Biomechanical Stress-induced Signaling in Smooth Muscle Cells: An Update

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Abstract: The vascular wall is an integrated functional component of the circulatory system that is continually remodelling or is developing atherosclerosis in response to hemodynamic or biomechanical stress. In this process mechanical force is an important modulator of Vascular Smooth Muscle Cell (VSMC) morphology and function, including apoptosis, hypertrophy and proliferation that contribute to the development of atherosclerosis, hypertension, and restenosis. How VSMCs sense and transduce the extracellular mechanical signals into the cell nucleus resulting in quantitative and qualitative changes in gene expression is an interesting and important research field. It has been demonstrated that mechanical stress rapidly induces phosphorylation of the platelet-derived growth factor (PDGF) receptor, activation of integrin receptor, stretch-activated cation channels, and G proteins, which might serve as mechanosensors. Once the mechanical force is sensed, protein kinase C and Mitogen Activated Protein Kinases (MAPKs) were activated, leading to increased transcription factor activation. Thus, mechanical stresses can directly stretch the cell membrane and alter receptor or G protein conformation, thereby initiating signaling pathways, usually used by growth factors. Based on the progress in this field, this article attempts to formulate a biomechanical stress hypothesis, i.e. that physical force initiates signal pathways leading to vascular cell death and inflammatory response followed by VSMC proliferation. These findings have provided promising information for designing new drugs or genes for therapeutic interventions for vascular diseases.

Keywords: Mechanical stress, smooth muscle cells, signal transduction, apoptosis, proliferation.

1. INTRODUCTION

Vascular Smooth Muscle Cells (VSMCs) are a major constituent of blood vessel walls and has a function in the maintenance of vessel structure[1]. VSMCs are exposed to mechanical stretch, a cyclic strain stress due to blood pressure. Along with other groups we have shown that hypertrophy, hyperplasia, and migration of VSMCs are the key events in the development of atherosclerosis, including hypertension-related atherosclerosis, angioplasty-induced restenosis, venous bypass graft arteriosclerosis, and spontaneous atherosclerosis [2-10]. Normally, adult arterial SMCs are contractile and not particularly responsive to growth factors or growth regulatory molecules that induce proliferation and cell migration [3]. In response to changes in mechanical stress several contractile proteins that are normally expressed in adult arterial walls gradually become undetectable. These proteins are smooth muscle myosin heavy chain isoforms, α-actin, h-caldesmon, and calponin. Non-muscle myosin heavy chain is mostly expressed in fetal vessel walls but is re-expressed in VSMCs that have been subjected to mechanical stress [1, 3, 11]. Mechanical stress leads to obvious structural reorganization with myofilament loss and cells developing extensive endoplasmic reticulum and large Golgi complexes and these cells show what is known as the synthetic phenotype. The cells lose their contractile ability, increase protein secretion and are more responsive to autocrine and paracrine growth factors produced in response to increased mechanical stress (high blood pressure). These growth factors further stimulate smooth muscle cell (SMC) hypertrophy and/or arterial wall intimal hyperplasia [3, 12, 13].

Hypertension increases biomechanical stress on the arterial walls by up to 30%, inducing VSMC hypertrophy and hyperplasia which leads to continuously elevated peripheral vascular resistance [14]. Spontaneous atherosclerotic lesions occur preferentially at bifurcation and curvatures, where hemodynamic force is disturbed [15, 16]. VSMC proliferation in large arteries and veins often occurs after surgical interventions such as angioplasty and vein bypass grafts. Increased biomechanical stress by disturbed local blood flow results in restenosis [17] while the increased biomechanical stress on vein grafts rapidly elevates blood pressure more than 10-fold (arterial vs. venous). We showed that this is a strong stimulus for SMC proliferation and leads to venous bypass graft-arteriosclerosis [18]. Spontaneous atherosclerotic lesions occur preferentially at bifurcation and curvatures, where hemodynamic force is disturbed [15, 16].

All tissues are subjected to physical forces originating either environmental or from tension, created by cells themselves [4, 5, 19, 20]. Only recently it has been recognised that mechanical force is an important regulator of structure and function for mammalian cells, tissues, and organs. Physical stimuli are sensed by cells before signals are transmitted via intracellular signal transduction pathways to the nucleus which result in physiological responses or pathological conditions. In this field, significant progress has

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recently been achieved, and this article will focus on the biomechanical stress-induced signaling events in VSMCs and attempt to develop a biomechanical stress hypothesis that physical force initiates signaling pathways leading to vascular cell death and an inflammatory response followed by smooth muscle cell proliferation.

Mechanical stress may initiate signaling by physically perturbing the cell surface or altering receptor conformation in a non-biochemical manner. These changes may initiate signaling pathways which lead to altered gene expression and release of autocrine and paracrine growth factors that may amplify the response. The change in gene expression and the action of growth factors released in response to biomechanical stress has profound consequences for VSMCs in vessel walls [21].

2. MECHANOSENSORS

2.1. Integrins

VSMCs are surrounded by basement membranes consisting largely of extracellular matrix (ECM). Integrins, a family of transmembrane receptors mediate cell attachment to ECM proteins at sites called focal contacts [22], are heterodimers composed of non-covalently bound transmembrane α and β subunits. There are over 15 α and 8 β subunits that can heterodimerise to produce more than 20 different receptors. Integrin αvβ3 has a particularly interesting expression pattern as it is expressed in vascular cells during angiogenesis and vascular remodelling [23, 24]. Integrin αvβ3 is a receptor for numerous ECM ligands including vitronectin, fibronectin, fibrinogen, thrombosponedin, proteolyzed collagens, and osteopontin. Integrins are not only cell adhesion mediators but also transmitters of extracellular stimuli to intracellular signaling events [3, 23]. These signaling events activate numerous downstream signals including cytoskeleton reorganization (leading to cell migration) and activation of the Ras-Raf-MAPK kinase (MEK)-MAPK pathways resulting in mitogenic responses [25-27]. Mechanical stress induced signaling from integrins is shown in Fig. (**2**).

Integrin β3 is upregulated by cyclic stretch in human umbilical endothelial cells [28]. When stretched human saphenous vein grafts show expression and activation of matrix metalloproteinase (MMP)-2, MMP-9 and integrin αv,

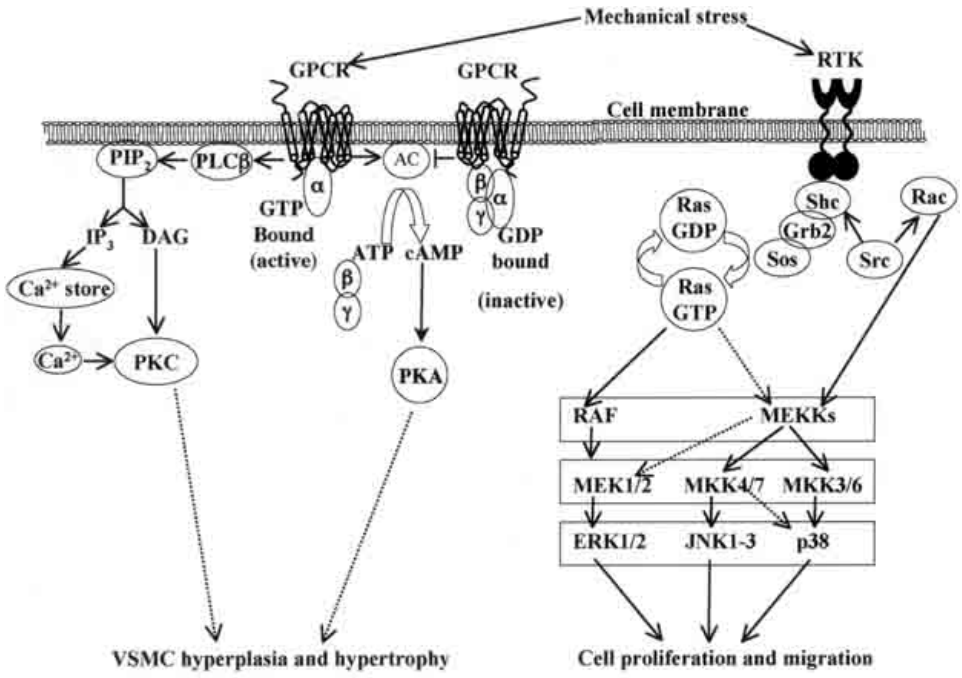

Fig. (1). Schematic diagram of receptor tyrosine kinase (RTK) and G protein coupled receptors (GPCR) activation by mechanical stress in VSMCs. In quiescent VSMCs, growth factor receptors and GPCRs are inactive. Ras and rac bind GDP and the G protein α, β, and γ subunits form heterotrimeric GDP bound G proteins that are inactive. In stretched VSMC, increased elongational and translational membrane mobility results in exposure of receptor kinase domains and/or conformational change of membrane-bound G proteins which leads to receptor autophosphorylation or facilitation of GDP exchange for GTP leading to activation of downstream targets. RTK activation leads to ras or rac activation via the adaptor proteins Shc, Grb2, Src and Sos and the consequent triggering of the MAPK cascade leading to cellular responses such as cell proliferation and migration. GPCR activation results in stimulation of adenyl cyclase (AC) to produce cAMP and subsequent protein kinase A (PKA) activation. Another aspect of GPCR activation is stimulation of phospholipase C (PLC) to cleave phosphatidylinositol 4,5-bisphoisphate (PIP_2) to forminositol-1,4,5-phosparte (IP_3) and diacylglycerol (DAG) which leads to protein kinase C (PKA) activation.

a receptor for active MMP-2 [29]. In cultured VSMCs mechanical stress increases DNA synthesis when the culture substrate is collagen, fibronectin, or vitronectin, but not elastin or laminin. The normal VSMC response to mechanical stress is also stopped by antibodies specific for β3 or αvβ5 integrin but not β1 integrins [30, 31]. This shows that mechanical stress sensed via specific cell-ECM interactions can alter myosin isoform expression [32]. Transcription and expression of the immediate-early genes, early-growth response gene-1 (Egr-1) and c-*jun* are modulated by mechanical stress in ECM-dependent manner [33]. It is probable that both the ability of cells to sense mechanical stress and the nature of the subsequent biochemical responses depend to some degree on the integrin-ECM interactions [34].

Integrin signaling is associated with the biogenesis of focal adhesion complexes consisting of clustered integrins and cytoskeletal proteins such as talin, paxillin, and phosphorylated signaling molecules including Focal Adhesion Kinase (FAK), Src, p130(CAS), Crk is recruited after shear stress, mechanical stress or cell [35, 36]. *In vitro* studies have shown that FAK binds directly to the cytoplasmic portions of integrin and to the cytoskeletal proteins such as paxillin, *in vitro* [37-39]. Integrin-mediated signal trasduction is an important step in a cells response to mechanical stress [36, 40-43]. FAK functions as part of a network of integrin-stimulated signaling pathways which can lead to the activation of the MAPK pathways as one example [24-26, 36]. FAK autophosphorylation can be mediated by integrins and this can result in tyrosine phosphorylation of cytoskeletal proteins like talin and paxillin with rapid cytoskeletal reorganisation after the application of mechanica. In this integrin mediated signaling network FAK also associates with Src-family protein tyrosine kinases (PTKs), p130Cas, Shc, Grb2, Phospho Inositol (PI)-3-kinase and paxillin.

Syndecan-4 is a membrane-associated heparan sulfate proteoglycan that occurs at the same location as integrin heterodimers in focal adhesion complexes. In response to mechanical stress Syndecan-4 is translocated to the cell surface, in contact with the substrate [44]. Dissociation of syndecan-4 and vinculin from focal adhesions may contribute to the promotion of cell motility because syndecan-4 overexpression retards mechanical stress-induced cell migration [44]. Because focal adhesions serve as part of a mechanotransduction system the physical forces sensed by focal adhesions may regulate the expression and intracellular distribution of syndecan-4 and modulate cell movement and orientation. It is not known if syndecan-4 alters, or is part of normal integrin mediated cell signaling in response to mechanical stress.

Unlike skeletal or cardiac muscle, VSMCs contain a reservoir of unpolymerised globular (G) actin. The cytosolic concentration of G-actin is lowered by increasing intravascular pressure implying a shift in the F:G equilibrium in favor of F-actin [45]. Inhibitors of actin polymerisation (cytochalasins and latrunculin) inhibit the development of myogenic tone and decrease the effectiveness of myogenic reactivity. Depolymerisation of F-actin with cytochalasin D causes VSMC relaxation and increased G-actin content, whereas polymerisation of F-actin with jasplakinolide causes VSMC contraction and decreased G-actin content [46]. These facts are consistent with observations in other cells in which actin dynamics are implicated in motility and contractility. Actin filament formation in VSMC may therefore be involved in mechanotransduction and by providing additional sites for myosin interaction, enhance force production in response to pressure or mechanical stress [46]. Actin polymerisation in VSMCs in response to the increased intravascular pressure (mechanical stress) could be a novel mechanism underlying arterial myogenic behavior. Although the mechanism by which actin polymerisation is stimulated by pressure is not known it probably occurs via integrin-mediated activation of signal transduction pathways previously associated with VSMC contraction (e.g., protein kinase C (PKC) activation, Rho A, and tyrosine phosphorylation).

