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The critical role of signal transducers and activators of transcription-3 in the proliferation of vascular smooth muscle cells

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ABSTRACT

The proliferation of vascular smooth muscle cells (VSMCs) is a key pathogenic characteristic of vascular remodeling diseases. The signal transducers and activators of transcription-3 (STAT3) signaling pathway is crucial for VSMCs growth, and its role in cytokine-induced VSMCs proliferation, calcification, and phenotypic switching is essential for developing new treatment targets. VSMCs can transform from contractile to synthetic phenotypes in response to stimuli, such as mechanical forces, growth factors, pro-inflammatory cytokines, and vascular injury. Understanding the mechanism of VSMCs proliferation triggered by the STAT3 pathway is important for identifying potential therapeutic targets for vascular remodeling diseases.

1. Introduction

Numerous cardiovascular disorders, such as atherosclerosis and restenosis, frequently exhibit vascular remodeling as a pathogenic feature.[1] Young, healthy blood arteries have VSMCs that are mostly contractile and have well-controlled blood pressure. However, VSMCs can change from a contractile phenotype, which is characterized by the expression of smooth muscle actin (SM-actin), smooth muscle myosin heavy chain (SMMHC), SM22, and Cadherin, to a synthetic phenotype in response to a variety of stimuli, including mechanical forces, growth factors, pro-inflammatory cytokines, and vascular injury.[1,2] In this case, VSMCs alter their morphology, proliferation, migration and synthesize large amounts of extracellular matrix components, leading to arterial remodeling.[2] Although these functional adjustments are required to repair vascular injury, they may also help to develop a number of vascular disorders, like: atherosclerosis, calcification, and hypertension. Therefore, it is still important to research the mechanism of VSMCs proliferation.

STATs are a significant class of transcription factors that are involved in numerous vital biological processes, including angiogenesis, survival, and proliferation. However, STATs are often found in the cytoplasm in an inactive state, and its activation is achieved through phosphorylation of Tyr-705 by receptor tyrosine kinases and receptor associated kinases. Among various STAT members, STAT3 is a classic transcription factor that mediates intracellular signal transduction in pathological vascular remodeling.[3] STAT3 activation in VSMCs is currently known to primarily increase cell proliferation, migration, and survival.[3] Therefore, understanding the mechanism of VSMCs proliferation triggered by the STAT3 signaling pathway is crucial for identifying potential therapeutic targets for illnesses involving vascular remodeling.

This article explores the role of STAT3-related signaling pathways in VSMCs proliferation, and its impact on calcification and phenotypic switching in VSMCs.

2. The mechanism of activation of the classical STAT3 signaling pathway

STAT3, initially identified as an acute phase response factor, is activated by cytokines from the IL-6 family.[4] Receptor-related kinases JAKs and non-receptor-related kinase Src are kinases that cytokine receptors must recruit since they lack intrinsic tyrosine kinase activity.[4,5] Tyr705 is phosphorylated by receptor tyrosine kinase to activate STAT3.[4] After being activated, STAT3 dimerizes, moves into the nucleus, and participates in target gene transcription. The target gene's DNA sequence-specificity of STAT is controlled by the amino terminus, DNA binding domain, SH2 domain, phosphorylation state, and sequence of the target gene's CIS regulatory elements.[6,7] Dimerization follows and activated STAT3 dimers translocate into the nucleus and regulate STAT3-regulated gene expression. It is currently understood that STAT3 might upregulate the production of proliferative genes such as CyclinD1, PCNA, and vascular endothelial growth factor (VEGF), as well as proto-oncogenes including Myc, c-Fos, and c-Jun, in addition to

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promoting the proliferation of VSMCs.[6,8–10]

The JAK/STAT3 signaling pathway can be triggered by a variety of protein ligands. JAK functions as the primary signaling hub of cytokine receptors while making up the majority of their intrinsic kinase activity. A single or three JAKs, which are cytoplasmically attached to the receptor and provide tyrosine kinase activity, are specially bound by each receptor.[3] JAK initiates receptor activation upon cytokine binding, triggers the release of tyrosine phosphorylation, encodes the specific STAT protein SH2, phosphorylates the tyrosine residue Y705 of the STAT ring domain, and causes the activated STAT protein to enter the nucleus as a dimer. These activated homodimers combine in the nucleus, attach to several gene promoter sites, and initiate transcription.[11] In addition to JAK, STAT3 can also be activated by other non-receptor kinases like members of the Src family, receptor tyrosine kinases like the cytokine angiotensin II (AngII) receptor, and growth factor receptors like epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) (Fig. 1).

