviations: Gab-1, grb2-associated binders; PKCθ, protein kinase Cθ; ATF2, activating transcription factor 2; MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cellular adhesion molecule-1; IP-10, induced protein 10; MAPK, mitogen-activated protein kinase; CREB, cyclic adenosine monophosphate response element–binding protein; IL-17, interleukin-17A; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated extracellular signal-regulated kinase; ERK1/2, extracellular signal-regulated kinases 1 and 2; PI3K, phosphatidylinositol 3-kinase; PDGF, platelet-derived growth factor; GATA4, GATA-binding protein 4; DNMT3A, DNA methyltransferases 3A; 5-mC, 5-methylcytosine; MAPK, mitogen-activated protein kinase; VEGFA, vascular endothelial growth factor A; VEGFR1, vascular endothelial growth factor R1; MCU, mitochondrial calcium uniporter; ROS, reactive oxygen species.

phosphorylation at the Tyr397 and Tyr925 sites and reducing Src phosphorylation (a downstream target of FAK) significantly attenuated VSMC migration and proliferation.[35,36]

Role of FAK Family Kinases in VSMC Proliferation

FAK interacts with nuclear transcription factors, including tumor suppressor P53, GATA-binding protein 4(GATA4), and TATA-box-binding protein-associated factor 9. Knockdown of GATA4 via shRNA attenuates endothelial hyperplasia induced by filamentous thread injury and promotes SMC proliferation by upregulating the transcription of the cell cycle protein D1[37] In healthy arteries, FAK is predominantly localized in the nuclei of smooth muscle cells. However, FAK is activated and redistributed to the cytoplasm after injury. Inhibition of FAK catalytic activity retains FAK in the nucleus, leading to reduced GATA4 protein expression in injured arteries. This inhibition also hampers GATA4-mediated transcription of the cell cycle protein D1, effectively blocking SMC proliferation and hyperplasia.[14]

Activation of FAK phosphorylation promotes cell-cell adhesion, cell-peripheral extracellular matrix adhesion, and cell proliferation, facilitating neointima formation. Deletion of Rac1, specifically in SMCs, leads to reduced cell proliferation and neointima formation following vascular injury. The Rho kinase family plays a role in focal adhesions and stress fiber formation, and inhibition of Rho kinase activity prevents neointima formation after vascular injury.[38] FAK governs the aforementioned cellular processes by activating the downstream small GTPases, Rac and Rho.

Pyk2 serves as an upstream regulator of multiple signaling pathways involved in Ang II–induced VSMC growth. The formation of the Pyk2-grb2/Src-shc-grb2 complex governs the ERK1/2 pathway by activating Ras. In addition,

Pyk2-dependent tyrosine phosphorylation, coupled with the interaction of p130 Cas, induces the activation of PI3K kinase.[39] By downregulating Pyk2 expression in Ang II–induced VSMCs, inhibition of both ERK1/2 and PI3K/Akt activation was achieved. Therefore, targeting Pyk2 phosphorylation is an essential molecular strategy for controlling VSMC growth.[8]

Role of FAK Family Kinases in VSMC Dedifferentiation

Under physiological conditions, VSMCs maintain a highly differentiated contractile resting state. However, in the presence of hypoxia and inflammation, VSMCs undergo dedifferentiation and transition from a contractile to a differentiated phenotype. This phenotypic switch is accompanied by reduced contractile capacity, enhanced proliferation and migration, and downregulated expression of SMC-specific contractile genes.[40] Inhibition of FAK leads to its nuclear translocation, resulting in reduced DNMT3A stability via ubiquitination and proteasomal degradation specificity. Consequently, this intervention reduces 5-mc levels, promotes SMC differentiation, and enhances the expression of SMC contractile genes.[41]

Role of FAK Family Kinases in VSMC Migration

Pyk2 downregulation hampers PDGF-induced AKT and ERK1/2 phosphorylation, inhibits cell cycle progression, and impedes VSMC proliferation.[42] Hepatocyte growth factor (HGF) within the plaques acts as a chemokine for VSMCs, thereby stimulating their migration. In VSMCs, HGF induces a time-dependent activation of FAK and Pyk2 phosphorylation, thereby facilitating VSMC migration.[43]

In conclusion, the FAK family kinases play an important role in regulating the phenotypic transition of VSMC during AS, making them a promising therapeutic target for AS.