The exact physical mechanism of signaling initiated by integrins activated by mechanical stress is not well defined. However, enhanced tyrosine phosphorylation is commonly observed in response to integrin receptor binding ECM proteins and to mechanical stress-induced integrin signaling in many cells [37, 40-43, 47-49].

2.2. Receptor Tyrosine Kinases

Platelet-Derived Growth Factors (PDGFs) are homo- or heterodimers of A and B polypeptide chains such that three different disulfide-bound dimers PDGF-AA, PDGF-BB, and PDGF-AB are formed [50]. Two distinct PDGF membrane receptors have been described; PDGF receptor-α binds all PDGFs while PDGF receptor-β binds PDGF-BB and PDGF-AB, but not PDGF-AA [51, 52]. Dimerisation is induced by ligand binding and leads to activation via autophosphorylation of tyrosines in the PDGF receptor kinase domain [53, 54]. This activation triggers sequential activation of MAPK cascades, which play a pivotal role in cell proliferation and differentiation [55].

We provided the first evidence that mechanical forces directly activate PDGF receptor-MAPK signal pathways [21]. In cultured VSMCs subjected to cyclic strain stress PDGF receptor-α is rapidly activated (phosphorylated). When the adaptor protein GRB2 is immunoprecipitated from stressed VSMCs subsequent Western blot analysis shows stress-induced phosphorylation of PDGF VSMCs [21], which shows that PDGF receptor-α is activated by biomechanical stress. Our work showed that suramin (a growth factor receptor inhibitor) does block PDGF receptor-MAPK activation in VSMCs after mechanical stress *in vitro*[56] and significantly inhibits mouse vein graft neointimal hyperplasia *in vivo* [57]. Mechanical stress also increases PDGF-B and PDGF receptor-α expression in VSMCs [58] and shear stress induces a rapid transient tyrosine phosphorylation of Flk-1/vascular endothelial growth factor receptor (VEGFR) and its concomitant association with the adaptor protein Shc [35]. Fig. (**1**) is a schematic of mechanical stress induced signaling from receptor tyrosine kinases.

The receptor tyrosine kinase ErbB2 is also activated by mechanical stress [59] and we have shown that it is expressed in VSMCs (unpublished data). This receptor, expressed in the heart, skeletal muscle and VSMCs, has widely been studied in the context of breast cancer but its role in muscle is not clear. ErbB2 has no known high affinity ligand but heterodomerises with other members of the ErbB family (including the epidermal growth factor (EGF) receptor, ErbB1) to enhance the potency and duration of signaling [60]. The EGF receptor has recently been shown to be activated by mechanical stress in alveolar epithelial cells with subsequent G protein and ERK1/2 activation [61]. Although signaling patways activated by autophosphorylation of ErbB2 heterodimers are well defined, it is not known which of these pathways are activated via ErbB2 in response to mechanical stress and what consequence they have for VSMCs.

More recently the expression of several receptors such as endothelin B receptor and the vascular endothelial growth factor (VEGF) receptor has been reported to be altered by mechanical stress. Endothelin-1 (ET-1) upregulation in venous bypass grafts in response to arterial blood pressure may play a major role in graft failure but endothelin-converting enzyme and endothelin A receptor expression is not pressure sensitive [62]. However, there is a mechanical stress dependent increase in expression of ET-1 and endothelin B receptor dependent on PKC but not c-Src activation [62]. In venous bypass stress induced changes in gene expression may contribute to graft failure by endothelin B receptor-mediated smooth muscle cell hyperplasia and graft occlusion [62]. In mechanically stressed VSMCs the level of VEGF mRNA is increased as compared to the unstressed cells and media from these cells (containing VEGF) increases endothelial cell migration [63]. Mechanical stress has also been shown to increase both VEGF and basic fibroblast growth factor (FGF) 2 expression in VSMCs [64]. The mechanism by which VEGF is induced has recently been determined to be mediated by phosphatidylinositol 3-kinase and PKC-zeta but not by mechanical stress induced extracellular signal regulated kinases (ERK) 1/2, Akt, Ras, or the classical/novel PKC pathways [65]. Mechanical stress activates (phosphorylates) the VEGF receptor and the implications of this are that physiological levels of mechanical stress stretch will induce an increase in VEGF secretion that might provide a stimulus for maintaining steady state VEGF levels essential for endothelial cell survival [63]. It has been shown that the VEGF recptor interacts with adherence junctions in the vascular endothelium and that this complex acts as shear-stress co-transducer, mediating the transduction of shear-stress signals into vascular endothelial cells [66]. The importance of the VEGF and other receptors as mechanosensors is becoming more widely recognised.

2.3. G Protein-Coupled Receptors and G Proteins

G proteins containing α, β, and γ subunits have a central role in coupling receptors to a plethora of intracellular enzymes and ion channels. VSMC hyperplasia and hypertrophy is a process of an adaptation to various mechanical and hormonal stimuli. Several independent signaling pathways are involved in activating the hypertrophic response [34, 67, 68]. Gq is a G protein and is q important in this process because various ligands including angiotensin II and endothelin 1, which are the activators of Gq-coupled receptors, will trigger hypertrophy and hyperplasia in cultured VSMCs [69-71]. Transgenic mice with cardiac restricted overexpression of Gαq, α1-adrenergic receptors and angiotensin II receptors develop cardiac hypertrophy [72-76]. Pressure overload (mechanical stress) was surgically induced and the transgenic mice developed much less ventricular hypertrophy than the controls [77] thus highlighting the importance of Gq in initiating cardiac hypertrophy in response to mechanical stress [78]. There are no reports about Gq-mediated VSMC responses to mechanical stress although Gq proteins in endothelial cells are activated by shear stress [77, 79-81]. Purified heterotrimeric G-protein subunits Gq/11 and Gi3/G0 are rapidly (within seconds) activated in endothelial cells after increased fluid flow and in cardiac fibroblasts by stretch-stress [79]. Antisense Gq oligonucleotides will inhibit shear stress-induced ras-GTPase activity [82]. Our own work has shown that pertussis toxin is a Gi inhibitor that will block p38 MAPK activation [83] this suggests that G proteins are involved in the primary mechanosensing response. Signaling from G proteins in response to mechanical stress is shown schematically in Fig. (**1**)

Protease activated receptors (PARs) are a recently characterised class of G-protein-coupled receptors that are activated by proteolytic cleavage of their NH2 termini by specific serine proteases [84]. After cleavage the new NH2 terminus acts as a tethered ligand, binds other extracellular receptor domains and initiates G-protein-dependent signaling. PAR-1, the thrombin receptor, was the first of four PARs that have been cloned [85, 86]. PAR-1 is found in diverse cell types including VSMCs [84, 87]. Mechanical stress increases PAR-1mRNA, protein levels and activity [88]. Antioxidants or an NADPH oxidase inhibitor block mechanical stress induced PAR-1 expression but protein kinase inhibitors enhance mechanical stress induced PAR-1 expression but inhibitors of nitric oxide (NO) synthase, tyrosine kinase, and mitogen-activated protein kinases have no effect [88]. Mechanical stress upregulates PAR-1 expression but shear stress downregulates this gene in VSMCs [88]. This provides an insight to elucidate signaling differences by which VSMCs respond to different mechanical forces.

2.4. Ion Channels

VSMCs subjected to mechanical stress exhibit a transient increase in intracellular calcium and divalent cations and depolarisation [68, 83], which are involved in maintenance of smooth muscle tension. Mechanical stress apparently causes these channels to open resulting in Ca^{2+} and Na^{+} influx and membrane depolarization contributing to the myogenic response to mechanical stress. Stretch-activated Ca^{2+} channels and Na^{+} channels in arterial SMCs are relatively non-selective for cations. Stretching the membrane of individual VSMCs using a patch electrode results in increased frequency of open Ca^{2+} channels [89]. Mechanical stress induces an increase in intracellular calcium from

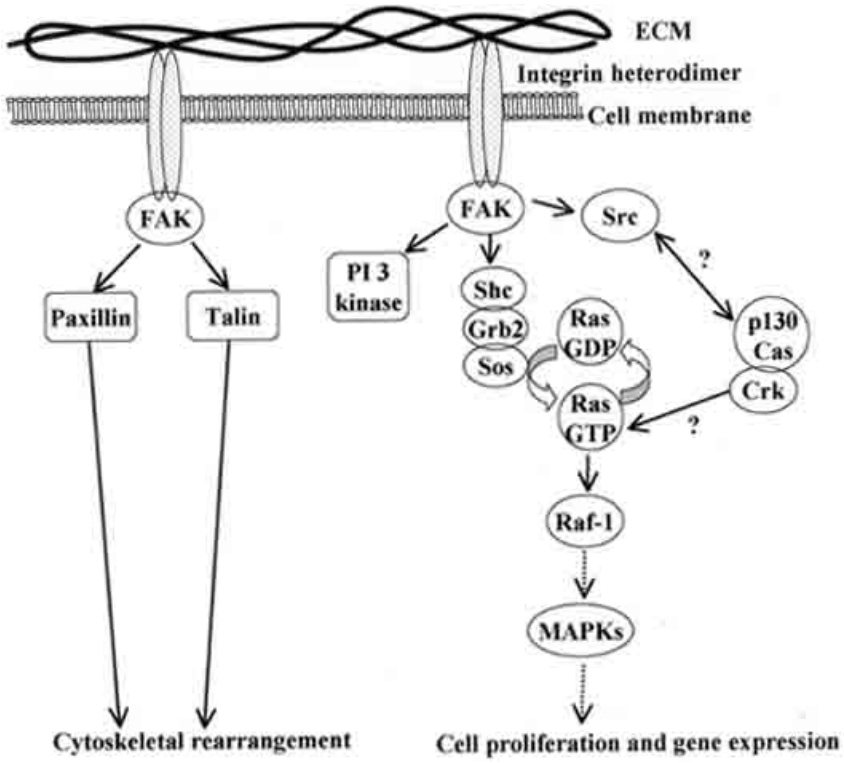

Fig. (2). Schematic representation of integrin-mediated mechanical signaling in VSMCs. Mechanical force directly disturbs the ECM resulting in integrin clustering and dimerisation which stimulates FAK autophosphorylation followed by activation of Src, p130CAS, Shc, and Grb2. This results in translocation of the GDP-GTP exchange protein Sos and subsequent stimulation of GTP exchange on ras or rac leading to cell proliferation. FAK autophosphorylation can also lead to phosphorylation of cytoskeletal proteins such as paxillin or talin which results in cytoskeletal rearrengment.

extracellular sources but partially dependent on Ca^{2+} influx from intracellular reserves. Calcium influx across the plasma membrane may occur via a stretch-activated channel or voltage-gated Ca^{2+} channel activation resulting from stretch-activated channel-induced depolarisation. The mechanical stress induced Ca^{2+} influx is blocked by gadolinium but not by nifedipine a classic calcium blocker [90-92]. Also, the stretch-activated channels show higher activity in arterial SMCs from spontaneously hypertensive rats as compared to Wistar-Kyoto rats [89]. Alteration of stretch-activated channels in arterial SMCs probably contributes to hypertrophy and remodelling of arterial tissue in hypertension.

Stretch-activated ion channels also regulate tyrosine phosphorylation of paxillin, focal adhesion kinase (pp125FAK), and pp130CAS, resulting in altered cell shape and cytoskeletal remodelling of endothelial cells in response to mechanical stress [93, 94]. Potassium channels other than ion non-selective gadolinium-sensitive channels also participate in signal transduction after mechanical stress[95]. A recent study has shown that mechanical stress induced Ca^{2+} influx can occur via an inositol 1,4,5-trisphosphate (IP_3) insensitive Ca^{2+} channel [96]. Inhibition of IP_3, ryanodine, and nicotinic acid adenine dinucleotide phosphate channels (NAADP) with heparin, ruthenium red or thio-NADP, respectively, does not block the increase in Ca^{2+} efflux in response to cyclical stretch [96]. However, lanthanum, gadolinium, and cytochalasin D but not nocodazole inhibit increased Ca^{2+} efflux [96]. This supports the existence of a novel stretch-sensitive intracellular Ca^{2+} store in VSMC that is distinct from the IP_3-, ryanodine-, and NAADP-sensitive stores but the channel has not been identified and so downstream signaling cannot yet be investigated.

