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REGULATION OF ENDOTHELIAL CELL FUNCTION BY FAK AND PYK2

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TABLE OF CONTENTS

1. ABSTRACT

Endothelial cells form a continuous single layer lining throughout the vascular tree. Such positioning allows the endothelium to monitor numerous environmental signals within the blood vessel, including blood composition, structural matrix, and blood flow dynamics. Following signal integration, endothelial cells then induce context-specific changes in vessel properties. The nonreceptor tyrosine kinases focal adhesion kinase (FAK) and proline-rich tyrosine kinase-2 (Pyk2) are activated by integrins, growth factors, and mechanical stimuli, suggesting a potentially important role in the integration of environmental stimuli. This review will explore the current understanding of FAK and Pyk2 signaling in endothelial regulation of vascular function.

2. INTRODUCTION

2.1. Endothelial cells in vascular biology

The endothelial monolayer performs a wide range of functions, affecting nearly every aspect of vascular biology (1-4). Changing environmental conditions stimulate endothelial cells to release certain growth factors and vasoregulatory molecules which modulate blood vessel structure by altering the function of underlying smooth muscle cells. Endothelial cells and their basement membrane form a barrier between blood-derived factors and the adjacent tissue regulating tissue perfusion. In addition, endothelial cells provide an anti-coagulant surface for the lining of the blood vessel, as exposure of the subendothelial matrix promotes clot formation. Inflammatory cell targeting to sites of inflammation requires endothelial cells to express specific adhesive proteins, such as ICAM-1 and VCAM-1. These proteins display a highly-regulated pattern of expression, allowing extravasation of inflammatory cells into adjacent tissue only when endothelial cells are exposed to pro-inflammatory stimuli. Lastly, the formation of new blood vessels from pre-existing vessels following tumor development or ischemic injury requires endothelial cell invasion into surrounding tissue and reorganization into tubular structures.

2.2. Signaling through FAK and Pyk2

Cell function is highly dependent upon environmental cues, such as growth factors, cell-cell and cell-matrix interactions, extracellular matrix proteins, and mechanical stimuli. The integrin class of matrix protein receptors “integrate” the cellular cytoskeleton with the extracellular matrix, by mechanically coupling the cell to its environment and by tranducing the composition and mechanical properties of the matrix through activation of specific signaling pathways (5,6). In addition, many

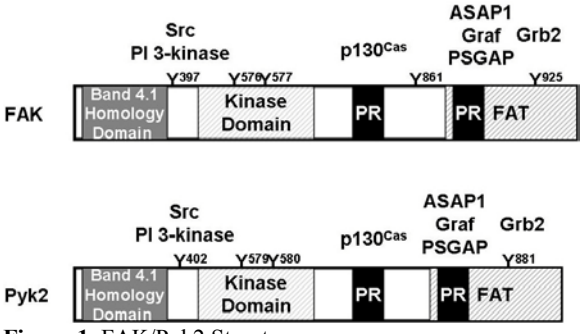

Figure 1. FAK/Pyk2 Structure.

growth factor-induced responses, such as Rac activation and ERK phosphorylation, require integrin-mediated adhesion (6,7). The ability of integrins to regulate cellular responses to such a wide variety of extracellular stimuli underscores the importance of cell-matrix interactions in cell physiology.

Upon activation, integrins bind to their matrix ligands resulting in integrin clustering. Structural and signaling proteins are recruited into these newly formed integrin clusters, allowing both structure-dependent signal regulation and signaling-induced changes in adhesion complex structure. This interplay allows for the formation of several different types of adhesions, each with distinct mechanical and signaling properties. Focal adhesions are highly-organized protein scaffolds which link integrins and bundled actin microfilaments termed stress fibers. Focal adhesions function as both structural and signaling units. Focal adhesion kinase (FAK) was first identified in 1992 as a nonreceptor tyrosine kinase which targets to sites of integrin clustering (8). While most non-receptor tyrosine kinases are recruited into signaling complexes through interactions between SH2 domains and SH3 domains with phosphotyrosines and proline-rich regions, respectively, FAK does not contain either SH2 or SH3 domains and appears to target to newly formed adhesions through interactions between FAK's C-terminal domain and the integrin-associated cytoskeletal anchor proteins paxillin and talin, although other interactions may also contribute to FAK's localization (9-11). FAK expression is ubiquitous and FAK is activated by numerous integrins, including β1, β2, and β3 integrins, suggesting FAK activation is a common adhesion-dependent signal. Upon localization to integrin clusters, FAK becomes autophosphorylated on Tyr397 which activates a number of downstream targets through recruitment of SH2 domain-containing proteins such as c-Src, PI 3-kinase, and Shc (Figure 1) (12-14). Src recruitment results in phosphorylation of several other residues on FAK, including Tyr576/577 required for maximal kinase activity and the proposed Grb2-binding site Tyr925 (15,16). In addition, FAK can also interact with SH3 domain-containing proteins, such as p130Cas, ASAP1, and PSGAP, through one of two proline-rich sequences in FAK's C-terminal domain (17-20). Regulation of these SH3 domain-dependent interactions is poorly understood, although phosphorylation of serine residues within FAK's proline-rich sequences could be involved (21).

