FAK Family Kinases

Subjects: Pathology
Contributor: Ssang-Taek Lim

In various vascular diseases, extracellular matrix (ECM) and integrin expression are frequently altered, leading to FAK or Pyk2 activation. In addition to the major roles of FAK and Pyk2 in regulating adhesion dynamics via integrins, recent studies have shown a new role of nuclear FAK in gene regulation in various vascular cells. In particular, FAK primarily localizes within the nuclei of vascular smooth muscle cells (VSMCs) of healthy arteries. However, vessel injury increased FAK localization back to adhesions and elevated FAK activity, leading to VSMC hyperplasia. The study suggested that abnormal FAK or Pyk2 activation in vascular cells may cause pathological condition in vascular diseases. Here we will review several studies of FAK and Pyk2 associated with integrin signaling in vascular diseases including restenosis, atherosclerosis, heart failure, pulmonary arterial hypertension, aneurysm, and thrombosis. Despite the importance of FAK family kinases in vascular diseases, comprehensive reviews are scarce. Therefore, we summarized animal models involving FAK family kinases in vascular diseases

Keywords: Pyk2 ; integrin ; vascular disease ; restenosis ; atherosclerosis ; heart failure ; pulmonary hypertension ; aneurysm ; thrombosis

1. Introduction

There have been a number of studies on the role of FAK and Pyk2 within vascular cells including VSMCs, ECs, cardiomyocytes (CMs), fibroblasts, macrophages, and platelets (Figure 2). Here, we focus on reviewing what is known about FAK or Pyk2 expression and activity within vascular cells and vascular diseases, with an emphasis on their connection to integrin signaling (Table 1). We will also discuss current FAK and Pyk2 genetic animal models used to study vascular diseases.

Table 1. Role of integrins on FAK family signaling in vascular cell types.

Cell Type	Integrin	Function	Reference
Vascular smooth muscle cells	α5β1	Binds fibronectin and promotes FAK activation	[1]
	αvβ3	Binds fibronectin and promotes FAK activation	[1]
		Promotes FAK activation upon binding to osteoprotegerin under hypoxic conditions	[2]
	α5 αv	Dual knockout using SM22α-Cre reduced FAK activity and tyrosine phosphorylation of downstream target proteins	[3]
Endothelial cells	α5β1	Promotes flow-induced FAK-mediated NF-κB transcriptional activation	[4]
		Promotes disturbed flow activation of FAK	[5]
		Mediates oxidized LDL activation of FAK	[6]
	α1β1	Increases FAK activation upon binding to semaphorin 7A	[7]
	αvβ3	Promotes flow-induced FAK-mediated NF-κB transcriptional activation	[4]
		Promotes high shear flow-induced FAK expression and inflammatory gene expression	[8]
Cardiomyocytes	β3	Increased association with FAK in pressure-overloaded hypertrophic hearts	[9]

Cell Type	Integrin	Function	Reference
Platelets	αIIbβ3	Activates FAK upon binding fibrinogen in conjunction with costimulatory molecules like ADP, epinephrine, and thrombin	[10]
		Activates Pyk2 upon binding fibrinogen to promote phosphorylation of c-Cbl	[11]
	α2β1	Activates Pyk2 to promote aIIbb3 inside-out signaling	[12]

2. Intimal Hyperplasia and Restenosis

Intimal hyperplasia is a type of vascular remodeling that arises from the proliferation and migration of VSMCs into the intima. This process occurs in various pathological conditions (i.e., atherosclerosis and pulmonary arterial hypertension) and during restenosis following clinical procedures such as angioplasty and vein graft [13][14][15][16]. Under healthy conditions, VSMCs are primarily surrounded by basement membrane ECMs such as collagen IV, laminin, and elastin which bind to α1β1, α2β1, and α3β1 integrins (reviewed in [17]). However, in pathological remodeling conditions, the ECM composition changes through the increased secretion of remodeling enzymes, such as matrix metalloproteinases (MMPs) and fibrotic ECM proteins including fibronectin, collagen I, vitronectin, and osteopontin which bind to α5β1 and αvβ3 integrins [17]. Early studies in vitro demonstrated that VSMCs proliferate more rapidly on fibronectin than on laminin [18]. Platelet-derived growth factor (PDGF) can induce VSMC proliferation via ERK MAPK activation in VSMCs plated on either fibronectin or laminin. However, PDGF can only activate FAK in VSMCs plated on fibronectin but not on laminin [18], suggesting that laminin binding integrins (α1β1, α2β1, α3β1) may not lead to PDGF-induced FAK activation. While PDGF was also shown to activate Pyk2 and promote VSMC proliferation through ERK and AKT activation [19], the role of integrins in Pyk2 activation in VSMCs has not been elucidated. Increased expression of α5β1 and αvβ3 integrins were found in VSMCs cultured on fibronectin compared to laminin or Matrigel [1]. This increase in α5β1 and αvβ3 integrins was also associated with increased FAK expression and activity, further supporting an important role for FAK signaling downstream of fibronectin-binding integrins in VSMCs. These studies indicate that basement membrane-binding integrins might maintain low FAK activity upon growth factor signaling and reduce VSMC proliferation and migration.

Differentiated VSMCs of healthy arteries are unique in that they abundantly express the endogenous FAK inhibitor FRNK [20][21][22]. Overexpression of FRNK decreases FAK activity, leading to reduced VSMC proliferation and migration [20]. Mutating L1034S (leucine to serine mutation) on FRNK reduced its localization to focal adhesions by decreasing the FRNK–paxillin interaction [22]. L1034S FRNK failed to inhibit FAK activation in response to angiotensin II in VSMCs when compared to wild-type (WT) FRNK, supporting the role for FRNK displacement of FAK at focal adhesions. Interestingly, FRNK expression is altered depending on the ECM species to which VSMCs are attached. FRNK expression was elevated in VSMCs plated on Matrigel or perlecan (a basement membrane proteoglycan), but was decreased in VSMCs plated on fibronectin [23]. ECM and integrin regulation of FRNK expression inversely correlates with FAK activity (pY397 FAK) within VSMCs.