Besides phosphorylating Y705, Serine/Threonine Kinase also phosphorylates Ser727 of STAT3.[3] Depending on phosphorylation kinases and cell types, Ser727 affects STAT3 transcription differently than Tyr705. Nonetheless, it is generally accepted that phosphorylated Ser727 and Tyr705 jointly activate STAT3-mediated transcription.[3]

3. Effect of STAT3 on VSMCs proliferation

The proliferation of VSMCs is regulated by Cyclin-dependent kinases (CDKs).[12] Prior to DNA synthesis and cell division, Cyclins and CDKs collaborate to create complexes. These complexes can be coupled with two CDK family inhibitors to inactivate the CDK complex and regulate cell cycle activity.[12] STAT3 regulates VSMCs proliferation by transcriptionally activating proliferative genes such as PCNA, OPN, survivin, MMP2, MMP9, and CyclinD. It also mediates the effect of cytokines and growth factors on VSMCs proliferation.[8] Because of the STAT3-mediated influence of cytokines and growth factors on VSMCs proliferation, VSMC migration and proliferation produced by AngII, PDGF-BB, Interleukin, and other stimuli can be successfully inhibited by blocking the JAK/-STAT3 pathway. Effect of STAT3 on VSMCs proliferation can be categorized into the following major subclasses: (a) Effect of STAT3 on cytokine-induced VSMCs proliferation, (b) Effect of STAT3 on VSMCs phenotypic switching, (c) Effect of STAT3 on VSMCs calcification. These subclasses are briefly discussed below.

3.1. Effect of STAT3 on cytokine-induced VSMCs proliferation

3.1.1. AngII

Vascular tone, VSMCs development and death, cell migration, extracellular matrix deposition, pro-inflammatory responses, and the induction of other growth factors and vasoconstrictors are all influenced by AngII.[13] By activating the ERK1/2 and S6 kinase signal pathways and increasing the production of TGF-β, ET-1, and osteopontin, AngII drives the cell cycle G0-G1 transition and thus promotes cell proliferation.[5] Under certain conditions, the STAT3 signaling pathway contributes