Smooth muscle exhibits mechanosensitivity independent of neural input and the native L-type calcium current recorded from human intestinal smooth muscle is modulated by stretch [97]. Lyford et al [97] cloned the α_{1C} L-type calcium channel subunit ($Ca_V1.2$) from human intestinal smooth muscle and expressed it in cultured cells. The channel retained mechanosensitivity when expressed alone or with a β_2 calcium channel. The heterologously expressed human cardiac α_{1C} splice form also showed mechanosensitivity. Inhibition of kinase signaling or truncation of the α_{1C} COOH terminus, which contains an inhibitory domain and a proline-rich domain thought to mediate mechanosensitive signaling from integrins, did not disrupt mechanosensitivity of the channel [97]. This strongly implies that mechanical regulation of Ca^{2+} influx occurs through L-type calcium channels in mammalian cells and suggests that mechanosensitivity resides within the pore forming α_{1C}-subunit. In contrast to this Chang et al[98] showed that increased levels of the activated transcription factor Heast shock factor 1 (HSF1) and heat-shock protein (HSP) 72 are observed in isolated rabbit hearts after

mechanical stress. This increase is observed when the hearts are perfused with a solution containing the specific L-type calcium channel inhibitor diltiazem but not with the general stretch-activated ion channel inhibitor gadolinium[98]. Stretch in aortas [99] or VSMCs [100] activates HSF1 and increases HSP70 mRNA through stretch-activated ion channels [98], which is a novel mechanism for HSF activation and may be an important signaling pathway for hemodynamic stress.

Vascular hypertrophy does occur during chronic hypertension and contributes to elevated peripheral vascular resistance. Acute pressure overloading (mechanical stress) of the vascular wall MAPKs in isolated rat aortas [101]. Increased perfusion pressure causes a pressure-dependent increase in MAP kinase activity in endothelium-intact and endothelium-denuded aortas is inhibited by the angiotensin receptor antagonist, losartan, the renin inhibitor, pepstatin A, and the angiotensin-converting enzyme inhibitor, captopril[101]. Ca^{2+} depletion and the Ca^{2+} channel antagonist, nifedipine, do not affect the mechanical stress-induced MAP kinase activation [101]. Mechanical stress of the vascular wall activates MAP kinases and that the MAP kinase activation is at least partly mediated via the vascular angiotensin system. But it is unlikely that the increase in MAP kinase activity is mediated by increased Ca^{2+} influx in VSMCs.

3. PKC ACTIVATION IN RESPONSE TO MECHANICAL STRESS

Protein kinase C (PKC) comprises a large family of serine/threonine kinases activated by lipid-derived second messengers that differ in substrate preferences, intracellular localization and activation mechanisms. The PKC family is subdivided into three groups: the conventional or cPKC, the novel or nPKC and the atypical or aPKC. cPKCs (α, β1, β2, γ) contain two conserved areas in their regulatory domains. The nPKCs (δ, ε, φ, η/L, and possibly μ or PKD) are activated by diacylglycerol or phorbol esters and have a C1 region. They are calcium independent as the C2-like region that is incapable of calcium binding. aPKCs (ζ, λ/ι) have no C2 region, only one cysteine-rich loop in the C1 region and do not bind diacylglycerol or calcium but are activated by phospholipids [102-104]. PKCμ is a novel PKC with a pleckstrin homology domain, an N-terminal hydrophobic domain and it is devoid of an inhibitory pseudosubstrate region found in other PKC family members [102]. PKCs play multiple roles in cellular signal processing as PKC isoenzymes are activated by large numbers of extracellular signals and modify the activities of numerous targets [102, 105, 106].

We have shown that p38 MAPK activation after mechanical stress is blocked by phorbol ester treatment before mechanical stress and this inhibition of p38 MAPK activation results in decreased VSMC proliferation and apoptosis [83, 107]. This is strongly suggestive of an important role for PKC in mediating p38 MAPK activation in mechanically stressed VSMCs. Mechanical stress increase PKC activity and in the case of cyclic strain this increased activity correlates with the formation of PKC particulates and VSMC proliferation[108, 109]. Staurosporine inhibition of PKC fails to alter these responses [109] highlighting the multifaceted nature of these responses and may be the participation of other staurosporine-insensitive PKC subfamilies. PKC inhibition results in the abrogation events upstream of NF-kappaB activation in the cytoplasm [110]. This leads to a marked repression of stretch-induced platelet-activating factor expression implicating a regulatory mechanism (direct or indirect) activation of transcription factors by PKC. There are probably many signaling pathways affected by PKC because PKC can activate many signal molecules [108-120]. Hypertrophic signaling is now a growing research area in the hope that specific PKC isoforms will be drug or gene therapy targets for studying or treating cardiovascular diseases associated with mechanical stress [121].

4. MAPK SIGNALING IN RESPONSE TO MECHANICAL STRESS

At the centre of MAPK signaling pathways are MAPKs from one of three possible subfamilies, ERK1/2, c-Jun NH_2-terminal kinase (JNK)/stress activated protein kinases (SAPK) or p38MAPK. MAPKs are phosphorylated (activated) by MAPK kinases (MEKs) which are typically dual-specificity kinases catalysing tyrosine and threonine phosphorylation of MAPKs. MEKs are in turn substrates for phosphorylation by MEK kinases (MEKKs) which are serine/threonine kinases. 12 members of the MAPK family have so far been identified in mammalian cells. These are grouped into five subfamilies based on function and sequence homology and function. Seven MEKs and 14 MEKKs are known in mammalian cells [122, 123]. Also small G proteins (i.e., Ras, Rac, Cdc 42) and specific kinases that may act as MAPK kinase kinase kinases (MEKKKs) regulate the activity of MEKKs (i.e., Raf, MEKK 1-4, transforming growth factor-beta activated kinase 1 (TAK1), and p21 activated kinase (PAK)) and control the activation of specific three-kinase MAPK modules [122, 124-131].

In atherosclerotic lesions we observed increased expression and activation of ERK1/2 [132] and it is known that shear stresses activates ERK1/2 in endothelial cells. In VSMCs we demonstrated that mechanical stress activates ERK1/2 in a time- and strength-dependent manner [56, 125]. Suramin (a growth factor receptor antagonist) will completely block ERK activation in response to mechanical stretch. Combined with the knowledge that mechanical stretch rapidly phosphorylates (activates) the PDGF receptor-α in VSMCs demonstrates that stress responsive ERK1/2 activation in VSMCs is a receptor-dependent pathway. ERK1/2 activation is regulated by the small G proteins ras and rac. When we expressed dominant negative ras (rasN17) or rac (rac-N17) mechanical stress responsive ERK activation was blocked in VSMCs[125]. Furthermore, we found that suramin and PD98059 inhibit ERK1/2 activation as well as blocking AP-1 DNA binding which suggests that ERK1/2 mediates stress-induced AP-1 activation and VSMC proliferation [56, 125].

The JNKs, (c-*jun* NH-terminal protein kinases) or SAPKs (stress-activated protein kinases) have two

similarities with ERK1/2. The other similarity is that phosphorylation on both the threonine and tyrosine is necessary for activation[127]. JNKs are activated in response to inflammatory cytokines (Tumor necrosis factor-α (TNF-α) and interleukin 1β (IL-1β)), heat-shock, osmotic stress, and UV light [127-131, 133, 134]. We showed that both JNK and ERK are activated in vascular walls by acute hypertension [19]. After balloon injury or angioplasty in animals both JNKs and ERKs were also activated in arterial walls [56, 58]. This suggests that mechanical stress is allied to JNK activation. In cultured VSMCs we saw that cyclic strain stress rapidly induces JNK activation [125] and both suramin and PD98059 have no effect on JNK activation after mechanical stress [125, 135]. This is indicative of separate means of JNK and ERK activation in mechanically stressed VSMCs

p38MAPK is homologous to the yeast HOG 1 kinase and is another member of the MAPK family. P38MAPK exits in four currently identified isoforms in mammalian cells α, β, γ, and δ[136]. p38MAPK isoforms α, γ and δ are activated upstream by MKK3 and MKK6 while isoform β is only activated by MKK6, which is the most potent p38 MAPK activation. p38 MAPK activation will also mediate actin reorganisation and migration of endothelial and VSMCs by increasing heat-shock protein 27 phosphorylation [137, 138]. Pharmacological compounds such as CSAIDs (cytokine suppressing anti-inflammatory drugs) bind to the p38α and β to inhibit their activity. Mechanical stress causes rapid p38 phosphorylation in VSMCs and results in both cell proliferation and apoptosis which is apparently mediated by PKC-ras/rac pathways [83, 107, 125]. In response to mechanical stress in cultured VSMCs p38 activation occurs in a time- and strength-dependent manner and is closely associated with PKC and G protein activation [83]. When p38 activation is inhibited VSMC migration response to PDGF-BB and mechanical stress induced proliferation were undetectable. Mechanical stress rapidly activates p38MAPKs by activation of PKC-ras/rac signal pathways. p38 MAPK activation is also closely associated with mechanical stress induced VSMC apoptosis [107]. Because the complexity of these signaling pathways are multiplied by the fact that there are four p38 subunits, the identification of specific subunits responsible for cell growth and/or apoptosis in stretched VSMCs will be a topic for further investigation.

We have seen that biomechanical stress induces apoptosis of VSMCs [107] but until recently the molecular mechanisms of mechanical stress-induced apoptosis were vague. When VSMCs are subjected to mechanical stress the tumour-suppressor p53 is activated [139]. This activation is largely attenuated by pretreatment with SB202190, a specific p38MAPK inhibitor, or stable transfection with dominant negative rac, an upstream signal transducer of p38MAPK pathways. p38MAPKs phosphorylate p53 within 30 min of mechanical stress [139]. Mechanical stress also results in oxidative DNA damage and antioxidant treatment abrogates p53 activation [139]. We showed that p53 activation is followed by expression and mitochondrial translocation of the proapoptotic protein Bax. Likewise, mechanical stress results in up-regulation of anti-apoptotic Bcl-2 proteins including Bcl-2 and Bcl-xL [139]. However, a marked loss of mitochondrial membrane potential occurred in wild-type, but not in $p53^{-/-}$ VSMCs. p53 deficient VSMCs lose their ability to express Bax and show no apoptosis in response to mechanical stress. These data provide the first evidence that VSMC apoptosis induced by mechanical stress is p53-dependent.

The atrial natriuretic peptide (ANP) is a cardiovascular hormone possessing anti-inflammatory potential due to its inhibitory action on the production of inflammatory mediators, such as tumor necrosis factor-alpha (TNFα) and is upregulated in response to hypertension (mechanical stress) [140, 141]. Activation of human vascular endothelial cells (HUVECs) with TNFα leads to an increase in macromolecule permeability and stress fiber formation while treatment of cells while ANP reduces stress fiber formation and elevates permeability [142]. ANP significantly reduces TNFα-induced p38 activation and attenuates HSP27 phosphorylation, a central target downstream of p38 MAPK [142]. The inhibitory action of ANP on TNFα-induced changes in endothelial cells involve the MAPK phosphatase-1 (MKP-1)-induced inactivation of p38 MAPK are clear and point to an anti-inflammatory and antiatherogenic potential of ANP. It remains to be determined if a similar effect may be observed with VSMCs.

5. NEGATIVE FEEDBACK REGULATION

MAPK phosphatase-1 (MKP-1) has a phosphatase activity for both phosphotyrosine and phosphothreonine and will inactivate ERKs and possibly JNKs/SAPKs [143-146]. Research from several groups, including ours, showed that MKP-1 is regulated at the transcriptional level and is induced in VSMCs by growth factors [147], oxidative stress [148], arachidonic acid [149], and phorbol esters[150]. MKP-1 is involved in a feedback loop to inactivate MAPKs after mitogenic stimulation in response to stress [137, 138, 144, 146, 151] the signal pathways leading to MKP-1 gene expression have not been fully elucidated. The MKP-1 pathway is a complex type of negative feedback-regulating pathway resulting in VSMC growth inhibitory response after mechanical stress [125]. Both MKP-1 protein and mRNA expression is increased in cultured VSMCs by mechanical stress and inhibition of either ERK kinase (MEK1/2) or p38 MAPKs accompanies a downregulation of MKP-1. Signaling to alter MKP-1 expression is likely to be mediated by MAPK pathways, which possibly share the same upstream signaling molecules such as ras, rac and raf. The negative feedback inhibition by MKP-1 is shown in Fig. (**3**).