Increased expression of fibronectin that occurs during intimal hyperplasia leads to increased vascular wall stiffness, which in turn activates FAK to promote VSMC proliferation and migration [24][25][26]. VSMCs plated on stiff hydrogels show elevated FAK activation and increased cyclin D1 expression compared to VSMCs on soft hydrogels, implicating that FAK can mediate mechanosignaling by sensing matrix stiffness [25][26]. The importance of FAK expression in vascular remodeling was examined by using a VSMC-specific Myh11-Cre mouse model to knockout (KO) FAK within VSMCs [24]. Loss of FAK expression in VSMCs significantly blocked wire injury-induced vascular remodeling compared to WT mice [24].

However, the differences between FAK kinase-dependent and -independent functions in VSMC proliferation have not been delineated. A recent study has investigated which of these FAK functions were critical for VSMC intimal hyperplasia by using either a pharmacological inhibitor or a VSMC-specific FAK KD knock-in mouse model during femoral wire injury [27]. Pharmacological or genetic FAK inhibition significantly blocked neointimal hyperplasia by forcing FAK localization to the nucleus and inhibiting its kinase activity when compared to the control mice, suggesting that both reduced FAK activity and increased FAK nuclear localization are important for reducing VSMC proliferation. Interestingly, the majority of FAK appeared to be within the nuclei of VSMCs of healthy femoral arteries and wire injury induced FAK activation and cytoplasmic relocalization [27]. This was the first study demonstrating that FAK may be sequestered inside the nucleus of VSMCs in vivo, which gives a glimpse into a previously unknown role of nuclear FAK. The study further showed that nuclear FAK limited VSMC proliferation by binding to and promoting the proteasomal degradation of GATA4 [27]. Following injury, GATA4 protein expression increased as FAK relocated from the nucleus to the cytoplasm, resulting in VSMC proliferation through direct regulation of cyclin D1 transcription [27]. Forced nuclear FAK localization through the use of a pharmacological FAK inhibitor or in VSMC-specific FAK-KD mice prevented GATA4-mediated cyclin D1 expression and VSMC hyperplasia [27]. These studies help to bridge the knowledge gap between FAK expression and activity in VSMC intimal hyperplasia that was previously unsolved in other studies [25][26]. In addition to promoting cyclin D1 expression, FAK activity has also been linked to the stability of S-phase kinase-associated protein 2 (Skp2), which promotes the degradation of the cell cycle inhibitors p21 and p27 [28]. It has been shown that inhibition of FAK by overexpression of either FRNK or the inactive FAK Y397F (tyrosine to phenylalanine) mutation reduced Skp2 expression and MG-132 (a proteasomal inhibitor) treatment prevented loss of the Skp2 protein. The study suggested that FAK activity may be important for regulating Skp2 protein stability [28]. We have found that nuclear FAK binds Skp2 and promotes Skp2 ubiquitination and proteasomal degradation (unpublished data). Taken together, these studies demonstrate that nuclear FAK suppresses VSMC proliferation by reducing expression of both cell cycle promoters (cyclin D1, Skp2) and increasing expression of cell cycle inhibitors (p21, p27). Although it will need further testing, it is plausible that the high levels of FRNK found in VSMCs may contribute to the increased nuclear FAK and inactive FAK that we observed within VSMCs of healthy arteries.

3. Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the vessel wall that results in the excess accumulation of lipids under the endothelium and VSMCs. As atherosclerosis tends to develop in areas of the vasculature that experience disturbed flow (usually in branch or bifurcated points and the inner aortic arch), several studies have investigated how this mechanical stimulation can lead to atherosclerosis progression [29]. The role of integrins in flow-induced signaling of ECs has been elucidated by investigating how different integrin heterodimers affect the EC response to different types of flow [5][8]. In early atherosclerotic lesions, changes in the ECM from collagen to fibronectin induces activation of both αvβ3 and α5β1 integrins [30], both of which lead to FAK activation and subsequent pro-inflammatory molecule expression [5][8]. Flow-mediated activation of integrins triggers activation of FAK and Src, which promote subsequent VEGFR2–Cbl complex formation leading to IKK-NF-κB activation in ECs [31][32]. Treatment with general tyrosine kinase inhibitors (genistein or AG82) reduced flow-induced NF-κB activation and nuclear localization [32], potentially through FAK and Src inhibition. The importance of FAK in flow-mediated signaling was further investigated by using FAK KO mouse aortic ECs [4]. Interestingly, this study found that β1 integrin activating antibodies did not promote transcriptional activation of NF-κB and ICAM-1 expression in FAK KO ECs [4], suggesting that FAK downstream of β1 integrin activation is important for flow-induced inflammation in ECs. It seems that α5β1, but not αvβ3, is required for disturbed flow-induced activation of FAK and NF-κB signaling in ECs [5]. Additionally, both α5β1 and FAK were activated in the inner aortic curvature (under disturbed oscillatory flow), but not in the outer aortic curvature (under linear flow) of low-density lipoprotein receptor (LDLR) KO mice fed a western diet [5]. Crosstalk between the mechanosensitive ion channel Piezo1 with disturbed-flow activation of α5β1 integrin was required for FAK activation and pro-atherogenic inflammatory signaling in ECs [5]. Disturbed flow was also shown to promote the activation of FAK in ECs through increased expression of semaphorin 7A, a transmembrane protein containing an RGD (Arg-Gly-Asp) motif which can serve as an α1β1 ligand [7]. Semaphorin 7A overexpression increased FAK activation and pro-inflammatory molecule expression [7]. On the contrary, integrin αvβ3, but not α5β1, was shown to be important for high shear flow-induced FAK and NF-κB activation [8]. High shear stress is typically found within occluding arteries, suggesting that different integrins are activated at different stages of atherosclerosis [8]. Together, these studies indicate that FAK is a key signaling mediator downstream of various integrins under differential flow conditions in ECs during the initiation and progression of atherosclerotic lesions, making it a potential candidate for the treatment of atherosclerosis.