The proline-rich tyrosine kinase-2 (Pyk2), also known as cell-associated kinase-beta (CAK-β), related adhesion focal tyrosine kinase (RAFTK), or calcium-dependent tyrosine kinase (CADTK), exhibits a considerable level of structural and sequence homology to FAK (22). However, unlike FAK, Pyk2 expression is highly cell type- and tissue specific. Pyk2 is generally located in the cytoplasmic compartment and perinuclear region, although Pyk2 can become localized to integrin clusters following activation (23). In addition, while FAK activation is closely tied to integrin-mediated adhesion, activation of Pyk2 is often independent of cell adhesion. Pyk2 is tyrosine phosphorylated in response to cellular stress (ex. UV irradiation, TNF-α, hyperosmotic shock), G protein-coupled receptor agonists (ex. angiotensin II, thrombin, lysophosphatidylcholine), and growth factors (VEGF, bFGF, PDGF) which induce an increase in intracellular calcium and activation of PKC (22,24). In some non-adherent cells, such as B cells and platelets, Pyk2 can be activated in response to integrin-mediated adhesion, although this may depend upon integrin-induced calcium transients (25,26). Pyk2 structure is very similar to FAK, containing a kinase domain and two proline-rich domains, as well as several phosphorylated residues homologous to FAK, including an autophosphorylation site (Tyr402), sites involved in kinase activation (Tyr579/580), and a site (Tyr881) homologous to the Grb2-binding site in FAK (22,24). As such, FAK and Pyk2 share many common downstream signaling partners, including Src, Shc, and p130Cas. However, some signaling partners are specific for Pyk2, such as Nirs (mammalian homologs of Drosophila rdgB) and gelsolin (27,28). Furthermore, Pyk2 phosphorylation is thought to be a critical mediator of G protein-mediated activation of the MAP kinases: extracellular signal regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38 MAP kinase (29,30). While FAK signaling in endothelial cells is well established, the role of Pyk2 in endothelial cell function has been given less attention. So while Pyk2 may play an important role in regulating endothelial cell function, there is considerably less information. Thus, this review will focus mainly on signaling through FAK, although relevant information on what is currently known about Pyk2 signaling in these systems will be discussed.

3. ENDOTHELIAL CELL TURNOVER

3.1. FAK regulation of endothelial cell proliferation

Cell proliferation in adherent cell types requires integrin-derived signals. FAK signaling appears to be involved in endothelial proliferation, since inhibiting FAK signaling in HUVECs results in lower proliferation rates (31). In addition, endothelial FAK signaling is implicated in the matrix specific activation of Rac, which drives cells through the G1-S transition (32). In this study, cells plated on fibronectin displayed FAK-dependent Rac activation, which stimulated expression of cyclin D1 inducing phosphorylation of cdk4 and cdk6 and transition to the S phase of the cell cycle. Interestingly, this same signal was not activated in cells plated on laminin. This is consistent with previous reports suggesting enhanced proliferation on α5β1 and αvβ3 ligands (ex. fibronectin), as compared to