The role of MKP-1 in negative regulation has been implicated *in vitro* and *in vivo* by our group and others [6, 125, 132, 150, 152-157]. MKP-1 overexpression in VSMC leads to dephosphorylation and inactivation of ERKs, JNKs/SAPKs and p38 MAPKs as well as inhibition of DNA synthesis in response to mechanical stress [125]. Acute hypertension and balloon injury induce MKP-1 expression in the vessel wall [6, 158]. In spontaneously hypertensive rats Insulin is more because MKP-1 expression is reduced in hypertensive rats compared to normotensive rats [157]. It is possible that mechanical stress-induced MAPK pathways mediate either proliferation or growth inhibition in human arterial VSMCs depending on a balance of the availability of

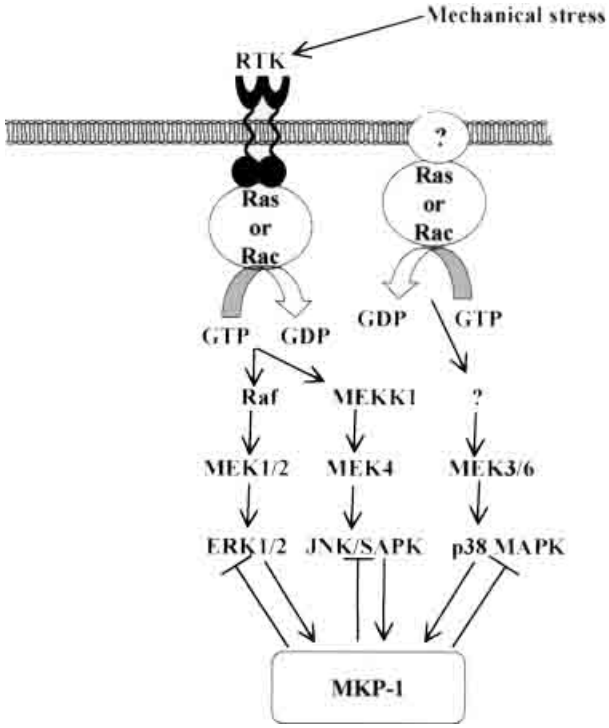

Fig. (3). A schematic representation of negative feedback regulation via MKP-1 expression in VSMCs in response to mechanical stress. Mechanical stress can activate growth factor-ras/rac-raf-MAPK pathways which lead to induction of MKP-1 expression. MKP-1, in turn, dephosphorylates and inactivates MAPKs leading to a blockade of stretch-induced signaling transduction.

specific downstream targets and the ability of the MAPK pathway to directly activate MKP-1. Recently the role of MKP-1 in cardiovascular disease has been studied in increasing detail with many groups confirming and extending our early work. Angiotensin II (Ang II) negatively regulates MAPKs through angiotensin receptor 1 (AT1) by increasing MKP-1 mRNA levels and through AT2 receptors by unknown mechanisms [159]. After cardiopulmonary bypass (CPB) in pigs the MEK/ERK pathway is inactivated in both ventricular and atrial myocardium by an increase in MKP-1 and the activities and protein levels of c-Src and Akt were not significantly modified before or after CPB, suggesting a specificity for the MEK/ERK pathway revealing a prevalence of inhibitory mechanisms in the MEK/ERK signal transduction machinery in myocardium subjected to CPB [160]. The interconnectivity between calcineurin-mediated cardiac myocyte hypertrophy and p38 MAPK signaling *in vitro* and *in vivo* was highlighted by the finding that calcineurin promotes down-regulation of p38 MAPK activity and enhances expression of MKP-1 in transgenic mice expressing activated calcineurin in the heart [161]. Insulin inhibition of PDGF-directed VSMC migration may be mediated in part by NO/cGMP/cGK Ialpha induction of MKP-1 and consequent inactivation of MAPKs [162]. Expression of MKP-1 is increased in a significant proportion of failing hearts and JNK1/2 and p38 MAPK activities are also decreased in failing human myocardium [163]. Increased expression of MKP-1 may therefore contribute to decreased MAPKs activity in failing human myocardium [163]. Constitutive MKP-1 expression in cardiomyocytes blocks p38 MAPK, JNK1/2, and ERK1/2 activation and prevents agonist-induced hypertrophy in transgenic mice providing further evidence to implicate MAPK signaling factors as obligate regulators of cardiac growth and hypertrophy and demonstrate the importance of MKP-1 as a counterbalancing regulatory factor in the heart [164].

Another candidate for negative feedback regulation is cyclic AMP-dependent protein kinase (PKA) which inhibits proliferation and migration [67, 109, 165-167]. Endothelial cells normally shield VSMCs from the circulating blood and synthesise agents that relax and inhibit VSMC growth. Removal of the endothelial cells leads to VSMC proliferation [165] after an increase in PKA activity. Increasingly from studies with VSMCs or other cell types it is becoming clear that PKA not only inhibits MAPK/ERK signaling pathways by Raf inhibition [166] but also inhibits cyclins (active in controlling cell cycle progression) possibly by downregulation cyclin D1 and cdk2 [109, 167]. Mechanical stress activates PKC and PKA squarely implicating PKA in mediating mechanical stress induced signal transduction in VSMCs [109].

6. CROSSTALK BETWEEN SIGNALING PATHWAYS

6.1 Crosstalk Between PKA and Growth Factor Receptor Signaling Pathways

Crosstalk between PKA and growth factor receptor signaling, Fig. (**4**), in relation to cardiovascular disease is a relatively new concept in signal transduction. Proliferation and migration of VSMCs contributes to blood vessel wall thickening in many types of cardiovascular disease. By antagonising major growth factor induced mitogenic signaling pathways PKA potently inhibits VSMC proliferation and migration. Crosstalk between PKA and the MAPK/ERK pathway, the p70 S6 kinase pathway and cyclin-dependent kinases is known but only crosstalk with growth factor receptors is discussed here, a comprehensive review was recently published by Bornfeldt and Krebs [67]. PKA also regulates expression of growth regulatory molecules. PKA activation in VSMCs results in inhibition of cell cycle progression and cell migration.

The MAPK cascade also known as the ERK pathway normally mediates responses induced by various growth factor receptors but also by mechanical stress (via the PDGF receptor and possibly others) in VSMCs [168-170]. Activation of the receptor by autophosphorylation leads to binding of adaptors like GRB2 and Shc to the receptor or proteins phosphorylated by the receptor. This leads to activation of Ras by a guanine nucleotide exchange factor (e.g., mammalian son-of-sevenless; mSOS). Sequential phosphorylation events follow and activate the protein kinases, Raf, MEK and MAPK. MAPK substrates include transcription factors, (e.g. Ets [171]), protein serine/threonine kinase p90rsk, cytosolic phospholipase A2 (cPLA2) and cytoskeletal proteins [172].

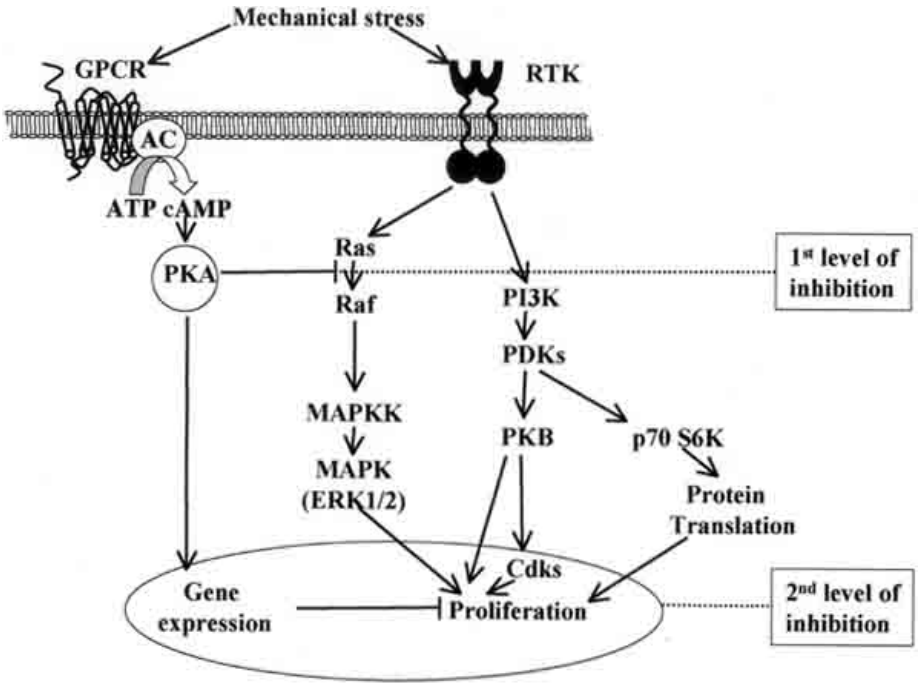

Fig. (4). Crosstalk between PKA (protein kinase A) and mitogenic signal transduction pathways in VSMCs. This schematic shows the points of antagonism between PKA and mitogenic signaling pathways in VSMCs. Cyclic AMP is generated following mechanical stress induced activation of membrane receptor coupled to one of several adenylate cyclases (ACs). The activation of PKA is determined by the extent of AC activation and the extent of cAMP hydrolysis by cAMP phosphodiesterases (PDEs). Active PKA inhibits mitogenic signaling pathways at several points. The first level of inhibition by PKA is close to the plasma membrane. Thus, signal transduction from a growth factor receptor like the PDGF receptor is inhibited at the level of Ras/Raf and at the level of and/or upstream of, phosphatidylinositol-3'kinase (PI3K). A second level of inhibition occurs in the nucleus where PKA inhibits several different cyclin-dependent kinases (Cdks and Cdc2) and induces gene responses via cAMP-responsive transcription factors. The products of PKA-regulated genes can inhibit VSMC proliferation for example, by reducing the responsiveness of the cell to growth factors

In human VSMCs PKA inhibits the MAPK/ERK cascade after activation of MAPK/ERK by growth factor receptors [173] (e.g., insulin receptor, epidermal growth factor receptor and PDGF receptor) although the receptors are apparently not targets of PKA-mediated inhibition of the MAPK cascade. The point of inhibition of the MAPK cascade by PKA is controversial but it is clear that the inhibition takes place upstream of MAPK and MAPKK but downstream of Ras activation because PKA doesn't inhibit the formation of active Ras and active PKA inhibits Ras induced MAPK activity [166, 174-179]. The point of MAPK/ERK cascade inhibition may be at Raf-1 [166]. PKA phosphorylates Raf-1 on Ser 43 [175, 180] and 621 [181]. B-Raf lacks serine 43 but is phosphorylated by PKA at Ser 429 and Ser 446, and this dramatically reduces GTP-Ras binding by PKA [182]. Therefore a possible mechanism for PKA inhibition of MAPK cascade is a reduced ability of active Ras to bind Raf [183, 184]. Another possible mechanism of MAPK pathway inhibition by PKA could be the inhibition of Raf activating kinases such as certain PKC isoforms or Src [185]. There are also other possibilities but the exact mechanism(s) of cAMP/PKA crosstalk with the MAPK/ERK cascade in SMCs remains to be defined.

MAPK/ERK activation often leads to proliferation although in some cases there is crosstalk between PKA and the MAPK/ERK resulting in growth inhibition [168]. VSMCs expressing inducible cyclooxygenase (COX-2) secrete cAMP-stimulating prostaglandins (e.g. PGE2) but this doesn't occur in VSMCs not expressing COX-2. PD 098059 (a MAPKK inhibitor) stops PDGF-induced proliferation of VSMCs not secreting PGE2 whereas in COX-2 expressing VSMCs MAPK activation negatively regulates proliferation. In these cells PDGF-induced MAPK activation leads to cPLA2 activation, PGE2 release and PKA activation, which inhibits VSMC proliferation [186]. Inhibition of either MAPKK signaling, COX-2 or PKA activation in VSMCs removes the effect of growth-inhibitory prostaglandins and resulting in cell cycle progression and proliferation [168]. This is a mechanism by which the growth factor induced MAPK pathway may lead to either proliferation or growth inhibition in VSMCs depending on the availability of certain downstream targets and indirect PKA activation. This mechanism is also an example of crosstalk between PKA and the PDGF receptor signaling pathway that shows the relevance of considering all stimuli that affect VSMC in a particular setting.

PKA antagonises stress-activated MAPK/SAPK signaling pathways that lead to JNK and p38 MAPK activation in VSMCs [187, 188]. The upstream target of PKA in this setting is not characterised but PDGF activates

JNK via PI_3K which is potential PKA target [189]. The role of SAPK signaling pathways in VSMC proliferation and migration is not completely understood. The p38 MAPK pathway exerts a negative feedback on the MAPK/ERK pathway by inhibiting MAPKK (MEK) in VSMCs [190]. The cAMP-mediated effects on ERK and SAPK pathways are very complex and it should always be considered that PKA crosstalk likely to be different in varying cell types because of the signaling components expressed, subcellular PKA localisation and varying stimuli.

6.2 Integration of Integrin Signaling by Crosstalk

Many biochemical steps in integrin signaling are shared with growth-factor receptors and integrins and are considered genuine signaling receptors. Integrins have three features that dramatically expand their range of functionality. First, integrins generally have immobilised ligands so signaling is usually restricted to discrete areas of the cell surface. Second, integrin cytoplasmic domains are essential anchoring points for the actin cytoskeleton so integrins provide physical connections between the intracellular and extracellular environments. Third, for many integrins ligand binding is regulated by signaling mechanisms by a process termed integrin activation. Integrins exist at an intersection between mechanical forces, cytoskeletal organisation, biochemical signals and adhesion. As discussed above mechanical stress is transmitted by integrins and influences the cytoskeleton. The cytoskeleton affects integrin activity and cell adhesion, and the cell adhesion regulates the cytoskeleton and the mechanical forces that cells exert on their surrounding extracellular matrix (ECM). All of these systems affect and are affected by biochemical signaling pathways. Integrins are therefore critical elements for specification of cell shape, polarity and motility are tied to gene expression and cell function.