Low-density lipoproteins (LDLs) that are trapped in the subendothelial layer can undergo several modifications, including becoming oxidized LDL (oxLDL). OxLDL can be endocytosed by several cell types within the vessel wall, such as macrophages, VSMCs, and ECs, which promotes a pro-inflammatory and atherogenic environment [33][34]. ECs more readily induce pro-inflammatory molecule expression in response to oxLDL stimulation when plated on fibronectin compared to basement membrane ECM [6]. This increased inflammatory response was found to be through integrin α5β1-mediated FAK activation [6]. Follow-up studies revealed that FAK activation by oxLDL led to ERK-RSK (ribosomal S6 kinase)-NF-κB activation to promote inflammatory VCAM-1 expression and monocyte recruitment [35]. Interestingly, FAK activity in the ECs of human atherosclerotic lesions is higher when compared to healthy arteries. An EC-specific FAK KD knock-in mouse model from a C57BL/6 background also reduced western diet-induced macrophage recruitment compared to EC FAK WT mice [35].

Atherosclerosis is a chronic inflammatory condition with elevated levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), secreted by activated ECs and macrophages within atherosclerotic lesions [36][37]. Recently, it was shown that dual inhibition of FAK and Pyk2 reduced TNF-α and IL-1β induced pro-inflammatory molecule expression in human ECs [36]. However, inhibition of FAK alone or siRNA knockdown of FAK or Pyk2 only reduced some pro-inflammatory molecules, suggesting that pan-inhibition of FAK family kinases is required to suppress TNF-α and IL-1β signaling in human ECs. By using a carotid ligation model in apolipoprotein E (ApoE) KO mice fed a high fat/high cholesterol (HF/HC) diet, it was shown that FAK activity was important for VCAM-1 expression and macrophage recruitment in vivo [36]. A more recent study showed that ApoE KO and LDLR KO mice fed a HF/HC diet laced with a FAK inhibitor had reduced atherosclerotic lesions and macrophage recruitment, implicating the potential effectiveness of FAK inhibition in treating atherosclerosis [38]. Taken together, these findings demonstrate the important role of FAK in promoting atherosclerosis and inflammation under various stimuli, and that inhibiting FAK activity could reduce atherosclerotic lesions.

4. Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a pathological form of high blood pressure in the lung due to vascular remodeling of distal pulmonary arteries, leading to hypertrophy of vascular media and intima SMCs, increased arterial pressure, resistance, and plexiform lesion formation. As the lung experiences a variety of mechanical stimulation, studies have focused on the role of the ECM, integrins and their downstream effectors in PAH [39][40]. FAK activation by αvβ3 integrins was shown to be important for pulmonary artery SMC (PASMC) proliferation [2]. Hypoxia triggers PASMC proliferation via increased expression of integrin αvβ3 and FAK activation [2]. Hypoxia also increased αvβ3 integrin binding to osteoprotegerin, a secreted protein upregulated in PAH patients, which in turn led to FAK activation [2]. Knockdown of FAK using siRNA reduced osteoprotegerin-induced PASMC proliferation in vitro. During hypoxic conditions, Pyk2 led to the activation of an ERK-NF-κB-Nox4-H_2O_2 pathway that reduced the expression of PPARγ, which in turn increased Pyk2 activation leading to a feed-forward loop that promoted PASMC proliferation [41]. In hypoxia-induced PAH in mice, Pyk2 KO mice showed reduced PASMC proliferation and reduced medial thickness in the lung compared to WT controls [42]. These studies indicate that both FAK and Pyk2 play important roles in the proliferation of PASMCs in hypoxic conditions, which drives PAH disease progression.

It has been shown that PASMCs isolated from PAH patients exhibited higher migratory activity upon PDGF stimulation compared to those from healthy patients [43]. Interestingly, PAH PASMCs express higher levels of the PDGF receptor and showed increased FAK activity. Pharmacological FAK inhibition efficiently reduced PAH PASMC migration as well as prevented downstream pathways including p21-activated kinase, p38, and JNK MAPK signaling, which are known signaling contributors to PAH progression. In a monocrotaline-induced PAH model in rats, treatment with a FAK inhibitor (PF-228) or siRNA against FAK significantly reduced symptoms of PAH [44]. Loss of either FAK activity or expression reduced nuclear localization of STAT3 and active pY705 STAT3, which blocked PASMC migration in a scratch wound assay. These studies showed the potential of FAK inhibitors in reducing PASMC proliferation, migration and survival in a PAH model. More studies are needed to fully elucidate the role of FAK in PAH progression.

5. Heart Failure

Several vessel narrowing and other systemic diseases that lead to increased blood pressure ultimately result in heart failure as the heart is unable to adequately supply the body with blood containing oxygen and nutrients. These vessel narrowing diseases overload the heart capacity and initially cause hypertrophy of cardiac muscle cells (i.e., CMs). If the underlying causes are not addressed, this increased cardiac hypertrophy will eventually result in cardiac dystrophy and heart failure. FAK and Pyk2 have been shown to play an important role in the progression of heart failure.

As increased blood pressure results in elevated mechanical stress on the heart, early studies investigated the role of β3 integrins in the progression of cardiac hypertrophy. It has been shown that FAK–β3 integrin association is elevated in pressure-overloaded hypertrophic hearts [9]. Increased expression of collagen type III, fibronectin, osteopontin, and β1, α3, and α1 integrin subunits has also been correlated with advancement of cardiac hypertrophy [45][46][47]. In a phenylephrine (PE)-induced hypertrophy model, FAK activation and hypertrophy is dependent on the ECM species to which the CMs are attached. While laminin and fibronectin promoted PE-induced FAK activation and CM hypertrophy, collagen type I and gelatin failed to activate FAK or promote hypertrophy, suggesting that certain ECM–integrin associations are required for hypertrophy [48]. On the contrary, overexpression of FRNK reduced PE-induced FAK activation and blocked hypertrophy, potentially through decreased ERK MAPK activation.