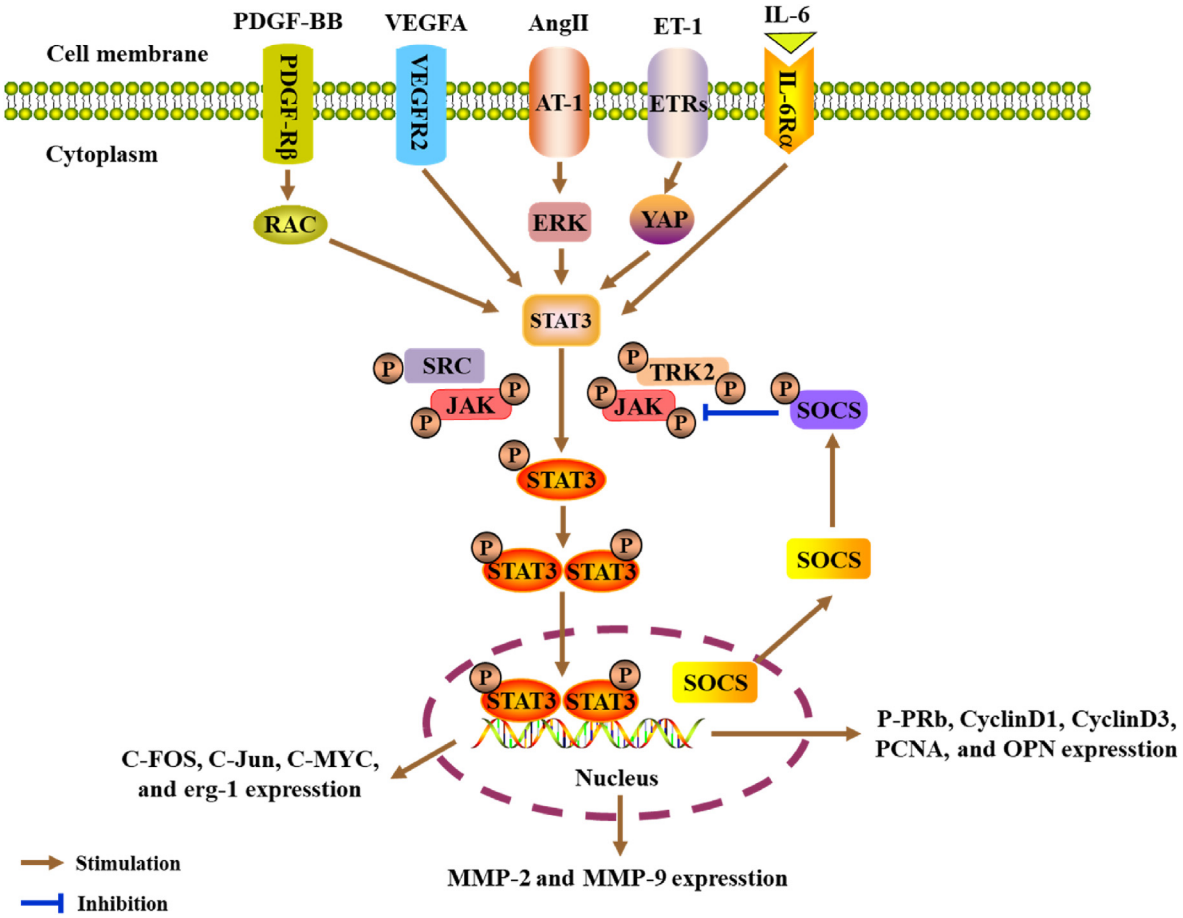

Fig. 1. The mechanism of activation of the classical STAT3 signaling pathway. STATs are often found in the cytoplasm in an inactive state, and its activation is achieved through phosphorylation of Tyr-705 by receptor tyrosine kinase such as EGFR, PDGFR, insulin-like growth factor receptor, receptor associated kinases such as JAK, and non-receptor kinases such as Src. Dimerization follows and activated STAT3 dimers translocate into the nucleus and regulate STAT3 target genes expression. STAT3 upregulate the production of proliferative genes such as CyclinD1, PCNA, and VEGF, metastatic genes such as MMP-2 and MMP-9, as well as proto-oncogenes including c-Myc, c-Fos, and c-Jun.

significantly to the increase in DNA synthesis of several proliferative proteins brought on by AngII. Studies have shown that glutathione S-transferase pi(GSTpi), calcitonin gene-related peptide, cucurbitacin I, and L-theanine can prevent STAT3 nuclear transfer and the expression of CyclinD1 by preventing Src, JAK2, and STAT3 phosphorylation, thereby preventing AngII-induced proliferation, migration, and hypertrophy in VSMCs.[9,14–16]

The proliferation of AngII in mammals is mediated by the two high-affinity plasma membrane receptors AT1 and AT2. The AT1 receptors, which are part of a family of seven membrane-coupled G protein-coupled receptors, typically activate phospholipase C via heterotrimeric Gq protein to mediate the physiological action of AngII.[5] And the AT1 receptor mediates AngII-induced vasoconstriction, growth, migration, formation of extracellular matrix, and inflammation. The combination of AngII and AT1 can activate p60 c-Src, the most representative member of the Src family, as well as JAK2, Tyk2 of the JAK family, phosphorylate tyrosine residues of STAT3 and it can also phosphorylate Ser727 in the STAT3 *trans*-activation domain through the ERK signaling pathway.[9]

Phosphorylated STAT forms homo- and heterodimers that translocate to the nucleus to form the CIS-inducing STAT protein complex (SIFs).[17] SIF is coupled with the target gene's CIS-inducible factor element (SIE) to stimulate the expression of c-Fos, c-Jun, c-Myc, erg-1, and other proto-oncogenes in VSMCs and promote cell proliferation.[5,17] AT2, a cross-model G protein-coupled receptor with 363 amino acids, suppresses cell proliferation, triggers death, and induces relaxation under physiological settings, and can also antagonizes the ability of AT1 in promoting VSMCs proliferation. The combination of AngII and AT2 inhibits the cell proliferation through PP2A's inhibition of ERK-induced dephosphorylation of Ser727 of STAT3 and PP2B's stimulation of dephosphorylation of Ser727 of STAT3 produced by the separation of SIF and SIE.[10,17]

3.1.2. PDGF-BB

PDGF-BB is one of the most effective stimulants for the proliferation and migration of VSMCs, functioning via the tyrosine kinase receptor PDGF-R.[18] The combination of PDGF-BB and PDGF-Rβ can activate the phosphorylation of PLCγ1, AKT, ERK1/2, and STAT3 to promote the proliferation of VSMCs.[19] PDGF induces the transcription of STAT3 target genes, and promotes the proliferation of VSMCs via activating the phosphorylation of Tyr705 and Ser727 of STAT3.[20] PDGF-BB-induced phosphorylation of STAT3 is mediated by JAK and Src, and PDGF-BB mediate the proliferative effects of VSMCs by activating the JAK2/-STAT3, which upregulating the expression of the retinoblastoma proteins *p*-pRb, CyclinD, survivin, and MMP-9.[21]

Rac signaling is involved in the regulation of cell adhesion, migration, cell cycle progression, and cell transformation, while PDGF-BB can promote the phosphorylation of STAT3 and ERK1/2 by activating Rac1, thereby promoting the nuclear translocation of STAT3, upregulating the expression of CyclinD1 and CyclinD3, as well as promoting the transition of G1/S phase and the proliferation of cells.[22] Reactive oxygen species take part in the PDGF-BB-induced phosphorylation of STAT3, and reactive oxygen source 12/15 lipoxygenase causes the phosphorylation of PDGF-Rβ tyrosine 579, which recruits Src to initiate STAT3 phosphorylation.[23] Proanthocyanidin A2 inhibits VSMCs proliferation by reversing the effects of PDGF-BB, which causes CDK inhibitors p27kip and p21waf1 to be downregulated and CDK2 and CyclinA to be increased. This is accomplished by disrupting the JAK2/STAT3/cPLA2 signaling pathway.[18]

3.1.3. Endothelin-1

Endothelin-1(ET-1) is one of the strongest vasoconstrictors in the body. When ET1 is synthesized and released from endothelial cells or VSMCs, it binds to ETA and ETB2 receptors on the surface of VSMCs to promote ET-1 signal transduction and regulate the contraction, proliferation and migration of VSMCs.[24,25] Furthermore, ET-1 can induce mitophagy of VSMCs, promote DNA synthesis and augment vasoconstriction.[26] Insulin and AngII promote the proliferation and migration of VSMCs by upregulating ET-1, which increases the stimulation of ET-1/ETAR on ERK signal activation.[25]