Integrin and growth factor mediated signaling share many common elements in their pathways so there are many instances where integrin signals may modulate growth-factor signals, Fig. (**5**), and *vice versa*. In reality normal growth factor mediated signaling only occurs when cells are in contact with the ECM or other cells via integrins. In the MAPK pathway numerous integrin and growth factor mediated signals converge along the pathway at several points [191].

If integrin-mediated adhesion is disrupted or lost then PDGF phosphorylation can be affected and p120Ras-GTPase activating protein (GAP) recruitment is increased concomitantly with reduced Ras activity[192], while Ras activation is not changed in the non-adherant cells although Raf or MEK activation is modulated. Raf is activated downstream of integrin-regulated p21-activated kinase (PAK), while MEK is activated downstream of FAK [193, 194]. Even under conditions that facilitate Erk activation in non-adherent cells, Erk will not translocate to the nucleus or phosphorylate transcription factors required for cell-cycle progression [195]. Disrupting the actin cytoskeleton and focal adhesions with cytochalasin closely mimics adhesion loss including decreased Erk, PAK and FAK activity. Erk is often a key mediator of the cell cycle because it is necessary for cyclin D expression [196]. It is clear that cellular outcomes of integrin and growth factor mediated signaling are affected by the strong synergy between soluble factors and cell adhesion.

Integrins are undoubtedly involved in the integration of mechanical stress and responses to soluble factors. As discussed above mechanical stress is sensed by integrins and cells sense tension and stress via ECM contacts and increase contractile force in response to either external mechanical stress or inflexible substrates that are not deformed by cell generated forces [197, 198]. Increased force results in accumulation of focal adhesion proteins at attachment sites so that cells subjected to mechanical stress form focal adhesions [199].

Integrins initiate many signaling pathways that regulate the function of other integrins and so there is a very large number of possible ways how one integrin may regulate the function of another. Mechanical stress does influence integrin activity. In addition to the examples discussed above, endothelial cells subjected to fluid shear stress also show rapid integrin $\alpha_v\beta_3$ activation [200]. The activated

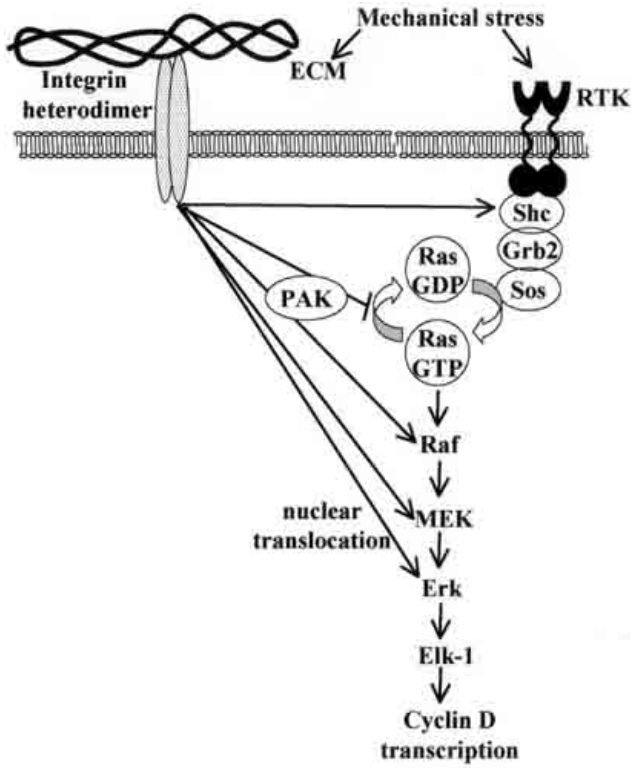

Fig. (5). Integrins can interact with the MAP kinase pathway at several points. Growth factor receptors initially trigger recruitment of the Grb2/Sos complex to the plasma membrane leading to sequential activation of Ras, Raf, MEK and Erk. Erk then translocates to the nucleus tp phosphorylate Elk-1 as well as other transcription factors. Integrin mediated signaling enhances this signal transduction at the level of Ras recruitment and Ras downregulation, Raf and MEK activation and nuclear translocation of Erk.

integrins subsequently bind ECM proteins and initiate signals, characteristic adhesion, such as transient Rho downregulation [200] as well as Shc and FAK [35] phosphorylation. Integrin-dependent signals are responsible for many responses to shear stress including cell and cytoskeletal alignment.

Adhesion via integrins results in ligand-independent activation (phosphorylation) of some growth-factor receptors including those for PDGF, which is also activated by mechanical stress, fibroblast growth factor (FGF), VEGF, hepatocyte growth factor (HGF) and epidermal growth factor (EGF) [201]. This *trans*-activation is mostly transient and not as potent as the signaling initiated by the cognate growth factor. Complexes containing both integrins and growth-factor receptors are formed in these cases and in some situations these complexes are necessary for maximal growth-factor-dependent receptor phosphorylation. Liganded integrin may be required for complete growth-factor-receptor activation and also for integrin mediated cell growth [202, 203].

The physiological significance of integrin *trans*-activation of growth factor receptors is unclear, especially in the context of vasculature but it may be a checkpoint in growth-factor signaling. However in most cells growth factors activate their receptors, leading to phosphorylation and then the recruitment of downstream effectors. Also, Src kinase family members are probably involved in *trans*-activation [203] but even in the absence of the three most abundant Src kinases, PDGF can induce PDGF receptor activation, downstream signaling and mitogenic responses [204]. So the main function of integrin *trans*-activation may be to modulate (not control) growth-factor signaling.

The emerging schema is that integrins function at intersections within complex networks of signaling pathways. These networks integrate signaling from integrin-mediated adhesion with highly regulated processes such as cell migration and proliferation. Understanding the connections between these signaling pathways will hopefully lead to an increased understanding of pathological conditions and the subtleties of homeostasis.

7. MECHANICAL STRESS INDUCED APOPTOSIS

Apoptosis of VSMCs and macrophages occurs in the atherosclerotic lesions of humans and in the animal models [205, 206]. Isolated VSMCs from human atherosclerotic plaques also have a higher propensity for both the spontaneous and induced apoptosis compared with VSMCs from the normal vessels [207]. Activation of MEKK1, a SAPK/JNK upstream kinase and SAPK/JNK pathways is implicated in triggering apoptosis in other cell types in response to the stress stimuli [208, 209]. We have demonstrated the coincidence of p53, pro-apoptotic protein BAX and BCL-Xs with selective activation of SAPK/JNK in atherosclerotic lesions of rabbits[210]. This indicates an *in vivo* SAPK/JNK mediation of apoptosis in the development of atherosclerosis. Some cells in the lesions may have higher SAPK/JNK activities and more p53. These cells may apoptose and SAPK/JNK-mediated signal transduction pathways (i.e., SAPK/JNK-activated p53) could be important *in vivo*.

In restenosis, acute medial cell loss is an initial event in response to vascular injury induced by balloon-catheter or angioplasty in animal modeles [211, 212]. Apoptotic cell death after vascular injury is a highly regulated process governed by activation of the MAPK signaling pathway and the relative expression of antiapoptotic genes. Angioplasty-induced vascular cell apoptosis due to mechanical stress is an important determinant of vascular remodeling and restenosis. It has recently been shown that in the animal models i.e. $Fas^{-/-}$ mice that VSMC apoptosis and neointimal hyperplasia after vascular injury are not Fas dependent [213, 214]. Fas is a death receptor that mediates apoptosis when it is activated by its ligand, FasL.

Although veins do not develop spontaneous arteriosclerosis, we have shown in the animal models that arteriosclerosis develops rapidly in venous bypass grafts and is exacerbated by hypercholesterolemia [215, 216]. Venous bypass grafts bear increased biomechanical forces due to increased blood pressure and are a very good model for studying the role of biomechanical stress in the pathogenesis of arteriosclerosis. We established the first mouse model of vein graft arteriosclerosis by grafting autologous jugular vein or vena cava to the carotid arteries [18]. The morphological features of our murine vascular graft model closely resemble those of human venous bypass graft disease [2, 18]. We have studied the role of biomechanical stress-induced apoptotic cell death in the development of vein graft arteriosclerosis and showed that the number of apoptotic cells in the vein wall increased for at least 8 weeks after grafting to an artery but not to any vein [2, 139]. Similarly when cultured VSMCs are subjected to mechanical stress an increase in apoptosis is observed [2, 139]. It is known that the mechanical stress results in an increase of endothelin receptor expression in VSMCs and that exposure of these cells to endothelin will induce apoptosis via an endothelin receptor dependent mechanism [217, 218]. This mechanism is sensitive to ECM interactions as it is observed in intact vessels in VSMCs cultured on fibronectin coated plates [217].

We initially showed the involvement of p38 activation in mechanical stress induced apoptosis [19, 83, 107]. VSMC lines stably transfected with dominant negative Rac, or overexpressing MKP-1 do not exhibit p38 activation by mechanical stress and the mechanical stress induced apoptosis is abolished [107, 125]. We have recently extended these studies to show that p53 is activated by mechanical stress in a Rac and p38 MAPK dependent manner [139]; We also showed that mechanical stress results in oxidative DNA damage in VSMCs and upregulation of the antiapoptotic proteins Bcl-2 and Bcl-xL [139], these results provide the first evidence that SMC apoptosis induced by mechanical stress is p53-dependent. Grafted veins are exposed to increased biomechanical forces in the form of stretch stress due to blood pressure [5]. The sudden elevation in the mechanical forces is a strong stimulus and is likely to result in activation of the intracellular signal pathways leading to gene expression and cell death [18]. At the same time a marked loss of VSMCs has been observed in the early lesions of human vein grafts [206]. One of the earliest events

in venous bypass grafts is apoptosis involving mechanical stress induced p38 MAPK activation as a transducing signal.

8. THE INFLAMMATORY RESPONSE AND THE MECHANICAL STRESS

Atherosclerosis is a disease that involves a continuous inflammatory response. Inflammation is important in all stages from initiation through progression and thrombotic complications. In the animal models of atherosclerosis, inflammation occurs concurrently with lipid accumulation in the artery wall. Atherosclerotic lesions occur mainly at branch points in arteries where endothelial cells experience disturbed flow which correlates with an increased mechanical stress [219]. The absence of normal shear stress reduces production of the endothelium-derived NO. NO is a vasodilator with anti-inflammatory properties and can also limit VCAM-1 expression [220]. The increased mechanical stress accompanying disturbed flow can increase the production of certain leukocyte adhesion molecules (e.g., intercellular adhesion molecule 1 (ICAM-1)) [221]. Increased mechanical stress also promotes VSMCs to produce proteoglycans that bind and retain lipoprotein particles, facilitating their oxidative modification which promotes inflammatory responses at the points of lesions formation [222]. Once leukocytes adhere to the endothelium in the first stages of atherosclerosis, they penetrate to the intima. Monocyte chemoattractant protein-1 (MCP-1) is responsible for the migration of monocytes to the intima at lesions [223, 224]. Similarly a family of T-cell chemoattractants may entice lymphocytes to the intima [225]. Once in the vascular wall, blood derived inflammatory cells perpetuate a local inflammatory response. Macrophages express receptors for modified lipoproteins allowing lipid ingestion and the formation of foam cells. Macrophage colony-stimulating factor (M-CSF) participates in the differentiation of monocytes into foam cells [226, 227]. T-cells also encounter signals that induce inflammatory cytokines such as γ-interferon and tumor necrosis factor (TNF)-ß that stimulate macrophages, endothelial cells and VSMCs. During the continued inflammatory process, various peptide growth factors promote VSMC proliferation and contribute to the formation of a dense extracellular matrix seen in the advanced atherosclerotic lesions [228].

Although mechanical stress may not be the primary cause for the processes described above, hypertension does increase the mechanical stress that a vessel must endure and follows closely behind lipids on the list of risk factors for atherosclerosis. Hemodynamic force does influence the location of spontaneous atherosclerosis and directly effects vascular remodeling in transplant arteriosclerosis. Inflammation may participate in hypertension and provide a pathophysiological link between the two diseases. Angiotensin II (AII) is a vasoconstrictor that can also trigger intimal inflammation. AII triggers superoxide anion production from the endothelial cells and VSMCs [229]. It will also increase expression of the proinflammatory cytokines such as interleukin (IL)-6 and monocyte chemotactic protein 1 (MCP-1) by VSMCs and VCAM-1 by the endothelial cells [230-232].