Interestingly, FAK and Pyk2 exhibit differential expression and activation status during the formation of cardiac hypertrophy and progression into heart failure in vivo. While both FAK and Pyk2 showed increased expression and activation in a pressure-overload model of heart failure, elevated Pyk2 expression preceded the development of left ventricular hypertrophy (LVH) [49]. On the other hand, FAK expression was highest during heart failure [49]. FAK expression was also shown to be critical for cardiac hypertrophy in both a CM-specific FAK KO model and mice treated with FAK siRNA [50][51]. CM-specific FAK KO mice was generated by crossing FAK flox/flox mice with MLC2v-Cre mice [52]. CM-specific FAK KO mice attenuated hypertrophy compared to WT mice after four weeks of transverse aortic constriction, suggesting that FAK expression may contribute to the initiation of cardiac hypertrophy [50]. In a related study, it has been shown that siRNA knockdown of FAK was able to prevent and reverse overload-induced LVH in vivo, suggesting that FAK expression is required for the progression of hypertrophy [51]. It is not yet known if FAK activity or cellular localization is important in the progression of cardiac hypertrophy.

Heart failure can lead to an inadequate supply of blood and oxygen to CMs, resulting in a heart attack. From studies that have tried to identify which proteins play a protective role during ischemia and reperfusion, FAK has been identified to play a protective role [53][54]. Using a CM-specific FAK KO model to evaluate the protective role of FAK during ischemia/reperfusion, it was found that mice lacking FAK in CMs had an increased infarct area and increased apoptosis [53]. In another study, a transgenic mouse expressing a super-activatable FAK mutant (K578E/K581E, termed SuperFAK) with increased FAK catalytic activity had a decreased infarct area following ischemia/reperfusion [54], suggesting that FAK activity within CMs is critical for protection against ischemia/reperfusion injury. In contrast to FAK, a recent study showed that Pyk2 activation may be detrimental to CMs following ischemia/reperfusion. Ischemia/reperfusion increased active pY402 Pyk2 in mouse hearts and was associated with increased inhibitory phosphorylation of Y656 of eNOS [55], a known Pyk2 target protein [56]. Mice treated with a dual FAK/Pyk2 inhibitor had decreased infarct areas following ischemia/reperfusion in WT but not eNOS KO mice [55], suggesting that Pyk2 inhibition of eNOS is what drives tissue damage following ischemia/reperfusion. These studies indicate that FAK and Pyk2 may have opposing roles during myocardial infarction; as such, more studies are needed to better understand their roles in heart failure.

6. Aneurysm

Aneurysm is an excessive enlargement caused by a weakening artery wall, often occurring in the vessels of the abdomen, brain, back of the knee, intestine, or spleen. Alterations to the ECM, such as elastin degradation, and decreased VSMC content are some common characteristics found in aneurysms (reviewed in [57]). Deletion of both integrin α5 and αv in VSMCs using SM22α-Cre led to the formation of large aneurysms within the brachiocephalic artery during embryogenesis [3]. VSMCs isolated from α5/αv integrin KO mice only form nascent adhesions instead of assembling into mature focal adhesions. As a result, α5/αv integrin deficient VSMCs showed decreased active pY397 FAK and reduced phosphorylation of paxillin and p130 Cas. However, a link between FAK activity and the formation of a brachiocephalic aneurysm has not been determined in vivo. In human and mouse abdominal aortic aneurysms (AAAs), increased expression of periostin, an ECM protein interacting with αvβ3 and αvβ5 integrins, was associated with inflammatory cell infiltration and degradation of elastin layers [58]. Rat VSMCs subjected to mechanical strain showed elevated FAK activity, which was reduced by pretreatment with a periostin-neutralizing antibody. Pharmacological FAK inhibition reduced the expression of monocyte chemoattractant protein-1, matrix metalloproteinase-9 (MMP-9), and MMP-2, in human AAA tissue samples ex vivo [58], suggesting that FAK activity in VSMCs may drive sustained inflammation in AAAs. In addition to VSMCs, FAK in macrophages have also been shown to play an important role in the progression of aneurysms. Increased FAK expression and activity were found within human AAA samples when compared to control aortas [59]. Immunostaining revealed that the increase in active FAK was primarily concentrated to the CD68+ macrophage population. FAK activity was required for TNF-α-induced expression of MMP-9 through NF-κB activation in murine macrophages. In a $CaCl_2$-induced AAA mouse model, pharmacological FAK inhibition reduced AAA formation through decreased macrophage recruitment and MMP-9 and MMP-2 expression [59]. These studies suggest that FAK inhibitors could be used in patients in which a potential aneurysm has been detected.

7. Thrombosis

Upon blood vessel injury, platelets adhere to subendothelial ECM proteins and become activated. If a thrombus breaks off from the vessel wall, it can lead to an embolism, which is an obstruction of an artery resulting in myocardial infarction and ischemic stokes. Platelets attach to von Willebrand factor in damaged vessels through αIIbβ3 integrin and the glycoprotein (GP) Ib-IX-V. Upon adhesion and activation, platelets spread out and secrete fibrinogen-containing granules, which also bind αIIbβ3 integrin and promote the adhesion of more platelets at the site of injury [60]. FAK activation in platelets is dependent on both occupancy of αIIbβ3 and a costimulatory molecule such as epinephrine, ADP, or thrombin [10][61]. These co-stimuli increase intracellular calcium and protein kinase C activation, which in turn activates integrin–FAK signaling. The role of FAK in platelets was tested using a megakaryocyte lineage-specific platelet factor 4 (Pf4)-Cre FAK KO mouse model [62]. Deletion of FAK led to prolonged tail bleeding and decreased platelet spreading, suggesting that FAK expression is required for proper platelet function and thrombus stability. However, it seemed that platelet aggregation in FAK KO mice was normal in response to thrombin and ADP [63]. Since both WT and FAK Pf4-Cre KO mice showed similar arterial occlusion times in a $FeCl_3$ model of thrombosis, this might have been due to compensatory Pyk2 upregulation and activity [62]. While the study showed that PF-228 (a FAK-specific inhibitor) had no effect on arterial occlusion times, PF-271 (a dual FAK/Pyk2 inhibitor) prevented $FeCl_3$-induced arterial occlusion [63]. This finding indicated that both FAK and Pyk2 activity may be important for thrombus formation.