Regulation of FAK Family Kinases in ECs

ECs are capable of producing vasodilators (such as NO, PG I2, and H_2S) as well as vasoconstrictor molecules, which contribute to the regulation of arterial structure and remodeling by ensuring a balance between vasodilators and vasoconstrictors, thereby sustaining vascular tone.[44,45] The microstructure of ECs, specifically the endothelial glycocalyx, plays a key role in maintaining their integrity.[46] Low endothelial permeability was observed under uniform flow conditions with high physiological shear stress in which the glycocalyx remained intact. Conversely, increased endothelial permeability has been observed in disturbed flow states with low physiological shear stress, leading to a reduction in the glycocalyx. Additionally, ECs exhibit metabolic activity and sustain their proliferation and vasodilatory functions through various metabolic pathways. Alterations in EC metabolism can induce EC proliferation and inflammation, thereby initiating AS.[47]

Role of FAK Family Kinases in EC Migration

Enhanced neovascularization within atherosclerotic plaques can worsen plaque instability. Overexpression of Sema7A in human umbilical vein ECs (HUVECs) markedly upregulates VEGFA/VEGFR2, stimulating EC migration and promoting neovascularization within the plaques. Mechanistic studies have shown that Sema7A increases FAK expression through a β1 integrin-dependent mechanism, thereby facilitating VEGFA/VEGFR2-mediated neovascularization. FAK inhibitors significantly inhibit EC migration and intraplaque neovascularization.[48]

Role of FAK Family Kinases in EC Senescence

The disturbed flow (d-flow) promotes ROS production, EC inflammation, and apoptosis, ultimately contributing to endothelial cell dysfunction.[49] d-Flow stimulates ROS production through NADPH oxidase activity. In addition, it activates p90RSK, which upregulates FAK K152 SUMOylation and subsequent phosphorylation of VaV2, promoting FAK phosphorylation. Introducing a mutation at the lysine 152 site of FAK sumoylation to replace it with arginine demonstrated a significant reduction in apoptosis and diminished levels of the cellular senescence marker β-galactosidase (SA-βgal) induced by d-flow. In addition, it mitigated EC activation and senescence.[15]

Role of FAK Family Kinases in EC Apoptosis

Increased EC apoptosis is evident in regions susceptible to AS, and this apoptotic process can further worsen AS progression. ECs form a lining on the interior surface of blood vessels and are vulnerable to apoptosis triggered by external environmental stimuli such as hypoxia, hyperoxia, oxidative stress, and inflammatory factors,[50] which subsequently aggravate AS progression.[51] The modulation of mitochondrial calcium uniporter (MCU) phosphorylation by Pyk2 participates in the regulation of mitochondrial Ca^{2+} uptake, ROS production, and EC apoptosis, thus contributing to the exacerbation of atherosclerotic disease progression. Pyk2/MCU protein expression is upregulated in a mouse model of AS. Similarly, increased levels of Pyk2/MCU were observed in a model of H_2O_2-induced HUVEC injury. Additionally, shRNA targeting Pyk2 effectively shielded ECs from H_2O_2-induced damage. Rosuvastatin exerted a protective effect in both the AS mouse model and the H_2O_2-induced HUVEC injury model by inhibiting the Pyk2/MCU pathway, reducing mitochondrial damage and ROS generation, and preventing EC apoptosis. In conclusion, targeting the Pyk2/MCU pathway may represent a novel approach to prevent AS progression.[52]

Taken together, these findings suggest that the FAK family kinases is capable of inducing EC dysfunction in response to a variety of stimuli and that inhibition of FAK activity reduces atherosclerotic lesions.