Biomechanical stress is likely to be a major cause of vein bypass graft arteriosclerosis which is also an inflammatory disease characterised by mononuclear cell infiltration followed by SMC proliferation [233, 234]. Mechanical stress may play a role in adhesion molecule expression via MAPK signaling pathways leading to NFkappa-B activation. Supporting this is the finding that neointimal lesions from vein grafts in ICAM-$1^{-/-}$ mice are reduced 30-50% compared to wild-type controls [235]. Increased ICAM-1 expression is seen in the endothelium and VSMCs of the grafted veins in wild type, but not ICAM-$1^{-/-}$ mice. Numbers of Mac-1 (CD11b/18)-positive cells adherent to the surface of vein grafts in ICAM-$1^{-/-}$ mice is much less and the positive cells are more abundant in the intimal lesions of vein grafts in wild-type mice [235]. ICAM-1 is critical in the development of venous bypass graft arteriosclerosis.

Exposure of endothelial cells to shear stress results in increased ICAM-1 and MCP-1 expression via activation of NFkappa-B and the transcription factor AP-1 [236]. These molecules are essential for leukocyte-endothelial cell interactions and subsequently cell infiltration that is characteristic of the early lesions in vein grafts subject to elevated blood pressure. Mechanical stress induces VSMCs to express ICAM-1 via NFkappa-B activation. Animal models expression of ICAM-1 by VSMCs is associated with monocyte/macrophage accumulation in the vein grafts. VSMCs of ICAM-$1^{-/-}$ mice do not express ICAM-1 and this correlates with the reduced neointimal lesions. ICAM-1 expression from VSMCs may be involved in the development of intimal hyperplasia in three ways. First, the interaction of MAC-1 and ICAM-1 on VSMCs may initiate intracellular signaling required for cytokine secretion by monocytes/macrophages. Support for this idea comes from the fact that macrophage inflammatory protein-1 (MCIP-1) production is induced in the cultured monocytes grown on ICAM-1 coated surfaces [237]. Second, binding of MAC-1 to ICAM-1 on VSMCs may be responsible for monocyte retardation in the vessel wall. Third, expression of ICAM-1 on VSMCs may be involved in the phenotypic change of VSMCs considered to be required for the migration and proliferation of VSMCs in the pathogenesis of atherosclerosis. The binding of MAC-1 to ICAM-1 on VSMCs may initiate signaling within VSMCs leading to changes in gene expression responsible for phenotypic change. Mechanical stress is a critical factor for initiating ICAM-1 expression in the vein grafts. Mechanical-stress-induced adhesion molecule and chemokine expression in the vessel wall are likely to be important for the inflammatory response.

9. SUMMARY

A large and growing body of evidence shows that mechanical stress significantly changes VSMC gene expression and both the proliferation and apoptosis. VSMCs sense and transduce mechanical stress signals to cellular responses via receptor-dependent and receptor-independent G protein-dependent pathways. In these pathways integrins, other receptors and small G proteins act as primary sensors. Integrins mediate cell adhesion and transduce signals induced by mechanical stress in VSMCs. Ras-dependent

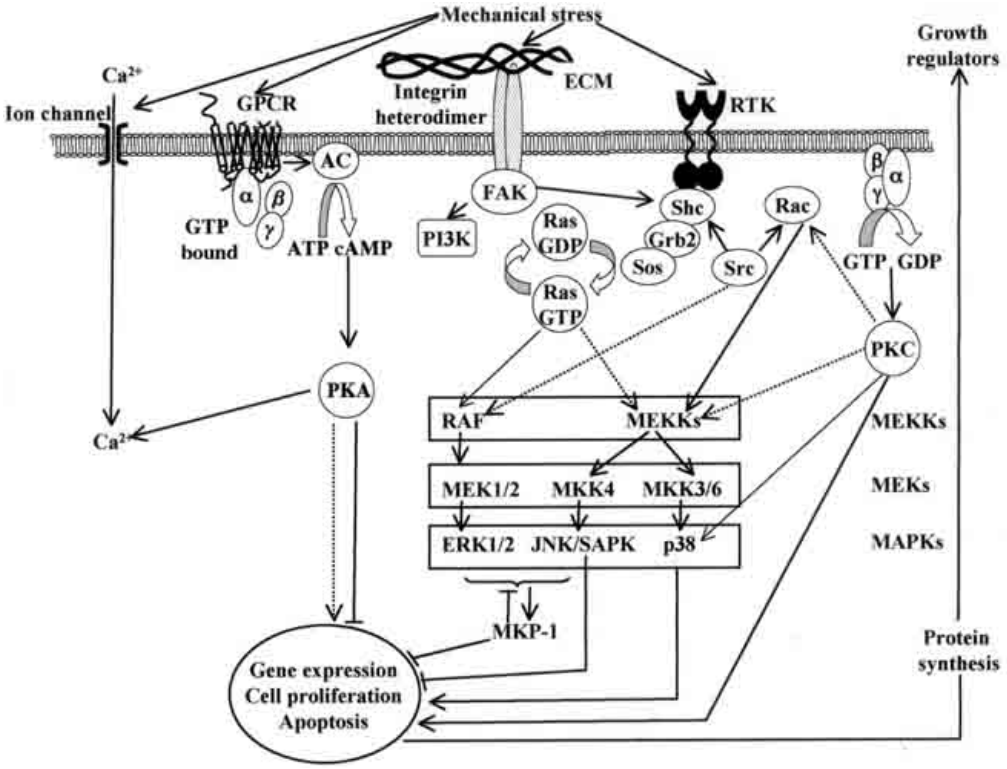

Fig. (6). A model for mechanical stress initiated signal transduction in VSMCs. Mechanical stress elongates the cell membrane and results in exposure of the kinase domain of receptors tyrosine kinases and/or conformational changes of G proteins (GPCRs and heterotrimeric G proteins) leading to receptor autophosphorylation or the facilitation of GDP exchange for GTP. Integrins and ion channels are also involved in response to mechanical stress in VSMCs. After receptor activation there is subsequent activation of Raf, MEKs, and PKCs resulting in MAPK activation which mediates gene expression, protein synthesis and cell proliferation or apoptosis. MAPK activation can simultaneously mediate MKP-1 expression and/or PKA activation both of which are negative regulators for MAPKs and cyclins

activation of signal pathway is also shared by several growth factor receptors. A number of intracellular signaling molecules including MAPKs, PKCs, MKP-1, and PKA are involved in the processes that lead to VSMC proliferation, growth inhibition or apoptosis. The cellular outcome depends on a balance between the availability of specific downstream targets such as PKC, MAPKs, as well as their targets (i.e., AP-1, NF-kappaB), and the ability of the signal to activate regulators of negative feedback such as MKP-1 and PKA.

The key events in our current knowledge of mechanical stress-induced signal transduction pathways in VSMCs are schematically presented in Fig. (**6**). Physical force initiates signal pathways that lead to various cellular fates including vascular cell death and an inflammatory response followed by smooth muscle cell proliferation. The first events, in the input layer include the sensing and conversion of extracellular physical force mediated by integrins, receptors, and G proteins. The second event in the signal processing layer include integration of intracellular signals mediated by PKCs, MAPKs, MKP-1, and PKA as well as crosstalk between pathways. The final events in the output layer are the cellular responses including transcription factor activation, gene expression, and cell proliferation or apoptosis. The different levels of mechanical stress-induced signaling in VSMCs provide enticing targets for therapeutic intervention in the cases where VSMC apoptosis, inflammatory response or proliferation contributes to the vascular diseases.

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REFERENCES

[1] Vinters HV, Berliner JA. Diabete Metab 1987; 13(3 Pt 2): 294-300.

[2] Xu Q. Trends Cardiovasc Med 2000; 10(1): 35-41.

[3] Ross R. Nature 1993; 362(6423): 801-9.

[4] Traub O, Berk BC. Arterioscler Thromb Vasc Biol 1998; 18(5): 677-85.

[5] Zou Y, Hu Y, Metzler B, Xu Q. Int J Mol Med 1998; 1(5): 827-34.

[6] Xu Q, Fawcett TW, Gorospe M, Guyton KZ, Liu Y, Holbrook NJ. Hypertension 1997; 30(1 Pt 1): 106-11.

[7] Hu Y, Cheng L, Hochleitner BW, Xu Q. Arterioscler Thromb Vasc Biol 1997; 17(11): 2808-16.

[8] Saul GD. Med Hypotheses 1999; 52(4): 349-51.

[9] Dethlefsen SM, Shepro D, D'Amore PA. J Vasc Res 1996; 33(5): 405-13.

[10] Standley PR, Obards TJ, Martina CL. Am J Physiol 1999; 276(4 Pt 1): E697-705.

[11] Aikawa R, Komuro I, Yamazaki T, Zou Y, Kudoh S, Tanaka M. J Clin Invest 1997; 100(7): 1813-21.

[12] Reidy MA. Thromb Haemost 1993; 70(1): 172-6.

[13] Meyrick B, Reid L. Clin Chest Med 1983; 4(2): 199-217.

[14] Molloy CJ, Taylor DS, Pawlowski JE. J Endocrinol 1999; 161(2): 179-85.

[15] Thubrikar MJ, Robicsek F. Ann Thorac Surg 1995; 59(6): 1594-603.

[16] DeBakey ME, Lawrie GM, Glaeser DH. Ann Surg 1985; 201(2): 115-31.

[17] Wang X, Li X, Yue TL, Ohlstein EH. Biochim Biophys Acta 2000; 1500(1): 41-8.

[18] Zou Y, Dietrich H, Hu Y, Metzler B, Wick G, Xu Q. Am J Pathol 1998; 153(4): 1301-10.

[19] Xu Q, Liu Y, Gorospe M, Udelsman R, Holbrook NJ. J Clin Invest 1996; 97(2): 508-14.

[20] Yamazaki T, Komuro I, Yazaki Y. Mol Cell Biochem 1996; 163-164: 197-201.

[21] Li C, Xu Q. Cell Signal 2000; 12(7): 435-45.

[22] Jockusch BM, Bubeck P, Giehl K, Kroemker M, Moschner J, Rothkegel M. Annu Rev Cell Dev Biol 1995; 11: 379-416.

[23] Aplin AE, Howe A, Alahari SK, Juliano RL. Pharmacol Rev 1998; 50(2): 197-263.

[24] Eliceiri BP, Cheresh DA. J Clin Invest 1999; 103(9): 1227-30.

[25] Schlaepfer DD, Broome MA, Hunter T. Mol Cell Biol 1997; 17(3): 1702-13.

[26] Schlaepfer DD, Hauck CR, Sieg DJ. Prog Biophys Mol Biol 1999; 71(3-4): 435-78.

[27] Guan JL. Matrix Biol 1997; 16(4): 195-200.

[28] Suzuki M, Naruse K, Asano Y, Okamoto T, Nishikimi N, Sakurai T. Biochem Biophys Res Commun 1997; 239(2): 372-6.

[29] Meng X, Mavromatis K, Galis ZS. Exp Mol Pathol 1999; 66(3): 227-37.

[30] Wilson E, Sudhir K, Ives HE. J Clin Invest 1995; 96(5): 2364-72.

[31] Sudhir K, Wilson E, Chatterjee K, Ives HE. J Clin Invest 1993; 92(6): 3003-7.

[32] Reusch P, Wagdy H, Reusch R, Wilson E, Ives HE. Circ Res 1996; 79(5): 1046-53.

[33] Morawietz H, Ma YH, Vives F, Wilson E, Sukhatme VP, Holtz J. Circ Res 1999; 84(6): 678-87.

[34] Lehoux S, Tedgui A. Hypertension 1998; 32(2): 338-45.

[35] Chen KD, Li YS, Kim M, Li S, Yuan S, Chien S. J Biol Chem 1999; 274(26): 18393-400.

[36] Oktay M, Wary KK, Dans M, Birge RB, Giancotti FG. J Cell Biol 1999; 145(7): 1461-9.

[37] Liu S, Thomas SM, Woodside DG, Rose DM, Kiosses WB, Pfaff M. Nature 1999; 402(6762): 676-81.

[38] Giancotti FG. Curr Opin Cell Biol 1997; 9(5): 691-700.

[39] Berton G, Lowell CA. Cell Signal 1999; 11(9): 621-35.

[40] Okuda M, Takahashi M, Suero J, Murry CE, Traub O, Kawakatsu H. J Biol Chem 1999; 274(38): 26803-9.

[41] Romer LH, McLean N, Turner CE, Burridge K. Mol Biol Cell 1994; 5(3): 349-61.

[42] Seko Y, Takahashi N, Tobe K, Kadowaki T, Yazaki Y. Biochem Biophys Res Commun 1999; 259(1): 8-14.

[43] Sai X, Naruse K, Sokabe M. J Cell Sci 1999; 112 (Pt 9): 1365-73.

[44] Li L, Chaikof EL. Arterioscler Thromb Vasc Biol 2002; 22(1): 61-8.