ET-1 also regulates growth, proliferation, and survival of VSMCs by activating PLC/DAG/IP3, MAPKs, and PI3K/PK pathways.[27] According to the recent research, ET-1 promotes the proliferation of astrocytes and regulates the STAT3 pathway in astrocytes.[27,28] ET-1 induces astrocyte proliferation by promoting 5′ side binding of STAT3 to CyclinD1 and SKP2 genes in rats.[26] These results imply that STAT3 might play a role in the proliferation of VSMCs that mediated by ET-1.

Yes associated protein (YAP), which is produced by the YAP1 gene, functions as a transcription co-activator and is the most important downstream effector on the Hippo signaling cascade. YAP promotes the phenotypic transformation, proliferation and differentiation of VSMCs by inhibiting MYO.[29,30] It's likely that YAP directly regulates STAT3, and YAP overexpression raises the phosphorylation of STAT3, which in turn promotes the expression of AngII and greatly promotes YAP-mediated angiogenesis.[31] YAP can prolong IL-6-mediated STAT3 aggregation and enhance STAT3 transcriptional activity via binding to STAT3 in the nucleus.[30] Meanwhile, ET-1 promotes the proliferation of cancer cells by activating YAP. Therefore, the ET-1/YAP, ET-1-STAT3, or ET-1/YAP/STAT3 signaling might contribute to the ET-1-induced VSMCs proliferation.[30]

3.1.4. Interleukins

After being triggered by various vascular agonists such as PDGF-BB, thrombin, and AngII, VSMCs can generate IL-6.[32] IL-6 mainly promotes the proliferation and migration of VSMCs by activating the JAK/STAT3 signaling pathway to promote the expression of MMP-2, MMP-9, CyclinD1, etc.[32,33] Following binding to the receptor, IL-6 triggers the formation of a hexamer signal complex composed of gp130 homodimer and two IL6Rβ hybrid dimers, which in turn activates JAKs that bind to the proline-rich proximal membrane box1 region of gp130. In turn, activated JAKs promote the phosphorylation of gp130, which causes STAT3 to be attracted to the cell and activated. The activated STAT3 translocates into the nucleus and then regulates the expression of target genes that involved in cell proliferation.[33]

STAT3 activity can be inhibited by suppressor of cytokine signaling (SOCS) family of inhibitors and protein inhibitor of activated STATs. SOCS3 is a classic JAK2/STAT3 negative feedback inhibitor, its increased levels have been shown to inhibit the migration and proliferation of VSMCs as well as inflammatory cytokine secretion.[34] [35]The sensitive SOCS3 subunit of the receptor, which is present in IL-6, has a high specific inhibition of the IL-6/STAT3 signaling pathway. SOCS3 competes with STAT3 for receptor docking sites, binds to JAK or phosphorylated receptors to attenuate JAK activity, and inhibits the IL/STAT3 pathway and the proliferation of VSMCs when STAT3 is activated by IL-6.[35] Since SOCS5 includes a MAPK interaction domain and is upregulated by IL-19 through the STAT3 pathway, it inhibits the expression of STAT3, P44/42, and p38 MAPK, thus inhibits the proliferation and migration of VSMCs.[34]

In conclusion, blocking AngII, PDGF-BB, Interleukin, and other stimuli may suppress VSMCs migration and proliferation. Thus, there are some therapeutic drugs regulating VSMCs proliferation through STAT3 signal pathway (Table 1).

3.2. Effect of STAT3 on VSMCs phenotypic switching

Mature VSMCs are static, with contraction phenotype and stable expression of contraction proteins, such as SM-actin, SMMHC, caloponin, SM myosin light chain kinase, etc.[30,36] VSMCs exhibit phenotypic plasticity under various stimuli such as growth factors, reactive oxides, and mechanical damage, switching between contraction and synthesis.[37] Phenotypic switching of VSMCs is associated with vascular remodeling diseases such as atherosclerosis, restenosis after angioplasty, and hypertension.[38]

Myocardin is a key regulator phenotypic switching of VSMCs.[39] The

Table 1
Some therapeutic drugs regulating VSMCs proliferation through STAT3 signal pathway.