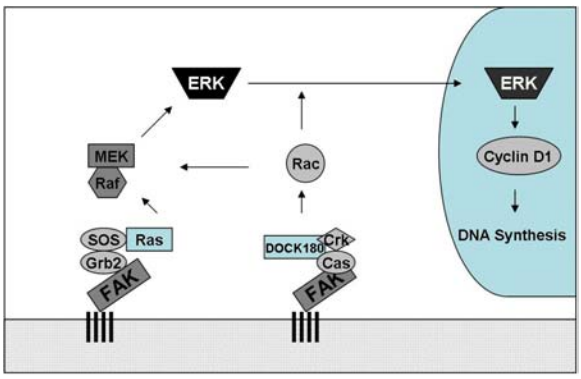

Figure 2. FAK Signaling in Endothelial Proliferation. FAK phosphorylation results in recruitment of the adapter proteins Shc and Grb2. These proteins recruit the Ras activating protein Sos leading to sequential activation of Raf, MEK, and ERK. Interactions between FAK and p130Cas are enhanced by FAK activation, leading to the recruitment of a Crk/DOCK180 complex, which activates Rac. Rac regulates ERK signaling by inducing Raf/MEK complex formation and by facilitating nuclear transport of active ERK. Nuclear ERK then stimulates the transcription of cyclin D1, a key initiator of S phase.

α2β1 ligands (ex. laminin) (33). In addition to Rac activation, FAK-induced ERK activation may also be a key mitogenic pathway (Figure 2). In fibroblasts, FAK signaling regulates cell cycle progression through Src-dependent activation of Grb2 and p130Cas (34). ERK activation downstream of Grb2 has been implicated in cell cycle progression, as has Rac activation downstream of p130Cas phosphorylation (32,35,36). In addition, Rac activation facilitates signaling through the ERK pathway, both by activation of upstream kinases and by regulating nuclear targeting (33,37). Consistent with this, induction of cyclin D1 is most often associated with activation of the ERK pathway (33,38).

3.2. FAK signaling in endothelial cell apoptosis

Cell survival requires specific matrix-derived signals, and loss of these signals results in a subset of apoptosis termed anoikis. Constitutively active FAK can rescue cells from anoikis, suggesting a key role for FAK signaling in matrix-induced cell survival (39). FAK signaling activates several anti-apoptotic pathways, including phosphorylation of JNK and activation of the PI 3-kinase/Akt pathway (40,41). In fibroblasts, FAK stimulates JNK activation through a Rac/PAK/MKK4 pathway, and this is the primary anti-apoptotic pathway in the absence of serum (40). However, the anti-apoptotic effect of serum is also mediated by FAK through activation of PI 3-kinase and Akt. VEGF-induced FAK phosphorylation on Tyr861 is postulated to mediate VEGF's anti-apoptotic function, although the mechanisms employed are unknown (42). An anti-apoptotic sequence (hep I) from the N-terminal domain of thrombospondin also stimulates FAK phosphorylation at Tyr397 and Tyr861, although the downstream effectors of this anti-apoptotic effect have not been determined (Orr *et al.*, unpublished; Elzie *et al.*, unpublished). Consistent with an anti-apoptotic function, several pro-apoptotic stimuli inhibit signaling through FAK. FAK is cleaved upon initiation of apoptosis by caspase 3, and this is thought to reduce the FAK's ability to inhibit apoptosis (43). Consistent with this, adenosine-homocysteine-induced endothelial cell apoptosis involves proteolytic degradation of FAK and is inhibited by FAK overexpression, but not by overexpression of a FAK construct incapable of PI 3-kinase activation (44,45). The snake venom disintegrin salmosin stimulates endothelial cell apoptosis concomitant with decreased FAK phosphorylation (46). Staurosporine, a microbial alkaloid known for its pro-apoptotic effects, stimulates porcine aortic endothelial cell apoptosis through FAK dephosphorylation at Tyr397 and Tyr861 (47). The staurosporine-induced pro-apoptotic effect was not associated with FAK proteolysis, suggesting FAK dephosphorylation is also an important pro-apoptotic signal.