[45] Low RB, Woodcock-Mitchell J, Evans JN. Adler KBAm Rev Respir Dis 1984; 129(2): 311-6.

[46] Cipolla MJ, Gokina NI, Osol G. FASEB J 2002; 16(1): 72-6.

[47] Hunter AJ, Ottoson N, Boerth N, Koretzky GA, Shimizu Y. J Immunol 2000; 164(3): 1143-7.

[48] Finkelstein LD, Shimizu Y. Biochem J 2000; 345 Pt 2: 385-92.

[49] Garcia AJ, Boettiger D. Biomaterials 1999; 20(23-24): 2427-33.

[50] Antoniades HN. Proc Natl Acad Sci USA 1981; 78(12): 7314-7.

[51] Matsui T, Heidaran M, Miki T, Popescu N, La Rochelle W, Kraus M. Science 1989; 243(4892): 800-4.

[52] Yarden Y, Ullrich A. Annu Rev Biochem 1988; 57: 443-78.

[53] Sasahara M, Fries JW, Raines EW, Gown AM, Westrum LE, Frosch MP, Cell 1991; 64(1): 217-27.

[54] Ullrich A, Schlessinger J. Cell 1990; 61(2): 203-12.

[55] Cano E, Mahadevan LC. Trends Biochem Sci 1995; 20(3): 117-22.

[56] Hu Y, Bock G, Wick G, Xu Q. FASEB J 1998; 12(12): 1135-42.

[57] Hu Y, Zou Y, Dietrich H, Wick G, Xu Q. Circulation 1999; 100(8): 861-8.

[58] Ma YH, Ling S, Ives HE. Biochem Biophys Res Commun 1999; 265(2): 606-10.

[59] Nguyen HT, Adam RM, Bride SH, Park JM, Peters CA, Freeman MR. Am J Physiol Cell Physiol 2000; 279(4): C1155-67.

[60] Yarden Y, Sliwkowski MX. Nat Rev Mol Cell Biol 2001; 2(2): 127-37.

[61] Correa-Meyer E, Pesce L, Guerrero C, Sznajder JI. Am J Physiol Lung Cell Mol Physiol 2002; 282(5): L883-91.

[62] Lauth M, Berger MM, Cattaruzza M, Hecker M. Arterioscler Thromb Vasc Biol 2000; 20(1): 96-103.

[63] Smith JD, Davies N, Willis AI, Sumpio BE, Zilla P. Endothelium 2001; 8(1): 41-8.

[64] Quinn TP, Schlueter M, Soifer SJ, Gutierrez JA. Am J Physiol Lung Cell Mol Physiol 2002; 282(5): L897-903.

[65] Suzuma I, Suzuma K, Ueki K, Hata Y, Feener EP, King GL. J Biol Chem 2002; 277(2): 1047-57.

[66] Shay-Salit A, Shushy M, Wolfovitz E, Yahav H, Breviario F, Dejana E. Proc Natl Acad Sci USA 2002; 99(14): 9462-7.

[67] Bornfeldt KE, Krebs EG. Cell Signal 1999; 11(7): 465-77.

[68] Marrero MB, Paxton WG, Schieffer B, Ling BN, Bernstein KE. Cell Signal 1996; 8(1): 21-6.

[69] Higashita R, Li L, Van Putten V, Yamamura Y, Zarinetchi F, Heasley L. J Biol Chem 1997; 272(41): 25845-50.

[70] Touyz RM, Deng LY, He G, Wu XH, Schiffrin EL. J Hypertens 1999; 17(7): 907-16.

[71] Bruce L, Nixon GF. Exp Physiol 1997; 82(6): 985-93.

[72] Milano CA, Dolber PC, Rockman HA, Bond RA, Venable ME, Allen LF, Proc Natl Acad Sci USA 1994; 91(21): 10109-13.

[73] Hein L, Stevens ME, Barsh GS, Pratt RE, Kobilka BK, Dzau VJ. Proc Natl Acad Sci USA 1997; 94(12): 6391-6.

[74] Mende U, Kagen A, Cohen A, Aramburu J, Schoen FJ, Neer EJ. Proc Natl Acad Sci USA 1998; 95(23): 13893-8.

[75] Sakata Y, Hoit BD, Liggett SB, Walsh RA, Dorn GW. Circulation 1998; 97(15): 1488-95.

[76] Dorn GW. 2nd, Brown JH. Trends Cardiovasc Med 1999; 9(1-2): 26-34.

[77] Gudi S, Nolan JP, Frangos JA. Proc Natl Acad Sci USA 1998; 95(5): 2515-9.

[78] Akhter SA, Luttrell LM, Rockman HA, Iaccarino G, Lefkowitz RJ, Koch WJ. Science 1998; 280(5363): 574-7.

[79] Gudi SR, Clark CB, Frangos JA. Circ Res 1996; 79(4): 834-9.

[80] Ishida T, Takahashi M, Corson MA, Berk BC. Ann N Y Acad Sci 1997; 811: 12-23; discussion 23-4.

[81] Gudi SR, Lee AA, Clark CB, Frangos JA. Am J Physiol 1998; 274(5 Pt 1): C1424-8.

[82] Gudi T, Lohmann SM, Pilz RB. Mol Cell Biol 1997; 17(9): 5244-54.

[83] Li C, Hu Y, Sturm G, Wick G, Xu Q. Arterioscler Thromb Vasc Biol 2000; 20(3): E1-9.

[84] Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Pharmacol Rev 2001; 53(2): 245-82.

[85] Vu TK, Hung DT, Wheaton VI, Coughlin SR. Cell 1991; 64(6): 1057-68.

[86] Vu TK, Wheaton VI, Hung DT, Charo I, Coughlin SR. Nature 1991; 353(6345): 674-7.

[87] Buresi MC, Schleihauf E, Vergnolle N, Buret A, Wallace JL, Hollenberg MD. Am J Physiol Gastrointest Liver Physiol 2001; 281(2): G323-32.

[88] Nguyen KT, Frye SR, Eskin SG, Patterson C, Runge MS, McIntire LV. Hypertension 2001; 38(5): 1038-43.

[89] Ohya Y, Adachi N, Nakamura Y, Setoguchi M, Abe I, Fujishima M. Hypertension 1998; 31(1 Pt 2): 254-8.

[90] Davis MJ, Donovitz JA, Hood JD. Am J Physiol 1992; 262(4 Pt 1): C1083-8.

[91] Davis MJ, Meininger GA, Zawieja DC. Am J Physiol 1992; 263(4 Pt 2): H1292-9.

[92] McCarron JG, Crichton CA, Langton PD, MacKenzie A, Smith GL. J Physiol 1997; 498 (Pt 2): 371-9.

[93] Naruse K, Yamada T, Sai XR, Hamaguchi M, Sokabe M. Oncogene 1998; 17(4): 455-63.

[94] Malek AM, Izumo S. J Cell Sci 1996; 109 (Pt 4): 713-26.

[95] Olesen SP, Clapham DE, Davies PF. Nature 1988; 331(6152): 168-70.

[96] Mohanty MJ, Li X. Am J Physiol Cell Physiol 2002; 283(2): C456-62.

[97] Lyford GL, Strege PR, Shepard A, Ou Y, Ermilov L, Miller SM. Am J Physiol Cell Physiol 2002; 283(3): C1001-8.

[98] Chang J, Wasser JS, Cornelussen RN, Knowlton AA. Circulation 2001; 104(2): 209-14.

[99] Xu Q, Fawcett TW, Udelsman R, Holbrook NJ. Hypertension 1996; 28(1): 53-7.

[100] Xu Q, Schett G, Li C, Hu Y, Wick G. Circ Res 2000; 86(11): 1122-8.

[101] Kubo T, Hosokawa H, Kambe T, Fukumori R. Eur J Pharmacol 2000; 391(3): 281-7.

[102] Gschwendt M. Eur J Biochem 1999; 259(3): 555-64.

[103] Blobe GC, Stribling S, Obeid LM, Hannun YA. Cancer Surv 1996; 27: 213-48.

[104] Ono Y, Fujii T, Igarashi K, Kuno T, Tanaka C, Kikkawa U. Proc Natl Acad Sci USA 1989; 86(13): 4868-71.

[105] Mochly-Rosen D, Gordon AS. FASEB J 1998; 12(1): 35-42.

[106] Yano K, Bauchat JR, Liimatta MB, Clemmons DR, Duan C. Endocrinology 1999; 140(10): 4622-32.

[107] Mayr M, Li C, Zou Y, Huemer U, Hu Y, Xu Q. FASEB J 2000; 14(2): 261-70.

[108] Igarashi M, Wakasaki H, Takahara N, Ishii H, Jiang ZY, Yamauchi T, J Clin Invest 1999; 103(2): 185-95.

[109] Mills I, Cohen CR, Kamal K, Li G, Shin T, Du W. J Cell Physiol 1997; 170(3): 228-34.

[110] Chaqour B, Howard PS, Richards CF, Macarak EJ. J Mol Cell Cardiol 1999; 31(7): 1345-55.

[111] Bowling N, Walsh RA, Song G, Estridge T, Sandusky GE, Fouts RL. Circulation 1999; 99(3): 384-91.

[112] Martelli AM, Sang N, Borgatti P, Capitani S, Neri LM. J Cell Biochem 1999; 74(4): 499-521.

[113] Pukac L, Huangpu J, Karnovsky MJ. Exp Cell Res 1998; 242(2): 548-60.

[114] Metzler B, Li C, Hu Y, Sturm G, Ghaffari-Tabrizi N, Xu Q. Arterioscler Thromb Vasc Biol 1999; 19(8): 1862-71.

[115] Buchner K. J Cancer Res Clin Oncol 2000; 126(1): 1-11.

[116] Keenan C, Kelleher D. Cell Signal 1998; 10(4): 225-32.

[117] Wang J, Frost JA, Cobb MH, Ross EM. J Biol Chem 1999; 274(44): 31641-7.

[118] Haller H, Maasch C, Lindschau C, Brachmann M, Buchner K, Luft FC. Acta Physiol Scand 1998; 164(4): 599-609.

[119] Songu-Mize E, Liu X, Hymel LJ. Am J Med Sci 1998; 316(3): 196-9.

[120] Pan J, Fukuda K, Saito M, Matsuzaki J, Kodama H, Sano M. Circ Res 1999; 84(10): 1127-36.

[121] Leitges M, Mayr M, Braun U, Mayr U, Li C, Pfister G. J Clin Invest 2001; 108(10): 1505-12.

[122] Garrington TP, Johnson GL. Curr Opin Cell Biol 1999; 11(2): 211-8.

[123] Widmann C, Gibson S, Jarpe MB, Johnson GL. Physiol Rev 1999; 79(1): 143-80.

[124] Widmann C, Gerwins P, Johnson NL, Jarpe MB, Johnson GL. Mol Cell Biol 1998; 18(4): 2416-29.

[125] Li C, Hu Y, Mayr M, Xu Q. J Biol Chem 1999; 274(36): 25273-80.

[126] Force T, Pombo CM, Avruch JA, Bonventre JV, Kyriakis JM. Circ Res 1996; 78(6): 947-53.

[127] Gutkind JS. J Biol Chem 1998; 273(4): 1839-42.

[128] Tibbles LA, Woodgett JR. Cell Mol Life Sci 1999; 55(10): 1230-54.

[129] Kyriakis JM, Avruch J. J Biol Chem 1996; 271(40): 24313-6.

[130] Clerk A, Harrison JG, Long CS, Sugden PH. J Mol Cell Cardiol 1999; 31(12): 2087-99.

[131] Reusch HP, Chan G, Ives HE, Nemenoff RA. Biochem Biophys Res Commun 1997; 237(2): 239-44.

[132] Hu Y, Dietrich H, Metzler B, Wick G, Xu Q. Arterioscler Thromb Vasc Biol 2000; 20(1): 18-26.

[133] Yujiri T, Sather S, Fanger GR, Johnson GL. Science 1998; 282(5395): 1911-4.

[134] Hu Y, Metzler B, Xu Q. J Biol Chem 1997; 272(14): 9113-9.

[135] Hu Y, Hochleitner BW, Wick G, Xu Q. Exp Gerontol 1998; 33(6): 601-13.

[136] Keesler GA, Bray J, Hunt J, Johnson DA, Gleason T, Yao Z. Protein Expr Purif 1998; 14(2): 221-8.

[137] Hedges JC, Dechert MA, Yamboliev IA, Martin JL, Hickey E, Weber LA. J Biol Chem 1999; 274(34): 24211-9.

[138] Rousseau S, Houle F, Landry J, Huot J. Oncogene 1997; 15(18): 2169-77.

[139] Mayr M, Hu Y, Hainaut H, Xu Q. FASEB J 2002; 16(11): 1423-5.