Recent studies showed that Pyk2 has multiple effects on platelet function and plays important roles downstream of both integrins and G-protein coupled receptors (GPCRs). Human platelet attachment to either monomeric type I collagen or GFOGER peptide, a specific ligand for integrin αIIb, activates Pyk2 [12]. Pyk2 activation was also dependent on phospholipase C γ-mediated intracellular calcium release. Pyk2 then promoted the activation of phosphatidylinositol-4,5-bisphosphate 3-kinase β to induce αIIbβ3 inside-out signaling [12]. Further, αIIbβ3 integrin outside-in signaling through fibrinogen activates Pyk2 in platelets [11]. Using Pyk2 KO platelets, they showed that Pyk2 was required for phosphorylation of c-Cbl, an SH2 domain-containing adapter protein. Taken together, these findings demonstrate that FAK and Pyk2 may share distinct and overlapping roles in regulating platelet functions, including platelet production, platelet activation, hemostasis, and thrombosis. The analysis of FAK/Pyk2 double conditional KO mice would certainly help to clarify the possible compensation effect and are needed for a more complete understanding of the regulation of platelet functions.

References

1. Cai, W.J.; Li, M.B.; Wu, X.; Wu, S.; Zhu, W.; Chen, D.; Luo, M.; Eitenmuller, I.; Kampmann, A.; Schaper, J.; et al. Activation of the integrins alpha 5beta 1 and alpha v beta 3 and focal adhesion kinase (FAK) during arteriogenesis. *Mol. Cell Biochem.* **2009**, *322*, 161–169, .
2. Jia, D.; Zhu, Q.; Liu, H.; Zuo, C.; He, Y.; Chen, G.; Lu, A; Osteoprotegerin Disruption Attenuates HySu-Induced Pulmonary Hypertension Through Integrin alphavbeta3/FAK/AKT Pathway Suppression. *Circ. Cardiovasc. Genet.* **2017**, , , .
3. Turner, C.J.; Badu-Nkansah, K.; Crowley, D.; van der Flier, A.; Hynes, R.O; alpha5 and alphav integrins cooperate to regulate vascular smooth muscle and neural crest functions in vivo. *Development* **2015**, *142*, 797–808, .
4. Tobias Petzold; A. Wayne Orr; Cornelia Hahn; Krishna A. Jhaveri; J. Thomas Parsons; Martin A. Schwartz; Focal adhesion kinase modulates activation of NF-kappaB by flow in endothelial cells. *American Journal of Physiology-Cell Physiology* **2009**, *297*, C814–C822, 10.1152/ajpcell.00226.2009.
5. Julián Albarrán-Juárez; András Iring; Shengpeng Wang; Sayali Joseph; Myriam Grimm; Boris Strilic; Nina Wettschureck; Till F. Althoff; Stefan Offermanns; Piezo1 and Gq/G11 promote endothelial inflammation depending on flow pattern and integrin activation. *Journal of Experimental Medicine* **2018**, *215*, 2655-2672, 10.1084/jem.20180483.
6. Yurdagul, A., Jr.; Green, J.; Albert, P.; McInnis, M.C.; Mazar, A.P.; Orr, A.W; alpha5beta1 integrin signaling mediates oxidized low-density lipoprotein-induced inflammation and early atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **2014**, *34*, 1362–1373, .
7. Hu, S.; Liu, Y.; You, T.; Heath, J.; Xu, L.; Zheng, X.; Wang, A.; Wang, Y.; Li, F.; Yang, F.; et al. Vascular Semaphorin 7A Upregulation by Disturbed Flow Promotes Atherosclerosis Through Endothelial beta1 Integrin. *Arterioscler. Thromb. Vasc. Biol.* **2018**, *38*, 335–343, .
8. Chen, J.; Green, J.; Yurdagul, A., Jr.; Albert, P.; McInnis, M.C.; Orr, A.W; alphavbeta3 Integrins Mediate Flow-Induced NF-kappaB Activation, Proinflammatory Gene Expression, and Early Atherogenic Inflammation. *Am. J. Pathol.* **2015**, *185*, 2575–2589, .
9. Dhandapani Kuppuswamy; Charlene Kerr; Takahiro Narishige; Vijaykumar S. Kasi; Donald R. Menick; G Cooper; Association of Tyrosine-phosphorylated c-Src with the Cytoskeleton of Hypertrophying Myocardium. *Journal of Biological Chemistry* **1997**, *272*, 4500-4508, 10.1074/jbc.272.7.4500.
10. S J Shattil; B Haimovich; M Cunningham; L Lipfert; J T Parsons; M H Ginsberg; J S Brugge; Tyrosine phosphorylation of pp125FAK in platelets requires coordinated signaling through integrin and agonist receptors. *Journal of Biological Chemistry* **1994**, *269*, 14738–14745, .
11. Cipolla, L.; Consonni, A.; Guidetti, G.; Canobbio, I.; Okigaki, M.; Falasca, M.; Ciraolo, E.; Hirsch, E.; Balduini, C.; Torti, M; et al. The proline-rich tyrosine kinase Pyk2 regulates platelet integrin alphaIIbbeta3 outside-in signaling. *J. Thromb. Haemost.* **2013**, *11*, 345–356, .
12. Consonni, A.; Cipolla, L.; Guidetti, G.; Canobbio, I.; Ciraolo, E.; Hirsch, E.; Falasca, M.; Okigaki, M.; Balduini, C.; Torti, M; et al. Role and regulation of phosphatidylinositol 3-kinase beta in platelet integrin alpha2beta1 signaling. *Blood* **2012**, *119*, 847–856, .