4.0 ENDOTHELIAL CELL MIGRATION AND ANGIOGENESIS

4.1. Regulation of angiogenesis

Angiogenesis, the formation of new capillaries from pre-existing blood vessels, is involved in vascular maturation during development and in tissue homeostasis following remodeling stimuli, such as tissue wounding or tumor progression. To accomplish this, endothelial cells move out of their monolayers, migrate and proliferate into the adjacent tissue, and form a lumen completing the capillary structure (48-50). Angiogenic growth factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), stimulate endothelial cells to loosen their cell-cell contacts and produce proteolytic enzymes which degrade the subendothelial basement membrane. The endothelial cells then utilize their matrix contacts to migrate through the basement membrane completing the cell sprouting process. Following sprouting, the leading edge of endothelial cells continues to migrate toward the angiogenic stimulus, while cells in the trailing edge display higher rates of proliferation. When this moving mass of endothelial cells forms a continuous connection between adjacent vessels, they reorganize into capillary tubes complete with lumen forming a new functional vessel. Although these are the minimal steps required, maturation of the newly formed vessel may involve more processes, such as smooth muscle recruitment and organization. The entire process is regulated at multiple levels, with progression determined by the balance of numerous pro-angiogenic and anti-angiogenic factors.

4.2. FAK signaling in growth factor-induced angiogenesis

FAK is activated downstream of both integrins and growth factors, suggesting FAK may be a key integrator of the matrix and growth factor signaling associated with angiogenesis. Consistent with this, FAK knockout mice die early in development due to severe developmental defects, such as in vasculogenesis (51). In addition, FAK expression is increased in angiogenic endothelial cells associated with malignant astrocytic tumors, and tumor-associated angiogenesis can be significantly diminished by transfecting the endothelial cells

with the endogenous FAK inhibitor FAK-related non-kinase (FRNK) (52). However, FAK appears to exert its pro-angiogenic effects through multiple mechanisms. For example, FAK is activated by angiopoietin-1 signaling through the Tie2 receptor, and this is required for angiopoietin-1-induced endothelial cell sprouting (53). Tumstatin, a cryptic fragment of collagen IV, binds to αvβ3 integrins and inhibits FAK activation, resulting in inhibition of protein synthesis (54). Sphingosine-1-phosphate (S1P) and vascular endothelial growth factor (VEGF) utilize FAK signaling to stimulate angiogenesis primarily through increased endothelial cell migration and possibly, increased proliferation (55,56). While the precise angiogenic FAK signals activated by angiopoietin-1 and S1P are unknown, the role of FAK in VEGF-induced angiogenesis is beginning to be defined. VEGF interacts with the Flk-1 receptor resulting in FAK phosphorylation, which stimulates FAK to bind and activate PI 3-kinase (55). FAK-dependent PI 3-kinase activation is required for VEGF-induced endothelial cell migration, and likely involves phosphorylation of the known PI 3-kinase-binding site (Tyr397). In addition, VEGF signaling through Flk-1 stimulates Src-dependent FAK phosphorylation at Tyr861, and VEGF-induced HUVEC migration is Src-dependent, suggesting FAK Tyr861 may be involved in VEGF-induced endothelial cell migration (42). However, Tyr861 does not appear to be involved in FAK-induced PI 3-kinase activation, suggesting VEGF utilizes multiple pro-migratory signaling events downstream of FAK activation. VEGF signaling through Flt-1 can stimulate tube formation in endothelial cells and fibroblasts, and this also occurs through a FAK-dependent process (57). Thus, growth factor-induced FAK signaling appears to be involved in multiple processes during angiogenesis, and downstream effects of FAK activation likely differ due to changes in signal context.

4.3. FAK in endothelial cell invasion

Invasion of endothelial cells into the surrounding tissue requires increased expression of proteolytic enzymes, such as plasmin and the matrix metalloproteases (MMPs), as well as increased cell migration (50,58). The initial barrier to endothelial invasion is the subendothelial basement membrane. Expression of the gelatinases MMP-2 and MMP-9 mediates degradation of gelatin, collagen I, and collagen IV, and is thus thought to be critical to invasion of basement membranes. In addition, these proteases serve to loosen endothelial tight junctions enabling cells to move out of the monolayer (59). FAK is involved in MMP-2 expression in small-cell lung carcinoma (60), and expression of MMP-2 in v-Src-transformed NIH 3T3 fibroblasts is attenuated by expressing the inhibitory FAK C-terminal fragment FAK-related non-kinase (FRNK) (61). In addition, EGF-induced MMP-9 expression and secretion was reduced in FRNK-expressing adenocarcinoma cells (62). Concanavalin A-induced MMP-2 and MMP-9 expression in FAK knockout fibroblasts was significantly increased following FAK reexpression (63). Despite this evidence, the role of FAK in MMP expression in endothelial cells has not been specifically addressed. In one study, angiopoietin-1 stimulated PI 3-kinase-dependent FAK phosphorylation and MMP-2 expression suggesting that FAK might similarly regulate MMPs in endothelial cells (53).