Stimuli	Therapeutic Drugs	Effects on VSMCs	Phosphorylation target	Mechanisms	References
AngII	GSTpi	Decrease AngII –induced VSMCs proliferation.	Src/STAT3	Arrest progression of cell cycle from G0/G1 to S phase and the expression of CyclinD1.	Chen D et al.[9]
	Cucurbitacin I	Decrease AngII–induced VSMCs proliferation.	Jak2/STAT3	Suppress Giα proteins expression.	Hossain E et al.[15]
	CGRP	Decrease AngII–induced VSMCs proliferation.	Src/STAT3	Suppress the level of ROS generated by NADPH oxidase in AngII-induced VSMCs.	Ye S et al.[13]
	L-theanine	Decrease AngII–induced VSMCs proliferation.	Jak2/STAT3	Arrest CyclinD1 expression.	Ben P et al.[16]
	Bazedoxifene	Decrease AngII–induced VSMCs proliferation.	IL6/gp130/STAT3	Arrest MMP2, MMP9 expression.	Yan D et al.[33]
PDGF	2-undecylsulfonyl-DMNQ	Decrease PDGF–induced VSMCs proliferation.	STAT3/Tyr	Suppress pRb phosphorylation and CyclinD1/E, CDK2/4 and PCNA expression.	Kim Y et al.[19]
	Scoparone	Decrease PDGF–induced VSMCs proliferation.	Non	Block the accumulation of STAT3 transported from the cytosol to the nucleus.	Park S et al.[21]
	TIPE2	Decrease PDGF–induced VSMCs proliferation.	Rac1-STAT3	Suppress CyclinD1 and D3 expression.	Zhang G et al.[22]
	I3MO	Decrease PDGF–induced VSMCs proliferation.	Src/STAT3	Interfere with PDGFR-Src-STAT3 signaling via impaired 12/15-LO activation.	Blazevic T et al.[23]
	Proanthocyanidin A2	Decrease PDGF–induced VSMCs proliferation.	JAK2/STAT3	Inhibite PDGF-mediated NADPH oxidase activation and intracellular ROS formation in VSMCs.	Zhang L et al.[18]
ET-1	Non	Progenitor stem cell proliferation and astr ocytic differentiation	JAK2/STAT3	Increase CyclinD1 and SKP2 genes expression.	Koyama Y et al.[26] and Cheng X et al.[28]

VSMCs vascular smooth muscle cells, *AngII* Angiotensin II, *PDGF-BB* platelet-derived growth factor two B subunits, *ET-1* endothelin-1, *ROS* reactive oxygen species, *NADPH* nicotinamide adenine dinucleotide phosphate, *SKP2* Sphase Kinaseassociated Protein 2, *MMP* matrix Metalloproteinases, *TIPE2* the tumor necrosis factor (TNF) α-induced protein 8-like 2, *GSTpi* glutathione S-transferase pi, *CGRP* calcitonin generelated peptide, *I3MO*, indirubin-3′-monoxime, *12/15-LO*,12/15-lipoxygenase.

connection between myocardin and serum reaction factor (SRF) plays a role in the transition between driving contraction and synthetic phenotypes.[39] The myocardin-SRF complex directly activates a variety of contracting smooth muscle genes by interacting with numerous CarG box promoters, and indirectly promotes contractile phenotypes by upregulating the expression of the cell cycle inhibitor gene Cyclin-dependent kinase inhibitor 1A and blocking NF–B-dependent cell cycle progression, thereby inhibiting VSMC proliferation.[39,40] However, STAT3 can promote the proliferation of VSMCs by preventing the formation of the myocardin-SRF-cArG box tertiary complex, decreasing myocardin synthesis, downregulate VSMCs contraction marker genes like SMA, SMMHC, and SM22 at the protein and mRNA levels, and upregulating the expression of CyclinD1, OPN, and PCNA.[39,41]

VEGF-activated VEGFR2 plays an important role in phenotypic switching of VSMCs.[40] Cyclin D1 and PCNA reduced in VEGF-treated HASMCs, while SMMHC and SMTN expression increased when VEGFR2 was knocked down.[40] Since STAT3 is a downstream regulator of VEGFA-induced phenotypic switching of VSMCs, its overexpression increases VEGFA-induced CyclinD1 and PCNA.[40] In eukaryotic cells, regulation of protein properties and function by post-translational modification is the central molecular mechanism that mediates signal transduction.[42] Myocardin can be induced by VEGF-induced NO through S-nitrosoglutathione (GSNO) s-nitrosylation and inhibition. S-nitrosylated myocardin inhibits the expression of ACTA2 and SM22 and regulates the proliferation of VSMCs. S-nitrosoglutathione reductase (GSNOR) blocks NO function by converting GSNO into NH3.[40] Myocardin can down-regulate GSNOR expression by combining with GSNOR's CarG Box to maintain the contraction phenotype of VSMCs. However, knockout STAT3 enhanced myocardin-mediated GSNOR promoter activity.[40] These results suggest that VEGFA-VEGFR2-STAT3 regulates the phenotypic transformation of VSMCs by interacting with myocardin, and that VEGFA-induced NO produces myocardin S-nitrosylation.