13. Andrew Newby; An overview of the vascular response to injury: a tribute to the late Russell Ross. *Toxicology Letters* **2000**, *112*, 519-529, 10.1016/s0378-4274(99)00212-x.

14. A W Clowes; M A Reidy; M M Clowes; Mechanisms of stenosis after arterial injury. *Laboratory Investigation* **1983**, *49*, 208–215, .

15. Chiraz Chaabane; Fumiyuki Otsuka; Renu Virmani; Marie-Luce Bochaton-Piallat; Biological responses in stented arteries. *Cardiovascular Research* **2013**, *99*, 353-363, 10.1093/cvr/cvt115.

16. Drachman, D.E.; Simon, D.I; Restenosis: Intracoronary Brachytherapy. *Curr. Treat. Options Cardiovasc.Med.* **2002**, *4*, 109–118, .

17. Alexandra C. Finney; Karen Y. Stokes; Christopher B. Pattillo; A. Wayne Orr; Integrin signaling in atherosclerosis. *Cellular and Molecular Life Sciences* **2017**, *74*, 2263-2282, 10.1007/s00018-017-2490-4.

18. Alex O. Morla; Jon E. Mogford; Control of Smooth Muscle Cell Proliferation and Phenotype by Integrin Signaling through Focal Adhesion Kinase. *Biochemical and Biophysical Research Communications* **2000**, *272*, 298-302, 10.1006/bbrc.2000.2769.

19. Jessica Pérez; Rebecca A. Torres; Petra Rocic; Mary J. Cismowski; David S. Weber; Victor Darley-Usmar; Pamela A. Lucchesi; PYK2 signaling is required for PDGF-dependent vascular smooth muscle cell proliferation. *American Journal of Physiology-Cell Physiology* **2011**, *301*, C242–C251, 10.1152/ajpcell.00315.2010.

20. Joan M. Taylor; Christopher P. Mack; Kate Nolan; Chris Regan; Gary K. Owens; J T Parsons; Selective Expression of an Endogenous Inhibitor of FAK Regulates Proliferation and Migration of Vascular Smooth Muscle Cells. *Molecular and Cellular Biology* **2001**, *21*, 1565-1572, 10.1128/mcb.21.5.1565-1572.2001.

21. Rebecca L. Sayers; Liisa J. Sundberg-Smith; Mauricio Rojas; Haruko Hayasaka; J. Thomas Parsons; Christopher P. Mack; Joan M. Taylor; FRNK Expression Promotes Smooth Muscle Cell Maturation During Vascular Development and After Vascular Injury. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2008**, *28*, 2115-2122, 10.1161/atvbaha.108.175455.

22. Yevgeniya E. Koshman; Taehoon Kim; Miensheng Chu; Steven J. Engman; Rekha Iyengar; Seth L. Robia; Allen Samarel; FRNK inhibition of focal adhesion kinase-dependent signaling and migration in vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2010**, *30*, 2226-2233, 10.1161/ATVBAHA.110.212761.

23. Heather A. Walker; John M. Whitelock; Pamela J. Garl; Raphael A. Nemenoff; Kurt R. Stenmark; Mary C.M. Weiser-Evans; Perlecan Up-Regulation of FRNK Suppresses Smooth Muscle Cell Proliferation via Inhibition of FAK Signaling. *Molecular Biology of the Cell* **2003**, *14*, 1941-1952, 10.1091/mbc.E02-08-0508.

24. Keeley L. Mui; YongHo Bae; Lin Gao; Shu-Lin Liu; Tina Xu; Glenn L. Radice; Christopher S. Chen; Richard K. Assoian; N-Cadherin Induction by ECM Stiffness and FAK Overrides the Spreading Requirement for Proliferation of Vascular Smooth Muscle Cells. *Cell Reports* **2015**, *10*, 1477-1486, 10.1016/j.celrep.2015.02.023.

25. Eric A. Klein; Liqun Yin; Devashish Kothapalli; Paola Castagnino; Fitzroy J. Byfield; Tina Xu; Ilya Levental; Elizabeth Hawthorne; Paul A. Janmey; Richard K. Assoian; et al. Cell-Cycle Control by Physiological Matrix Elasticity and In Vivo Tissue Stiffening. *Current Biology* **2009**, *19*, 1511-1518, 10.1016/j.cub.2009.07.069.

26. YongHo Bae; Keeley L. Mui; Bernadette Y. Hsu; Shu-Lin Liu; Alexandra Cretu; Ziba Razinia; Tina Xu; Ellen Puré; Richard K. Assoian; A FAK-Cas-Rac-Lamellipodin Signaling Module Transduces Extracellular Matrix Stiffness into Mechanosensitive Cell Cycling. *Science Signaling* **2014**, *7*, ra57, 10.1126/scisignal.2004838.

27. Kyuho Jeong; Jung-Hyun Kim; James M. Murphy; Hyeonsoo Park; Su-Jeong Kim; Yelitza A.R. Rodriguez; Hyunkyung Kong; ChungSik Choi; Jun-Lin Guan; Joan M. Taylor; et al. Nuclear Focal Adhesion Kinase Controls Vascular Smooth Muscle Cell Proliferation and Neointimal Hyperplasia Through GATA4-Mediated Cyclin D1 Transcription. *Circulation Research* **2019**, *125*, 152-166, 10.1161/circresaha.118.314344.

28. Bond, M.; Sala-Newby, G.B.; Newby, A.C; Focal adhesion kinase (FAK)-dependent regulation of S-phase kinase-associated protein-2 (Skp-2) stability. A novel mechanism regulating smooth muscle cell proliferation. *J. Biol. Chem.* **2004**, *279*, 37304–37310, .