FAK signaling is also involved in the dynamic regulation of adhesion and cytoskeletal organization during cell migration (64). FAK knockout fibroblasts and fibroblasts transfected with FRNK demonstrate an increase in the number and size of focal adhesions, and a subsequent decrease in cell migration (31,65). The first evidence for FAK's role in endothelial cell migration came from experiments using wounded endothelial cell monolayers. Romer *et al.* demonstrated that FAK phosphorylation and kinase activity was increased in wounded monolayers, where migration is high (66). Loading of the FAK C-terminus in HUVECs resulted in decreased FAK phosphorylation and decreased cell migration (31). In addition, VEGF and sphingosine-1-phosphate stimulate endothelial cell migration through a FAK-dependent mechanism (55,56). The hep I sequence of thrombospondin stimulates focal adhesion disassembly through a FAK-dependent pathway involving both ERK and PI 3-kinase (Orr *et al.*, unpublished). Thrombospondin-mediated focal adhesion disassembly is critical for its pro-migratory effect in endothelial cells, suggesting a role for FAK in thrombospondin-induced endothelial cell migration, although this has not been specifically addressed (67). Thus, FAK appears to be a critical component of the cell migration machinery, and a key transducer of both integrin-derived and growth factor-derived promigratory signals.

4.4. Pyk2 signaling in angiogenesis

Tang *et al.* found that Pyk2 is preferentially expressed in endothelial cells derived from pulmonary tissue in both mouse and human, underscoring the heterogeneity of endothelial cells depending upon the site of origin (68). Overexpression of a catalytically inactive form of Pyk2, Pyk2-K457A, in pulmonary endothelial cells resulted in defects in cell adhesion, spreading, and migration (68). This also translated into decreased capacity for angiogenesis, as measured by observing the formation of capillary-like structures on Matrigel and by assaying vessel sprouting in *ex vivo* explants of pulmonary artery and pulmonary vein in Matrigel. The ability of catalytically inactive Pyk2 to inhibit adhesion and migration was not observed in HUVECs or NIH3T3 fibroblasts, which express very low levels of Pyk2. Expression of FAK, p130CAS, and Hef1 were significantly reduced in pulmonary vein endothelial cells expressing catalytically inactive Pyk2, suggesting that a Pyk2-dependent pathway is vital for regulation of protein expression in pulmonary endothelial cells (68). These data indicate that Pyk2 signaling plays an important role in pulmonary angiogenesis, and at least a portion of this affect appears to involve regulation of endothelial gene expression. The reasons behind the apparent preferential importance of Pyk2 in pulmonary endothelium remain unknown.

5. ENDOTHELIAL BARRIER FUNCTION

5.1. Modulating vascular permeability

The main function of the vasculature is the delivery of oxygen and nutrients to all the tissues of the body. While this occurs along the entire vascular tree, the

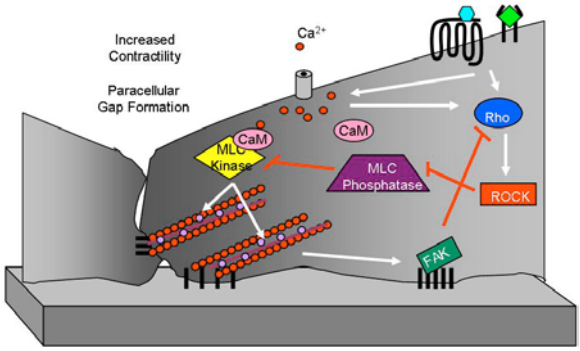

Figure 3. Regulation of Endothelial Contractility by FAK. Myosin light chain phosphorylation regulates endothelial cell contractility and monolayer permeability. Contractility is activated through Ca2+/calmodulin-dependent stimulation of myosin light chain kinase, and Rho/Rho kinase-dependent inhibition of myosin light chain phosphatase. Increased contractility stimulates FAK phosphorylation, which limits Rho activity, providing a break for the contractile and permeability responses.