3.3. Effect of STAT3 on VSMCs calcification

VSMCs are mesenchymal-derived cells that can differentiate into other mesenchymal-derived cells such as osteoblasts and chondrocytes under the stimulation of oxidative stress and inflammation. They express osteoblast morphological factors, bone morphogenetic proteins (BMP) 2 and BMP4, osteocalcin and transcription factor alkaline phosphatase (ALP), osteric, etc., that promote the release of vesicle structures containing hydroxyapatite, which in turn causes calcium deposition and mineralization in the blood vessel wall.[43–45] STAT3 is involved in VSMCs calcification, it binds to the promoter of osteoblast differentiation and bone formation transcription factor to promote osteoblast transcription and osteoblast differentiation.[46] For example, activation of the JAK/-STAT3 signaling pathway promotes the expression of IL-6-RUNT-related transcription factor 2 (Runx2), osteoprotegerin(OPG), and osteocalcin of osteoblast genes.[44,45] Adiponectin can reduce the osteogenic differentiation of VSMCs by inhibiting the phosphorylation of STAT3 and downregulating the osteogenic transcription factor osteric.[46]

Inflammation is closely related to vascular calcification, and inflammatory factors such as TNF-α, IL-6, and IL-17 are involved in the calcification of VSMCs.[46–49] The osteogenic transformation and mineralization of VSMCs are induced by IL-6 and are dependent on STAT3.[46] IL-6s/sIL-6R accelerates VSMCs calcification in a STAT3-dependent manner by activating STAT3 signaling pathway, it activates the transcription of Runx2, ALP and OPN.[44,50] Runx2 is a crucial factor to the osteoblast differentiation and bone formation, and it has the ability to induce the transcription of the OPN, PARP1, and CoIIA1 genes.[45] Direct interaction between Runx2 and SRF disrupted the formation of myocardin-SRF complex and induced the phenotypic transformation of VSMCs into osteochondral tissue.[45] Activated Runx2 enlists JMJD2B into the STAT3 targeting site in the Runx2 promoter region to demethylate H3K9me3 and enhance osteoblast differentiation.[50] Moreover, PARP1 also inhibits miRNA-204 expression through the IL-6/STAT3 pathway, which increases the expression of Runx2 and promotes calcification of VSMCs.[45] The binding of receptor tyrosine kinase-like orphan receptor 2 (ROR2) to WNT5A activates the typical WNT pathway and plays an important role in osteoblast differentiation.[44] IL-6/sIL-6R stimulation activates the ROR2/WNT5A pathway in a STAT3-activated manner to promote calcification.[44] Vascular calcification is also associated with cellular senescence, and senescence-promoted vascular calcification in VSMCs can also be mediated by IL-6, which activates the IL-6/sIL-6R/p53/p21 pathway to induce senescence-associated calcification in VSMCs.[47] High levels of IL-17 inhibit autophagy by activating JAK2/STAT3, enhancing the RANKL,

promoting calcification and osteoclast formation, reducing OPG.[51]

Krupple like factor (KLF4) is a zinc finger transcription factor that regulates osteogenic differentiation and the transcription of osteogenic genes in VSMCs,[43] while miR-135a may reduce VSMCs calcification by down-regulating STAT3 expression through KLF4.[43] Additionally, Cathepsin S is capable of breaking down the extracellular matrix surrounding the artery wall and the basement membrane, as well as participate in the calcification and degradation of elastin.[52] The absence of membrane elastin in the blood vessels promotes the osteogenic transformation of smooth muscle cells. Research showed that the inhibition of STAT3 attenuates Cathepsin S expression and reverses loss of elastin, whereas hydrogen sulfide attenuates vascular calcification by upregulating elastin expression through inhibition of STAT3/CAS signaling.[52]

4. Signal pathways of STAT3 promoting VSMCs proliferation

As discussed above, the transcription factor STAT3 plays a critical role in cytokine-induced VSMCs proliferation, phenotypic switching, and calcification. And STAT3 involves in the regulation of multiple signaling pathways, such as TGF-β/STAT3/FoxO1 signal pathways, STAT3/Pim1/NFAT signal pathways, STAT3/cPLA2 signal pathways (Fig. 2).

4.1. TGF-β/STAT3/FoxO1 signal pathways

TGF-β signal is crucial for maintaining vascular integrity and homeostasis.[53] There are three types of TGF-β receptors, namely TβRI, TβRII, and TβRIII. TGF-β delivers intracellular signals mostly via the Smad-dependent signaling pathway after forming a polymer with the receptor.[54] Smads family proteins play a key role in the transmission of TGF-β signals from cell surface receptors to the nucleus. TGF-β functions as a ligand to build a receptor complex that activates Smads into the nucleus, where they jointly either stimulate or inhibit the transcription of the target genes they regulate.[54]

The STAT3 signaling pathways play a partially mediating role in TGF-β-mediated proliferation and differentiation. TGF-β can promote cell proliferation by inducing phosphorylation of STAT3 in human hepatoma cells.[55] TGF-βRI bound to Smad phosphorylates STAT3 and JAK1, which increases TGF-β-mediated transcription of target genes.[55] Histone acetyltransferase p300 acts as a bridge between TGF-β-induced Smad3 and STAT3, forming the STAT3-P300-Smad3 complex which is vital for neural progenitor cells to differentiate into astrocytes.[56]