29. Jeng-Jiann Chiu; Shu Chien; Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiological Reviews* **2011**, *91*, 327-387, 10.1152/physrev.00047.2009.

30. A. Wayne Orr; Mark H. Ginsberg; Sanford J. Shattil; Hans Deckmyn; Martin A. Schwartz; Matrix-specific Suppression of Integrin Activation in Shear Stress Signaling. *Molecular Biology of the Cell* **2006**, *17*, 4686-4697, 10.1091/mbc.E06-04-0289.

31. Yingxiao Wang; Joann Chang; Yi-Chen Li; Yi-Shuan Li; John Y.-J. Shyy; Shu Chien; Shear stress and VEGF activate IKK via the Flk-1/Cbl/Akt signaling pathway. *American Journal of Physiology-Heart and Circulatory Physiology* **2004**, *286*, H685-H692, 10.1152/ajpheart.00237.2003.

32. Wang, Y.; Flores, L.; Lu, S.; Miao, H.; Li, Y.S.; Chien, S; Shear Stress Regulates the Flk-1/Cbl/PI3K/NF-kappaB Pathway Via Actin and Tyrosine Kinases. *Cell Mol. Bioeng.* **2009**, *2*, 341–350, .

33. Jane E. Murphy; Philip R. Tedbury; Shervanthi Homer-Vanniasinkam; John H. Walker; Sreenivasan Ponnambalam; Biochemistry and cell biology of mammalian scavenger receptors. *Atherosclerosis* **2005**, *182*, 1-15, 10.1016/j.atherosclerosis.2005.03.036.

34. Jillian P. Rhoads; Amy S. Major; How Oxidized Low-Density Lipoprotein Activates Inflammatory Responses. *Critical Reviews in Immunology* **2018**, *38*, 333-342, 10.1615/CritRevImmunol.2018026483.

35. Yurdagul, A., Jr.; Sulzmaier, F.J.; Chen, X.L.; Pattillo, C.B.; Schlaepfer, D.D.; Orr, A.W; Oxidized LDL induces FAK-dependent RSK signaling to drive NF-kappaB activation and VCAM-1 expression. *J. Cell Sci.* **2016**, *129*, 1580–1591, .

36. Murphy, J.M.; Jeong, K.; Rodriguez, Y.A.R.; Kim, J.H.; Ahn, E.E.; Lim, S.S; FAK and Pyk2 activity promote TNF-alpha and IL-1beta-mediated pro-inflammatory gene expression and vascular inflammation. *Sci. Rep.* **2019**, *9*, 7617, .

37. Ssang-Taek Steve Lim; Nichol L.G. Miller; Xiao Lei Chen; Isabelle Tancioni; Colin T. Walsh; Christine Lawson; Sean Uryu; Sara M. Weis; David A. Cheresh; David D. Schlaepfer; et al. Nuclear-localized focal adhesion kinase regulates inflammatory VCAM-1 expression. *Journal of Cell Biology* **2012**, *197*, 907-919, 10.1083/jcb.201109067.

38. Takeshi Yamaura; Tatsuhiko Kasaoka; Naoko Iijima; Masaaki Kimura; Shinji Hatakeyama; Evaluation of therapeutic effects of FAK inhibition in murine models of atherosclerosis. *BMC Research Notes* **2019**, *12*, 200, 10.1186/s13104-019-4220-5.

39. Thenappan Thenappan; Stephen Y. Chan; E. Kenneth Weir; Role of extracellular matrix in the pathogenesis of pulmonary arterial hypertension. *American Journal of Physiology-Heart and Circulatory Physiology* **2018**, *315*, H1322-H1331, 10.1152/ajpheart.00136.2018.

40. Paul B. Dieffenbach; Marcy Maracle; Daniel J. Tschumperlin; Laura E. Fredenburgh; Mechanobiological Feedback in Pulmonary Vascular Disease. *Frontiers in Physiology* **2018**, *9*, 951, 10.3389/fphys.2018.00951.

41. Kaiser M. Bijli; Bum-Yong Kang; Roy L. Sutliff; C. Michael Hart; Proline-rich tyrosine kinase 2 downregulates peroxisome proliferator-activated receptor gamma to promote hypoxia-induced pulmonary artery smooth muscle cell proliferation. *Pulmonary Circulation* **2016**, *6*, 202-210, 10.1086/686012.

42. Fukai, K.; Nakamura, A.; Hoshino, A.; Nakanishi, N.; Okawa, Y.; Ariyoshi, M.; Kaimoto, S.; Uchihashi, M.; Ono, K.; Tateishi, S.; et al. Pyk2 aggravates hypoxia-induced pulmonary hypertension by activating HIF-1alpha. *Am. J. Physiol. Heart Circ. Physiol.* **2015**, *308*, H951–H959, .

43. Jamie L. Wilson; Chamila Rupasinghe; Anny Usheva; Rod Warburton; Chloe Kaplan; Linda Taylor; Nicholas Hill; Dale F. Mierke; Peter Polgar; Modulating the dysregulated migration of pulmonary arterial hypertensive smooth muscle cells with motif mimicking cell permeable peptides. *Current Topics in Peptide & Protein Research* **2015**, *16*, 1-17, .

44. Roxane Paulin; Jolyane Meloche; Audrey Courboulin; Caroline Lambert; Alois Haromy; Antony Courchesne; Pierre Bonnet; Steeve Provencher; Evangelos D. Michelakis; Sébastien Bonnet; et al. Targeting cell motility in pulmonary arterial hypertension. *European Respiratory Journal* **2013**, *43*, 531-544, 10.1183/09031936.00181312.

45. K Graf; Yung S. Do; Naoto Ashizawa; Woerner P. Meehan; Cecilia M. Giachelli; Charles C. Marboe; Eckart Fleck; W Hsueh; Myocardial osteopontin expression is associated with left ventricular hypertrophy. *Circulation* **1997**, *96*, 3063-3071, 10.1161/01.cir.96.9.3063.