Involvement of the FAK Family Kinases in the Inflammatory Response to Atherosclerosis

AS is a progressive inflammatory disease of the blood vessel wall driven by innate immunity. During the early stages of AS, the inflammatory process begins with EC activation. These cells express inflammatory factors, including interleukin-8 (IL-8), ICAM-1, and monocyte chemoattractant protein-1 (MCP-1). During the advanced stages of AS, macrophages and other inflammatory cytokines infiltrate the vessel wall. They degrade collagen fibers within the extracellular matrix of plaques by secreting matrix metalloproteinases, resulting in plaque rupture and hemorrhage.[53] Consequently, modulation of inflammation mitigates the development of AS and its associated complications.[54]

The suppression of FAK or Pyk2 expression results in decreased TNF-α-induced activation of ERK and JNK and reduced expression of CAMs. In a carotid artery ligation model using ApoE-/- mice, activation of FAK/Pyk2 promoted the migration of macrophages to the vascular endothelium. Administration of dual inhibitors targeting FAK/Pyk2 resulted in decreased expression of inflammatory cell adhesion factors and reduced the recruitment of macrophages.[55]

FAK facilitates NF-kB activation, which leads to upregulation of VCAM-1 expression and promotes monocyte adhesion through the ERK-RSK-IKKβ pathway.[13] Inhibition of FAK activation in ECs exposed to oxidized low-density lipoprotein (ox-LDL) inhibits atherosclerosis by downregulating VCAM-1 and ICAM-1 expression and reducing monocyte adhesion.[56]

Cyclic adenosine monophosphate response element–binding protein (CREB) plays a vital role in orchestrating the inflammatory response and facilitating THP1 cell migration and endothelial cell adhesion. CREB activation depends on the essential function of Pyk2 activity. 15(S)-HETE induces THP1 cell migration and adhesion, thereby initiating the activation of the Pyk2 signaling pathway that ultimately results in CREB activation and the production of IL-17.[57]

Taken together, these findings suggest an important role for FAK family kinases in promoting AS and inflammation, and that inhibition of FAK activity reduces atherosclerotic lesions.

FAK Family Kinases are Involved in Lipid Accumulation in Atherosclerosis

Lipid uptake is facilitated by scavenger receptors such as CD36, lectin-like ox-LDL receptor-1 (LOX-1), and scavenger receptor A1.[58] Upon endothelial injury, LDL traverses the endothelial monolayer and undergoes modification, resulting in the formation of ox-LDL. Macrophages take up ox-LDL through scavenger receptor-mediated phagocytosis and cytophagocytosis at the cell membrane, leading to increased lipid accumulation and foam cell formation.[59] Strategies aimed at modulating lipid levels constitute mainstream therapies for AS, and targeting the reduction in foam cell formation by blocking lipid uptake holds promise for the treatment of this condition.

Prothrombin-induced foam cell formation was observed in peritoneal macrophages of ApoE-/- mice. Immunofluorescence staining of cross-sections of mouse aortic roots showed increased Pyk2 phosphorylation and

CD36 expression in ApoE-/- mice. In vitro studies showed that thrombin-induced CD36 expression in RAW264.7 cells, in a clock-dependent pattern resulted in foam cell formation. PYK2 activation plays a key role in thrombin-induced CD36 expression and foam cell formation. Thrombin promotes tyrosine phosphorylation of Pyk2 (Tyr402), and Pyk2 inhibition attenuates thrombin-induced activation of CD36 expression and foam cell generation.[60]

In conclusion, regulation of lipid metabolism by inhibition of FAK family kinase activity is also a potential therapeutic strategy for AS.

FAK Family Kinases Inhibitors Find Various Applications

Currently, the development of FAK inhibitors is a research hotspot in academia and pharmaceutical companies, with a primary focus on oncology, where small molecules inhibit FAK phosphorylation, block intracellular signaling in the cell membrane, and inhibit cancer cell proliferation. To date, two compounds, namely GSK2256098 (NCT02523014, NCT02428270) and Defactinib (NCT01951690) are in Phase II trials, while Conteltinib (NCT05580445) is in Phase IB/II trials; VS-4718 (NCT01849744), PF-562271(NCT00666926), IN10018 (NCT05327231), and APG-2449 (NCT03917043) are in Phase I trials. Many inhibitors are being investigated in preclinical studies, such as BJG-03-025, PF-573228, and Y15.[61–63] Although clinical trials on the association between FAK inhibitors and AS have not been widely conducted, the relevant basic research provides corresponding evidence for this. We reason that drug research targeting the regulation of FAK for the treatment of AS and cardiovascular diseases will progress significantly.