FoxO1 is considered as a tumor-suppressor protein which has pleiotropic functions including inhibiting cell proliferation, inducing apoptosis, protecting cells from oxidative stress and DNA damage, and regulating immune responses.[57,58] In VSMCs, FoxO1 transcriptional activation promotes the up-regulation of cell cycle arrest protein p21Cip1/Waf and p15INK4B and promotes G1 phase arrest.[58] Meanwhile, FoxO1 is a downstream factor of STAT3, and its expression is negatively correlated with the expression of p-STAT3. However, the inhibition of STAT3 increased both mRNA and protein level of FoxO1.[57,59]

TGF-β induced upregulation of P-STAT3 and P-FoxO1 in VSMCs, and

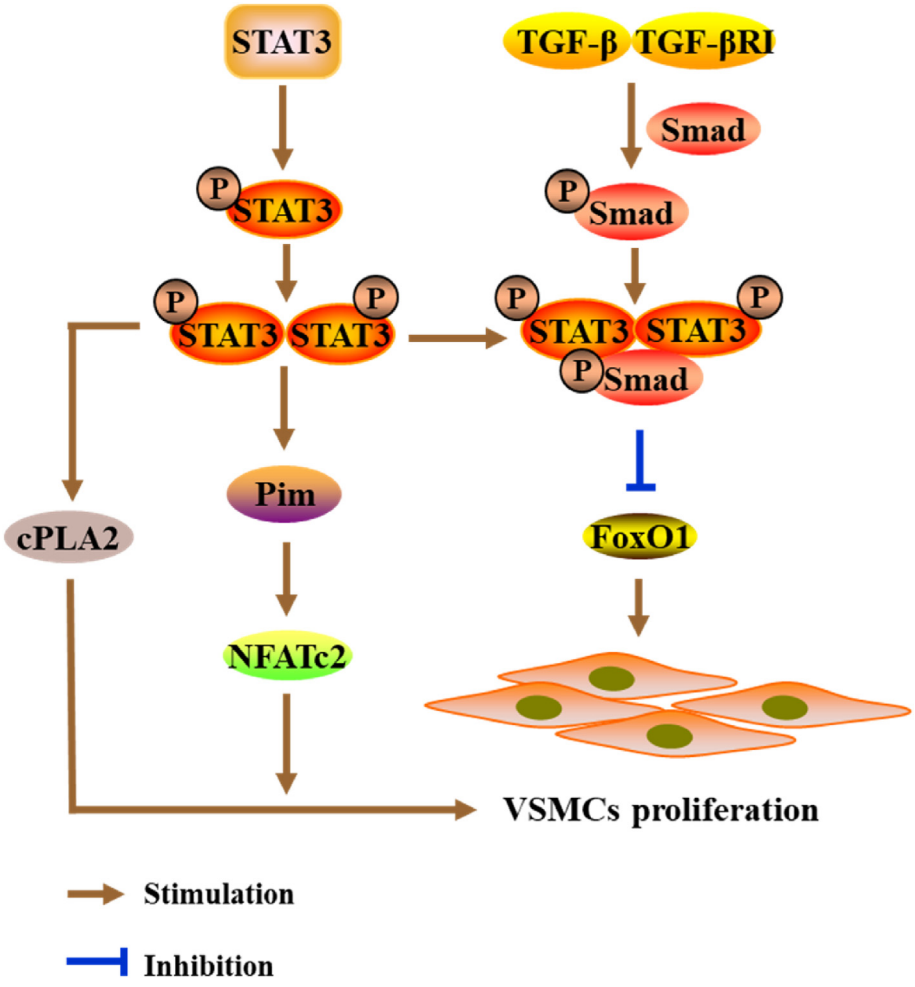

Fig. 2. STAT3 involves in the regulation of multiple signaling pathways and promotes VSMCs proliferation. After being activated, STAT3 dimerizes, and participates in the transcription of Pim, which iniduce the transcription of cPLA2 and NFATc2. TGF-βRI bound to Smad phosphorylates STAT3 and JAK1, which increases TGF-β-mediated transcription of target genes. FoxO1 is a downstream factor of STAT3, and its expression is negatively correlated with the expression of p-STAT3.

its mechanism is that TGF-β induced rapid translocation of P-STAT3 into the nucleus, while FoxO1 is induced to enter the cytoplasm and inactivate the cytoplasm.[53] Phosphorylation of FoxO1 in HASMCs stimulated by TGF-β1 was not induced by the STAT3 inhibitor BP-1-102, suggesting that TGF-β induces inactivation of FoxO1 by P-STAT3.[53] Taken together, TGF-β upregulates STAT3 phosphorylation in VSMCs, negatively regulates FoxO1, and promotes cell proliferation.