46. W. Mamuya; A. Chobanian; Peter Brecher; Age-related changes in fibronectin expression in spontaneously hypertensive, Wistar-Kyoto, and Wistar rat hearts. *Circulation Research* **1992**, *71*, 1341-1350, 10.1161/01.res.71.6.1341.

47. L Terracio; K Rubin; D Gullberg; E Balog; W Carver; R Jyring; T K Borg; Expression of collagen binding integrins during cardiac development and hypertrophy. *Circulation Research* **1991**, *68*, 734–744, .

48. Joan M. Taylor; Joshua D. Rovin; J. Thomas Parsons; A Role for Focal Adhesion Kinase in Phenylephrine-induced Hypertrophy of Rat Ventricular Cardiomyocytes. *Journal of Biological Chemistry* **2000**, *275*, 19250-19257, 10.1074/jbc.m909099199.

49. Allison L. Bayer; Maria C. Heidkamp; Nehu Patel; Michael J. Porter; Steven J. Engman; Allen Samarel; PYK2 expression and phosphorylation increases in pressure overload-induced left ventricular hypertrophy. *American Journal of Physiology-Heart and Circulatory Physiology* **2002**, *283*, H695-H706, 10.1152/ajpheart.00021.2002.

50. Laura A. DiMichele; Jason T. Doherty; Mauricio Rojas; Hilary E. Beggs; Louis F. Reichardt; Christopher P. Mack; Joan M. Taylor; Myocyte-restricted focal adhesion kinase deletion attenuates pressure overload-induced hypertrophy. *Circulation Research* **2006**, *99*, 636-645, 10.1161/01.RES.0000240498.44752.d6.

51. Carolina F.M.Z. Clemente; Thaís F. Tornatore; Thais H. Theizen; Ana C. Deckmann; Tiago Campos Pereira; Iscia Lopes-Cendes; José Roberto M. Souza; Kleber G. Franchini; Targeting Focal Adhesion Kinase With Small Interfering

RNA Prevents and Reverses Load-Induced Cardiac Hypertrophy in Mice. *Circulation Research* **2007**, *101*, 1339-1348, 10.1161/circresaha.107.160978.

52. J Chen; S W Kubalak; K R Chien; Ventricular muscle-restricted targeting of the RXRalpha gene reveals a non-cell-autonomous requirement in cardiac chamber morphogenesis. *Development* **1998**, *125*, 1943–1949, .
53. Zeenat S. Hakim; Laura A. DiMichele; Mauricio Rojas; Dane Meredith; Christopher P. Mack; Joan M. Taylor; FAK regulates cardiomyocyte survival following ischemia/reperfusion. *Journal of Molecular and Cellular Cardiology* **2008**, *46*, 241-248, 10.1016/j.yjmcc.2008.10.017.
54. Zhaokang Cheng; Laura A. DiMichele; Zeenat S. Hakim; Mauricio Rojas; Christopher P. Mack; Joan M. Taylor; Targeted focal adhesion kinase activation in cardiomyocytes protects the heart from ischemia/reperfusion injury. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2012**, *32*, 924-933, 10.1161/ATVBAHA.112.245134.
55. Sofia-Iris Bibli; Zongmin Zhou; Sven Zukunft; Beate Fisslthaler; Ioanna Andreadou; Csaba Szabo; Peter Brouckaert; Ingrid Fleming; Andreas Papapetropoulos; Tyrosine phosphorylation of eNOS regulates myocardial survival after an ischaemic insult: role of PYK2. *Cardiovascular Research* **2017**, *113*, 926-937, 10.1093/cvr/cvx058.
56. Loot, A.E.; Schreiber, J.G.; Fisslthaler, B.; Fleming, I; Angiotensin II impairs endothelial function via tyrosine phosphorylation of the endothelial nitric oxide synthase. *J. Exp. Med.* **2009**, *206*, 2889–2896, .
57. Raymundo Alain Quintana; W. Robert Taylor; Cellular Mechanisms of Aortic Aneurysm Formation. *Circulation Research* **2019**, *124*, 607-618, 10.1161/circresaha.118.313187.
58. Osamu Yamashita; Koichi Yoshimura; Ayako Nagasawa; Koshiro Ueda; Noriyasu Morikage; Yasuhiro Ikeda; Kimikazu Hamano; Periostin Links Mechanical Strain to Inflammation in Abdominal Aortic Aneurysm. *PLOS ONE* **2013**, *8*, e79753, 10.1371/journal.pone.0079753.
59. Takasuke Harada; Koichi Yoshimura; Osamu Yamashita; Koshiro Ueda; Noriyasu Morikage; Yasuhiro Sawada; Kimikazu Hamano; Focal Adhesion Kinase Promotes the Progression of Aortic Aneurysm by Modulating Macrophage Behavior. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2017**, *37*, 156-165, 10.1161/atvbaha.116.308542.
60. S J Shattil; H Kashiwagi; N Pampori; Integrin signaling: the platelet paradigm. *Blood* **1998**, *91*, 2645–2657, .
61. B Haimovich; N Kaneshiki; P Ji; Protein kinase C regulates tyrosine phosphorylation of pp125FAK in platelets adherent to fibrinogen. *Blood* **1996**, *87*, 152-161, 10.1182/blood.v87.1.152.bloodjournal871152.
62. Ian S. Hitchcock; Norma E. Fox; Nicolas Prévost; Katherine Sear; Sanford J. Shattil; Kenneth Kaushansky; Roles of focal adhesion kinase (FAK) in megakaryopoiesis and platelet function: studies using a megakaryocyte lineage–specific FAK knockout. *Blood* **2008**, *111*, 596-604, 10.1182/blood-2007-05-089680.
63. M. E. Roh; M. Cosgrove; K. Górski; Ian S. Hitchcock; Off-targets effects underlie the inhibitory effect of FAK inhibitors on platelet activation: studies using Fak-deficient mice. *Journal of Thrombosis and Haemostasis* **2013**, *11*, 1776-1778, 10.1111/jth.12343.