[140] Sugawara A, Nakao K, Sakamoto M, Morii N, Yamada T, Itoh H. Lancet 1985; 2(8469-70): 1426-7.

[141] Arendt RM, Gerbes AL, Ritter D, Stangl E, Bach P, Zahringer J. J Hypertens Suppl 1986; 4(2): S131-5.

[142] Kiemer AK, Weber NC, Furst R, Bildner N, Kulhanek-Heinze S, Vollmar AM. Circ Res 2002; 90(8): 874-81.

[143] Sun H, Tonks NK, Bar-Sagi D. Science 1994; 266(5183): 285-8.

[144] Misra-Press A, Rim CS, Yao H, Roberson MS, Stork PJ. J Biol Chem 1995; 270(24): 14587-96.

[145] Liu Y, Gorospe M, Yang C, Holbrook NJ. J Biol Chem 1995; 270(15): 8377-80.

[146] Lai K, Wang H, Lee WS, Jain MK, Lee ME, Haber E. J Clin Invest 1996; 98(7): 1560-7.

[147] Duff JL, Marrero MB, Paxton WG, Charles CH, Lau LF, Bernstein KE. J Biol Chem 1993; 268(35): 26037-40.

[148] Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ. J Biol Chem 1996; 271(8): 4138-42.

[149] Metzler B, Hu Y, Sturm G, Wick G, Xu Q. J Biol Chem 1998; 273(50): 33320-6.

[150] Bokemeyer D, Lindemann M, Kramer HJ. Hypertension 1998; 32(4): 661-7.

[151] Begum N, Ragolia L, Rienzie J, McCarthy M, Duddy N. J Biol Chem 1998; 273(39): 25164-70.

[152] Valledor AF, Xaus J, Marques L, Celada A. J Immunol 1999; 163(5): 2452-62.

[153] Koyama H, Olson NE, Dastvan FF, Reidy MA. Circ Res 1998; 82(6): 713-21.

[154] Franklin CC, Kraft AS. J Biol Chem 1997; 272(27): 16917-23.

[155] Lavoie JN, L'Allemain G, Brunet A, Muller R, Pouyssegur J. J Biol Chem 1996; 271(34): 20608-16.

[156] Kusari AB, Byon J, Bandyopadhyay D, Kenner KA, Kusari J. Mol Endocrinol 1997; 11(10): 1532-43.

[157] Begum N, Song Y, Rienzie J, Ragolia L. Am J Physiol 1998; 275(1 Pt 1): C42-9.

[158] Fawcett TW, Xu Q, Holbrook NJ. Cell Stress Chaperones 1997; 2(2): 104-9.

[159] Hiroi Y, Hiroi J, Kudoh S, Yazaki Y, Nagai R, Komuro I. Hypertens Res 2001; 24(4): 385-94.

[160] Araujo EG, Bianchi C, Sato K, Faro R, Li XA, Sellke FW. J Thorac Cardiovasc Surg 2001; 121(4): 773-81.

[161] Lim HW, New L, Han J, Molkentin JD. J Biol Chem 2001; 276(19): 15913-9.

[162] Jacob A, Molkentin JD, Smolenski A, Lohmann SM, Begum N. Am J Physiol Cell Physiol 2002; 283(3): C704-13.

[163] Communal C, Colucci WS, Remondino A, Sawyer DB, Port JD, Wichman SE. J Card Fail 2002; 8(2): 86-92.

[164] Bueno OF, De Windt LJ, Lim HW, Tymitz KM, Witt SA, Kimball TR. Circ Res 2001; 88(1): 88-96.

[165] Griendling KK, Alexander RW. FASEB J 1996; 10(2): 283-92.

[166] Cook SJ, McCormick F. Science 1993; 262(5136): 1069-72.

[167] Vadiveloo PK, Filonzi EL, Stanton HR, Hamilton JA. Atherosclerosis 1997; 133(1): 61-9.

[168] Bornfeldt KE, Campbell JS, Koyama H, Argast GM, Leslie CC, Raines EW. J Clin Invest 1997; 100(4): 875-85.

[169] Servant MJ, Giasson E, Meloche S. J Biol Chem 1996; 271(27): 16047-52.

[170] Nelson PR, Yamamura S, Mureebe L, Itoh H, Kent KC. J Vasc Surg 1998; 27(1): 117-25.

[171] Whitmarsh AJ, Shore P, Sharrocks AD, Davis RJ. Science 1995; 269(5222): 403-7.

[172] Davis RJ. J Biol Chem 1993; 268(20): 14553-6.

[173] Graves LM, Bornfeldt KE, Raines EW, Potts BC, Macdonald SG, Ross R. Proc Natl Acad Sci USA 1993; 90(21): 10300-4.

[174] Sevetson BR, Kong X, Lawrence JC. Jr. Proc Natl Acad Sci USA 1993; 90(21): 10305-9.

[175] Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ, Sturgill TW. Science 1993; 262(5136): 1065-9.

[176] Burgering BM, Pronk GJ, van Weeren PC, Chardin P, Bos JL. EMBO J 1993; 12(11): 4211-20.

[177] Calleja V, Ruiz Enriquez P, Filloux C, Peraldi P, Baron V, Van Obberghen E. Endocrinology 1997; 138(3): 1111-20.

[178] Vaillancourt RR, Gardner AM, Johnson GL. Mol Cell Biol 1994; 14(10): 6522-30.

[179] Chen J, Iyengar R. Science 1994; 263(5151): 1278-81.

[180] Chuang E, Barnard D, Hettich L, Zhang XF, Avruch J, Marshall MS. Mol Cell Biol 1994; 14(8): 5318-25.

[181] Mischak H, Seitz T, Janosch P, Eulitz M, Steen H, Schellerer M. Mol Cell Biol 1996; 16(10): 5409-18.

[182] Peraldi P, Frodin M, Barnier JV, Calleja V, Scimeca JC, Filloux C. FEBS Lett 1995; 357(3): 290-6.

[183] Hafner S, Adler HS, Mischak H, Janosch P, Heidecker G, Wolfman A. Mol Cell Biol 1994; 14(10): 6696-703.

[184] Feig LA, Schaffhausen B. Nature 1994; 370(6490): 508-9.

[185] Magnuson NS, Beck T, Vahidi H, Hahn H, Smola U, Rapp UR. Semin Cancer Biol 1994; 5(4): 247-53.

[186] Graves LM, Bornfeldt KE, Sidhu JS, Argast GM, Raines EW, Ross R. J Biol Chem 1996; 271(1): 505-11.

[187] Rao GN, Runge MS. J Biol Chem 1996; 271(34): 20805-10.

[188] Pyne NJ, Pyne S. Cell Signal 1997; 9(3-4): 311-7.

[189] Lopez-Ilasaca M, Li W, Uren A, Yu JC, Kazlauskas A, Gutkind JS. Biochem Biophys Res Commun 1997; 232(2): 273-7.

[190] Daum G, Kalmes A, Levkau B, Wang Y, Davies MG, Clowes AW. FEBS Lett 1998; 427(2): 271-4.

[191] Schwartz MA, Ginsberg MH. Nat Cell Biol 2002; 4(4): E65-8.

[192] DeMali KA, Balciunaite E, Kazlauskas A. J Biol Chem 1999; 274(28): 19551-8.

[193] Juliano RL, Aplin AE, Howe AK, Short S, Lee JW, Alahari S. Methods Enzymol 2001; 333: 151-63.

[194] Schwartz MA, Assoian RK. J Cell Sci 2001; 114(Pt 14): 2553-60.

[195] Aplin AE, Stewart SA, Assoian RK, Juliano RL. J Cell Biol 2001; 153(2): 273-82.

[196] Assoian RK, Schwartz MA. Curr Opin Genet Dev 2001; 11(1): 48-53.

[197] Choquet D, Felsenfeld DP, Sheetz MP. Cell 1997; 88(1): 39-48.

[198] Wang HB, Dembo M, Hanks SK, Wang Y. Proc Natl Acad Sci USA 2001; 98(20): 11295-300.

[199] Schoenwaelder SM, Burridge K. Curr Opin Cell Biol 1999; 11(2): 274-86.

[200] Tzima E, del Pozo MA, Shattil SJ, Chien S, Schwartz MA. EMBO J 2001; 20(17): 4639-47.

[201] Giancotti FG, Ruoslahti E. Science 1999; 285(5430): 1028-32.

[202] Moro L, Venturino M, Bozzo C, Silengo L, Altruda F, Beguinot L. EMBO J 1998; 17(22): 6622-32.

[203] Moro L, Dolce L, Cabodi S, Bergatto E, Erba EB, Smeriglio M. J Biol Chem 2002; 277(11): 9405-14.

[204] Klinghoffer RA, Sachsenmaier C, Cooper JA, Soriano P. EMBO J 1999; 18(9): 2459-71.

[205] Mayr M, Xu Q. Exp Gerontol 2001; 36(7): 969-87.

[206] Kockx MM. Arterioscler Thromb Vasc Biol 1998; 18(10): 1519-22.

[207] Bennett MR, Evan GI, Schwartz SM. J Clin Invest 1995; 95(5): 2266-74.

[208] Wernig F, Xu Q. Progress Biophys Mol Biol 2002; 78: 105-137.

[209] Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Science 1995; 270(5240): 1326-31.

[210] Metzler B, Hu Y, Dietrich H, Xu Q. Am J Pathol 2000; 156(6): 1875-86.

[211] Perlman H, Maillard L, Krasinski K, Walsh K. Circulation 1997; 95(4): 981-7.

[212] Malik N, Francis SE, Holt CM, Gunn J, Thomas GL, Shepherd L. Circulation 1998; 98(16): 1657-65.

[213] Sata M, Sugiura S, Yoshizumi M, Ouchi Y, Hirata Y, Nagai R. Arterioscler Thromb Vasc Biol 2001; 21(11): 1733-7.

[214] Sata M, Suhara T, Walsh K. Arterioscler Thromb Vasc Biol 2000; 20(2): 309-16.

[215] Dietrich H, Hu Y, Zou Y, Dirnhofer S, Kleindienst R, Wick G. Arterioscler Thromb Vasc Biol 2000; 20(2): 343-52.

[216] Dietrich H, Hu Y, Zou Y, Huemer U, Metzler B, Li C. Am J Pathol 2000; 157(2): 659-69.

[217] Cattaruzza M, Berger MM, Ochs M, Fayyazi A, Fuzesi L, Richter J. Cell Death Differ 2002; 9(2): 219-26.

[218] Cattaruzza M, Dimigen C, Ehrenreich H, Hecker M. FASEB J 2000; 14(7): 991-8.

[219] Topper JN, Cai J, Falb D, Gimbrone MA, Jr. Proc Natl Acad Sci USA 1996; 93(19): 10417-22.

[220] De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA, Jr. J Clin Invest 1995; 96(1): 60-8.

[221] Nagel T, Resnick N, Atkinson WJ, Dewey CF, Jr Gimbrone MA. Jr. J Clin Invest 1994; 94(2): 885-91.

[222] Lee RT, Yamamoto C, Feng Y, Potter-Perigo S, Briggs WH, Landschulz KT. J Biol Chem 2001; 276(17): 13847-51.

[223] Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P. Mol Cell 1998; 2(2) 275-8.

[224] Boring L, Gosling J, Cleary M, Charo IF. Nature 1998; 394(6696): 894-7.

[225] Mach F, Sauty A, Iarossi AS, Sukhova GK, Neote K, Libby P. J Clin Invest 1999; 104(8): 1041-50.

[226] Smith JD, Trogan E, Ginsberg M, Grigaux C, Tian J, Miyata M. Proc Natl Acad Sci USA 1995; 92(18): 8264-8.

[227] Qiao JH, Tripathi J, Mishra NK, Cai Y, Tripathi S, Wang XP. Am J Pathol 1997; 150(5): 1687-99.

[228] Ross R. N Engl J Med 1999; 340(2): 115-26.

[229] Griendling KK, Ushio-Fukai M, Lassegue B, Alexander RW. Hypertension 1997; 29(1 Pt 2): 366-73.

[230] Kranzhofer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kubler W. Arterioscler Thromb Vasc Biol 1999; 19(7): 1623-9.

[231] Hernandez-Presa M, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M. Circulation 1997; 95(6): 1532-41.

[232] Tummala PE, Chen XL, Sundell CL, Laursen JB, Hammes CP, Alexander RW. Circulation 1999; 100(11): 1223-9.

[233] Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. J Am Coll Cardiol 1990; 15(7): 1667-87.

[234] Motwani JG, Topol EJ. Circulation 1998; 97(9): 916-31.

[235] Zou Y, Hu Y, Mayr M, Dietrich H, Wick G, Xu Q. Circ Res 2000; 86(4): 434-40.

[236] Resnick N, Gimbrone MA, Jr. FASEB J 1995; 9(10): 874-82.

[237] Couffinhal T, Duplaa C, Moreau C, Lamaziere JM, Bonnet J. Circ Res 1994; 74(2): 225-34.