4.2. STAT3/Pim1/NFAT signal pathways

Pim proteins are a family of serine/threonine kinases with short half-life period that are highly evolutionarily conserved in multicellular organisms.[60] Pim plays a role in cells proliferation by accelerating the cell cycle and preventing apoptosis,[61] and its mechanisms are as follows: (a) Pim can phosphorylate CDC25A, which promotes G1 and S phase progression, and CDC25C, which promotes G2/M phase progression. (b) Pim also regulates the phosphorylation of cell cycle inhibitors p21Cip1/WAF1 and p27Kip to promote cell cycle and proliferation.[61] (c) Pim interacts with the apoptosis factor BAD to phosphorylate the Ser112 site, which releases the pro-apoptotic protein BAX and the anti-apoptotic proteins Bcl-xl and Bcl-2 to form a heterodimer and neutralizes the pro-apoptotic activity.[61]

Pim's proliferative and anti-apoptotic effects are mediated via the STAT3 signaling pathway.[62] After treatment with a STAT3 inhibitor, the expression of cyclin-dependent kinase inhibitors and the senescence-related markers p21 and p16 are considerably increased.[62] In addition to upregulate the expression of BCL-2 and promote the transcription of the Pim1 gene, STAT3 also activates nuclear factor of activated T cells (NFATc2), a downstream transcription factor.[60,63] NFATc2 has the ability to depolarize cells, increase Ca^{2+} concentrations, down-regulate K^{+} channels like Kv1.5, and promote cell survival, proliferation, and resistance to mitochondrial apoptosis.[64] High glucose promotes VSMCs proliferation by activating the STAT3/Pim1 signaling pathway, while the CML promotes the proliferation of VSMCs by activating its receptor RAGE and upregulating STAT3.[60,65] At the same time, the activation of the STAT3/Pim1/NFATc2 signaling pathway promotes the proliferation of PASMCs and may be a new target for the treatment of pulmonary hypertension.[63]

4.3. STAT3/cPLA2 signal pathways

The release of arachidonic acid mediated by intracellular phospholipase A2 (cPLA2) is an important factor of VSMCs proliferation.[66,67] Arachidonic acid is a polyunsaturated fatty acid and an important component of membrane phospholipids. Once released, it is metabolized by cyclooxygenase, lipoxygenase, or cytochrome p450 monooxygenase pathways or to synthetic membrane phospholipids that play a role in cell growth and survival by the arachidonic acyl coenzyme a synthetase and arachidonic acyl lysophospholipids transferase esterification pathways.[66] cPLA2 promotes the proliferation of VSMCs may also be related to the induction of CyclinD1 expression.[68] The cPLA2 inhibitor PYR inhibits the proliferation of VSMCs by increasing G1 cell cycle arrest and increasing TGF-β-mediated mitotic inhibition.[68]

The protrusion and contraction of cell membranes are essential for cell movement, including the remodeling of cell membrane phospholipids.[66] The expression of cPLA2 mediated by STAT3 may be involved in the remodeling of membrane phospholipids during cell movement, proliferation, and survival. In adipocytes, lipopolysaccharide increases the proliferation and fat formation of preadipocytes through the JAK/STAT3-dependent cPLA2 expression, thus promotes obesity.[67] In astrocytes, leptin upregulates the expression of caveolin-1 through the JAK/STAT3 pathway to block the interaction between Src and EGFR, and inhibits the expression of cPLA2 to reduce the synthesis and release of arachidonic acid.[69] ERK1/2 and PI3K are essential for the expression of cPLA2 in VSMCs.[70] PDGF-BB-induced growth and motility of VSMCs require the expression of cPLA2, which depends on JAK/STAT3.[18] PDGF-BB promotes cPLA2 expression in a time- and concentration-dependent manner, and the cPLA2 inhibitor PYR significantly inhibits PDGF-BB-induced proliferation of VSMCs.[18] Proanthocyanidin A2 inhibits the proliferation of VSMCs by inhibiting the JAK2/STAT3/cPLA2 signaling pathway, reversing the PDGF-BB-induced downregulation of CDK inhibitors p27kip and p21waf1 and upregulating of CDK2 and CyclinA.[18]

5. Conclusion

VSMCs are cellular components in the middle layer of normal vascular walls. The primary role of the VSMCs is to maintain appropriate blood pressure through dynamically contract and relax in response to vasoactive stimulation to regulate the diameter of the lumen and maintain a healthy blood pressure. Under pathological conditions, the increase or decrease of various cytokines and growth factors can stimulate the proliferation of VSMCs. Abnormal proliferation of VSMCs leads to increased thickness of vascular cell walls, which damages the smoothness of blood flow and ultimately leads to increased blood pressure. STAT3 plays an important role in mediating VSMCs proliferation, it can be regulated by a variety of cytokines and growth factors, including AngII, PDGF-BB, ET-1, Interleukin, etc. And STAT3 is involved in the regulation of multiple signaling pathways, including TGF/STAT3/FoxO1 signal pathways, STAT3/cPLA2 signal pathways, and STAT3/Pim1/NFAT signal pathways. Finally, further research needs to be done with the involvement of STAT3 in the proliferation of VSMCs in vascular diseases. Understanding the mechanism of VSMCs proliferation triggered by the STAT3 pathway is important for identifying potential therapeutic targets for vascular diseases.

CRediT authorship contribution statement

Shuai Li: Writing – original draft. **Chan Liu:** Writing – review & editing.

Declaration of competing interest

These authors have no conflicts of interest to declare.

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