majority of molecular exchange occurs within capillaries where endothelial cells comprise the main cellular component. The endothelial monolayer and its underlying basement membrane serve as a barrier between factors and cells within the bloodstream and the surrounding tissue. While vessels maintain a basal level of permeability, certain situations, such as inflammation and ischemia, require a localized increase in vascular permeability (69). Localized increases in permeability are both vital, serving to correct the inciting stimuli, and potentially deleterious, as the body's immune response can injure the surrounding tissue. Thus, a tight regulation of vascular permeability is necessary to maintain vascular homeostasis, while retaining the ability to react to acute remodeling stimuli. Endothelial monolayers utilize three mechanisms for increasing vascular permeability: cell-cell junction disassembly, cellular contraction, and fenestration, or formation of transcellular pores (70-72). While FAK signaling may affect all of these functions, the best characterized role for FAK in vascular permeability is through the regulation of endothelial cell contractility.

5.2. Endothelial contractility and FAK signaling

The factors that regulate paracellular gap formation can be though of as contractile forces stimulating gap formation and adhesive forces generated at sites of cell-cell and cell-matrix adhesions opposing gap formation. Contractility is generated through interactions between activated myosin and actin filaments. Endothelial cells contain an abundance of these contractile components, with actin and myosin comprising ~16 % of total cellular protein (73). Myosin, the contractile element in this machinery, becomes activated following phosphorylation of the myosin light chain (MLC) regulatory subunit (74). The classic signaling pathway for MLC phosphorylation involves Ca^{2+}/calmodulin-dependent MLC kinase (MLCK) autophosphorylation, resulting in enhanced kinase activity (Figure 3) (75). In addition, unlike smooth muscle cells, endothelial cells express an isoform of MLC kinase containing a unique NH2-terminal domain which is sensitive to phosphorylation by Src (76,77). Consequently, stimulation of endothelial cell permeability by phosphatase inhibitors is blocked by inhibiting Src signaling (78,79). The inactivating enzyme, MLC phosphatase, is inhibited by phosphorylation allowing for prolonged MLC phosphorylation and sustained contraction (80,81). The best characterized MLC phosphatase inactivating pathway occurs through the small GTPase Rho, which stimulates the Ser/Thr kinase ROCK to phosphorylate and inactivate MLC phosphatase (80). Consistent with this, preventing activation of Rho and ROCK results in enhanced endothelial cell barrier function (82-85). Thus, contractility-induced permeability occurs in response to growth factors and bioactive molecules that induce an increase in intracellular calcium and that stimulate Rho activation, such as thrombin, LPA, and histamine (82,85-87).

FAK signaling appears to influence the vascular permeability response primarily through its relationship with cellular contraction. Rho activity stimulates enhanced cellular contraction, focal adhesion maturation, and FAK phosphorylation (88,89). FAK signaling is postulated to limit Rho activation, since FAK knockout fibroblasts have enhanced levels of active Rho (Figure 3) (90). The target for FAK's regulation of Rho activity is unknown, however FAK can bind directly to the RhoGAPs Graf and PSGAP and may be involved in Src-dependent activation of p190 RhoGAP following integrin ligation (20,90-92). Thrombin, a well characterized permeability mediator, stimulates cellular contractility through the classic Rho-dependent pathway, resulting in enhanced focal adhesion and stress fiber formation and increased FAK phosphorylation (82,85). Reducing FAK expression results in an enhanced and prolonged permeability response in response to thrombin, consistent with the proposed negative feedback role for FAK in the contractility response (93). Thus, FAK signaling appears to be involved in a negative feedback loop in which the cell limits its contractile responses.

5.3. Endothelial cell interactions with inflammatory cells

Inflammatory cells use the endothelial cell monolayer to target areas of active inflammation. Various inflammatory cytokines and growth factors stimulate nearby endothelial cells to express adhesion markers which inflammatory cells use to adhere to the endothelium and migrate between adjacent endothelial cells (94,95). The initial interactions between inflammatory cells and endothelial cells are mediated through the selectins, cell surface glycoproteins varying in length. The propulsive force of blood flow combined with the adhesive force between inflammatory cells and endothelial cells cause the inflammatory cell to roll along the endothelial monolayer until adhesive forces overcome the propulsive forces of flow (96,97). Strong adhesive events mostly involve endothelial cell Ig-like proteins, including ICAM-1 and VCAM-1, and β2 integrins (LFA-1, MAC-1) on inflammatory cells. Regulation of this interaction occurs at endothelial expression and activation of ICAM-1 and VCAM-1 and at integrin activation in the inflammatory cells. As the inflammatory cell stops rolling and sticks to

the endothelium at sites of inflammation, subsequent transendothelial migration requires loosening of endothelial cell-cell adhesions and formation of intercellular gaps for inflammatory cells to migrate through (98).