Pf-562271

PF-562271 is a dual inhibitor of FAK/Pyk2 that reduces VCAM-1 and ICAM-1 expression, monocyte migration, and macrophage recruitment in ApoE-/- mice and HAOEC ECs. The FAK/Pyk2 inhibitor, PF-562271, has anti-inflammatory properties and shows promise as a potential therapeutic agent for atherosclerotic disease.[55]

Vs-4718

GATA4 plays a role in SMC differentiation and vascular endothelial hyperplasia. The FAK inhibitor VS-4718 is currently in clinical development as a cancer therapy. In the femoral arterial wire injury model, VS-4718 induced a loss of FAK activity and increased nuclear FAK accumulation, thereby blocking neointimal hyperplasia via reduced GATA4 and cyclin D1 expression. These research findings suggest that VS-4718 may also be evaluated as a potential therapy for vessel wall-narrowing diseases.[14]

Acarbose

In a rabbit AS model, acarbose dose-dependently inhibited FAK phosphorylation, downregulated the PI3K/Akt signaling pathway, inhibited aberrant proliferation and migration of VSMCs, and attenuated the extent of aortic AS lesions.[64]

Isatinidilol

The novel third-generation β-adrenergic receptor blocker, isatinidilol, demonstrated a dose-dependent inhibition of PDGF-induced proliferation in ECs. It also exhibited inhibitory effects on intimal hyperplasia and VSMC proliferation and migration in a rat carotid artery balloon injury model. Mechanistic investigations revealed that isatinidilol hindered Pyk2 phosphorylation in a concentration-dependent manner, reduced the coupling of PKC-ERK1/2 to Pyk2, reduced the intracellular calcium ion concentration, and attenuated intimal thickening by suppressing VSMC proliferation.[65]

Pelargonidin

Pelargonidin is a novel FAK inhibitor that has demonstrated a dose-dependent inhibition of PDGF-BB-induced cell proliferation in HASMCs. Mechanistic studies have revealed that pelargonidin directly targets FAK and binds to it in an adenosine triphosphate-competitive manner. This binding inhibits the phosphorylation of FAK at the Tyr397, Tyr576, and Tyr577 sites induced by PDGF-BB. Consequently, pelargonidin effectively inhibits FAK activity and shows potential preventive effects against AS.[66] Similarly, petunidin, another anthocyanin, has a preventive effect on AS. In vivo studies showed that petunidin inhibited neointima formation induced by carotid balloon injury. In vitro studies showed its inhibitory effect on PDGF-BB-induced FAK phosphorylation and abnormal migration of HASMCs.[12]

Alginate Oligosaccharides

Alginate oligosaccharides (AOS) are polysaccharide polymers extracted from seaweed that inhibit the FAK/PI3K signaling pathway and protect HUVECs from H_2O_2-induced oxidative stress damage, providing a new alternative strategy for the prevention of AS.[67]

Ginsenoside Rg3

Ginsenoside Rg3 effectively inhibited AS plaque formation and improved lipoprotein abnormalities in ApoE-/- mice. In vivo studies revealed that ginsenoside Rg3 may protect ECs and inhibit AS by up-regulating PPARγ via repressing FAK-mediated pathways, thus inhibiting the expression of VCAM-1 and ICAM-1 in intima, protect ECs, and inhibit AS.[56]

Rubiarbonone C

Rubiarbonone C, the primary active ingredient of Lobelia philippinensis, belongs to the triterpenoid class. Recent studies have demonstrated that rubiarbonone C inhibits VSMC migration by suppressing FAK activation and reducing F-actin reorganization. Thus, rubiarbonone C shows great potential as a therapeutic candidate for the treatment of AS.[68]

Mulberry Aqueous Extracts

Mulberry aqueous extracts contain polyphenolic compounds that have been shown to possess anti-hyperglycemic and lipid-modulating effects that help in the prevention of cardiovascular disease. The aqueous extract of mulberry was found to reduce the interaction between integrin-β3 and FAK, inhibit VSMC migration, and prevent the development of high-cholesterol diet-induced AS in a rabbit model.[69]

1-Deoxynojirimycin

1-Deoxynojirimycin (DNJ) is the active ingredient found in Morus alba that effectively inhibits lipid accumulation. In A7r5 cells cultured under a diabetic hyperglycemic condition, DNJ was found to reduce FAK phosphorylation and VSMC migration in a dose-dependent manner. DNJ was also found to inhibit glucose-stimulated migration of VSMCs by activating the AMPK/RhoB pathway and suppressing FAK pleiotropy.[70]