Stable lymphocyte-endothelial association induces remodeling of the endothelial cell cytoskeleton concomitant with an increase in FAK phosphorylation (99). Binding of T-cells to P-selectin and E-selectin stimulates Src-dependent FAK phosphorylation, similar to that seen during hyperpermeability responses (100). Consistent with this, remnant-like lipoprotein particles stimulate increased monocyte binding to endothelial cells through the previously described permeability pathway involving Rho-induced contractility leading to FAK phosphorylation (101). Rho activation also regulates clustering of leukocyte receptors on endothelial cells, such as E-selectin, ICAM-1, and VCAM-1, suggesting a role for Rho activation in stimulation of leukocyte homing (102). Furthermore, ICAM-1 ligation is associated with Rho activation, and inhibiting Rho signaling reduces ICAM-1 expression (103-104). While the role of FAK in this pathway has not been determined, one can infer from the vascular permeability data that Rho-induced FAK activation may be a negative feedback loop to limit permeability responses and inflammatory cell recruitment.

6. HEMODYNAMIC FORCE TRANSDUCTION

6.1. Endothelial cell mechanotransduction

An important aspect of endothelial biology is the ability to sense various aspects of blood vessel mechanical properties, including pressure, stretch, and shear stress, and to regulate blood vessel biology accordingly (105-108). The importance of mechanical stimulation on blood vessel biology is most apparent with shear stress and its atheroprotective properties (109-111). Laminar shear stress suppresses endothelial cell proliferation and apoptosis, maintaining the endothelial monolayer in a quiescent state (112,113). In addition, laminar flow induces production of anti-coagulant molecules such as prostaglandins and nitric oxide and reduces the expression of adhesive proteins involved in leukocyte recruitment, such as ICAM-1 and VCAM-1 (114-116). In contrast, turbulent blood flow enhances endothelial cell proliferation and apoptosis, processes incompatible with maintenance of a functional monolayer (110). Furthermore, turbulent flow conditions result in increased expression of ICAM-1 and VCAM-1 stimulating increased monocyte recruitment (116-117). Consistent with this, atherosclerotic plaques preferentially arise in areas of the vascular tree where turbulent flow patterns predominate, such as in large arteries with curvatures and bifurcations (109-111).

Mechanotransduction refers to the conversion of mechanical forces into biochemical signals. Several mechanoresponsive elements within the cell, including the apical endothelial glycocalyx, adherens junctions, and integrin-mediated matrix adhesions, are linked to the actin cytoskeleton, suggesting a potential connection between cytoskeletal elements and mechanosensory signaling (105,108). Consistent with this, actin stress fibers and focal adhesions align following exposure to laminar flow patterns, and this cytoskeletal alignment is followed by total cellular alignment, as the cell elongates in the direction of flow (118). Cellular alignment is postulated to be an adaptive response, as aligned cells show reduced cytoskeletal deformation in response to flow. Cyclic strain, such as that induced by pulsatile blood flow, also induces endothelial cytoskeletal remodeling, although unlike shear stress, cyclic strain induces cytoskeletal alignment perpendicular to the axis of stretch (119). However, since the axis of stretch is perpendicular to the axis of flow, both mechanical stimuli within the blood vessel stimulate cells to align in the same direction. Consistent with an atheroprotective role for shear stress, endothelial cells in atherosclerotic prone areas of vasculature display a cobblestone-like morphology (120,121).