In summary, current researchers have conducted a large number of studies on FAK inhibitors, but there are still gaps and shortcomings. First, the dose-response relationship of FAK inhibitors has yet to be explored, and some studies have shown that FAK inhibitors can promote angiogenesis and antagonize their own antitumor effects at low concentrations.[71] Second, single-target FAK inhibitor therapy suffers from insufficient efficacy because the disease is associated with multiple abnormal mechanisms. In future, researchers should focus on multi-target drug development, such as FAK/Pyk2, FAK/IGF-1 receptor, FAK/EGF receptor, and FAK/CDK4/6 pathways. The application of computer-aided drug design, pharmacophore modeling, and molecular docking approaches to develop multi-target FAK inhibitors are other new directions in drug discovery. Finally, future studies still need to conduct in-depth research on regulatory mechanisms of the FAK family of kinases to assist in the development of novel and more selective inhibitors.

Discussion

The FAK family of kinases plays a crucial role in regulating many aspects of normal cellular behavior and has emerged as a promising therapeutic target for a wide range of diseases. This review summarizes the progress of research on FAK family kinases in AS, where activation of FAK phosphorylation has been found to exacerbate the progression of AS to various pathogenetic outcomes. However, there are some limitations to the current review. First, most animal models employed in current basic research only involve the early developmental stages of AS, and it is not clear whether FAK can regulate AS after plaque formation. There is also a lack of reports on the clinical application of FAK kinase inhibitors in AS, and the clinical efficacy of FAK kinase inhibitors needs to be verified by large-scale randomized controlled trials. Although the mechanism of action of FAK family kinases in AS has been extensively investigated, further studies are needed to address these open questions and overcome existing barriers. FAK is predominantly present in the nucleus of VSMCs in healthy arteries, and injury promotes FAK cytoplasmic relocalization and FAK activation in vivo. Little is known about how FAK re-localization occurs. Second, integrin signaling regulates FAK localization, and how integrins regulate FAK localization in different types of vascular cells in healthy and diseased states needs to be further

investigated and considered. Third, ER stress induces ox-LDL-mediated phenotypic transformation of VSMCs that involves reciprocal regulation between FAK and ER stress. Whether FAK is involved in the phenotypic transformation of VSMCs via reciprocal regulation with ER stress remains to be investigated. In conclusion, FAK family kinases are potential therapeutic targets for AS, and we hope to see more FAK and Pyk2 research within the AS field in the future.

Abbreviations

FAK, Focal adhesion kinase; Pyk2, proline-rich tyrosine kinase 2; VSMC, vascular smooth muscle cells; EC, endothelial cell; CVD, Cardiovascular disease; NLS, nuclear localization sequence; NEWS, nuclear export sequence; PRRs, proline-rich regions; VEGF, Vascular endothelial growth factor; SMC, smooth muscle cell; HGF, Hepatocyte growth factor; Sema7A, Signal protein 7A; ROS, Reactive oxygen species; d-flow, disturbed flow; IL, interleukin; MCP-1, Monocyte chemoattractant protein-1; HUVECs, human umbilical vein endothelial cells; ox-LDL, Oxidized low-density lipoprotein; LOX-1, lectin-like oxLDL receptor-1; AOS, Alginate oligosaccharides; DNJ, 1-deoxynojirimycin; Gab-1, grb2-associated binders; PKCθ, protein kinase Cθ; ATF2, activating transcription factor 2; MCP-1, monocyte chemoattractant protein-1; ICAM-1, Intercellular adhesion molecule 1; VCAM-1, Vascular cellular adhesion molecule-1; IP-10, induced protein 10; MAP, mitogen-activated protein kinase; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated extracellular signal-regulated kinase; ERK1/2, extracellular signal-regulated kinases 1 and 2; PI3K, phosphatidylinositol 3-kinase; PDGF, platelet-derived growth factor; GATA4, GATA-binding protein 4; DNMT3A, DNA methyltransferases 3A; 5-mC, 5-methylcytosine; MARK, mitogen-activated protein kinase; MCU, mitochondrial calcium uniporter.

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Disclosure

The authors report no conflicts of interest in this work.

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