6.2. Mechanotransduction through FAK

Shear stress stimulates integrin activation and subsequent ligation-dependent signaling, including FAK phosphorylation (122-124). FAK phosphorylation recruits the Grb2/SOS complex, and inhibiting FAK signaling by transfection with FAK (F397Y) prevents shear stress-mediated activation of ERK2 and JNK1 (124). Although FAK phosphorylation is not the only signal to be implicated in shear stress-induced MAP kinase activation, the requirement for FAK signaling in shear stress-mediated MAP kinase activation suggests integrin activation is a critical event in this pathway. MAP kinase activation is required for induction of several pro-inflammatory cytokines, including IL-8 and MCP-1, suggesting FAK is involved in shear stress-induced alterations in endothelial cell gene expression (125). In addition, integrin activation drives the cytoskeletal and cellular alignment associated with shear stress through regulating the activity of the Rho family GTPases (126-128). Li *et al.* demonstrated that shear stress stimulates cell migration in the direction of flow due to lamellipodia extension in the downstream edge of endothelial cells leading to polarized focal adhesion formation and FAK phosphorylation (129). However, the effect of inhibiting FAK signaling was not addressed in this study. While the role of FAK signaling in these morphological changes has yet to be determined, one could predict from previous data that FAK signaling may regulate shear stress-induced alignment through regulation of Rac and Rho activity.

Like shear stress, cyclic strain also stimulates integrin reorganization, FAK phosphorylation, and cellular elongation (130,131). This appears to involve activation of stretch-activated calcium channels, since removal of extracellular calcium or addition of the stretch-activated calcium channel inhibitor gadolinium (Gd3+) both inhibit this response (132,133). Suppressing FAK expression also prevented the strain-induced paxillin phosphorylation and morphological changes, suggesting FAK signaling is critically involved in strain-induced cellular alignment (133).

6.3. Pyk2 in Mechanical Signaling

Pulsatile flow results in rhythmic distension in endothelial cells, and this cyclic strain modulates the

endothelial cell gene expression profile (134,135). While hemodynamic mechanotransduction is known to stimulate calcium mobilization, evidence is mounting suggesting that mechanosensitive-modulation of endothelial gene expression is redox sensitive. Cyclic strain stimulates NADPH oxidase activity through a calcium- and PKCα-dependent mechanism (136,137). Inhibition of reactive oxygen species production blocks cyclic strain-induced Pyk2 phosphorylation, while addition of H2O2 stimulates Pyk2 phosphorylation (136). Like cyclic strain, acute onset of flow results in PLC- and calcium-dependent generation of reactive oxygen species, which are required for shear stress-mediated Pyk2 phosphorylation (137). Furthermore, shear stress-dependent p130Cas phosphorylation requires Pyk2, suggesting a role for Pyk2 in shear stress-induced Rac activation and JNK phosphorylation (137). While the precise role of Pyk2 phosphorylation in mediating endothelial cell responses to cyclic strain and shear stress is currently unknown, reactive oxygen species production in response to cyclic strain and shear stress leads to the expression of several pro-inflammatory proteins, such as ICAM-1 and MCP-1 (138-140).

7. SUMMARY AND PERSPECTIVE

A wide variety of stimuli result in endothelial FAK and Pyk2 activation, and this activation propagates an equally immense array of signals. To gain functional insight into FAK and Pyk2 signaling, a working knowledge of how signals become integrated and the effects of specific signal combinations must be established. While our current understanding of FAK- and Pyk2-dependent signal integration is lacking, specific patterns of signal consequences are becoming apparent. For example, FAK is a critical player in the adhesion-dependent survival and propagation of endothelial cells, preventing apoptosis through activation of JNK and PI 3-kinase and stimulating the G1-S transition through a Rac-ERK-cyclin D1 dependent pathway. In addition, FAK appears to be critically involved in migration of adherent cells, and mediates pro-angiogenic signaling downstream of a variety of growth factors. Contractility-dependent permeability responses, such as following thrombin treatment or inflammatory cell recruitment, appear to be limited by FAK signaling, most likely through the downregulation of Rho activity. Finally, hemodynamic forces, such as cyclic stretch and shear stress, modulate endothelial cell shape and gene expression through FAK-related and FAK-dependent pathways respectively.

Several growth factors and cellular stresses stimulate Pyk2 activation, however the functional consequences of these signals have not been conclusively demonstrated. Given the ability of Pyk2 to activate the MAP kinase cascades downstream of a variety of stimuli, the most obvious function for Pyk2 in endothelial cells is the regulation endothelial gene expression. Consistent with this, pulmonary endothelial cells express high levels of Pyk2, and inhibiting Pyk2 signaling in these cells results in defective angiogenesis through altered gene expression, including expression of FAK. However, given the diversity of signals downstream of FAK, Pyk2 will likely be found to induce additional cellular functions in endothelial cells following a more in-depth examination of Pyk2 signaling.

8. ACKNOWLEDGEMENTS

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