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Activation and inflammation of the venous endothelium in vein graft disease

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Abstract:

The long saphenous vein is the most commonly used conduit in coronary artery bypass graft (CABG) surgery when bypassing multiple diseased arteries; however, its use is complicated by the development of vascular inflammation, intimal hyperplasia and accelerated atherosclerosis leading to compromised graft efficacy. Despite refinement of surgical techniques to improve graft patency late vein graft failure remains a significant problem. Moreover, there is a lack of pharmacological interventions proven to be effective in the treatment of late vein graft failure. A greater understanding of the molecular nature of the disease and the interactions between endothelial and smooth muscle cells as a result of alterations in local haemodynamics may assist with designing future beneficial pharmacological interventions.

Venous endothelial cells (ECs) are physiologically adapted to chronic low shear stress; however, once the graft is implanted into the arterial circulation, they become suddenly exposed to acute high levels of shear stress. A small number of *in vitro* and *ex vivo* studies have demonstrated that acute high shear stress is associated with the activation of a pro-inflammatory profile in saphenous vein ECs, which may be mediated by mitogen-activated protein kinase (MAPK) and nuclear factor- κ B (NF- κ B) signalling pathways. The impact of acute changes in shear stress on venous ECs and the role of ECs in the development of intimal hyperplasia remains incomplete and is the subject of this review.

1. Introduction:

Ischaemic heart disease (IHD) is a leading cause of morbidity and mortality in the Western world [1]. It occurs as a result of mismatch in oxygen demand and supply usually due to atherosclerosis in one or more of the coronary arteries leading to significant stenosis in the coronary circulation. Management of IHD can be non-invasive, through modulating risk factors and using pharmacological agents, or invasive, including percutaneous coronary intervention (PCI) and/or coronary artery bypass grafting (CABG), aimed towards unblocking or bypassing the occlusion, respectively [2,3]. CABG remains the gold standard intervention in many patients in the presence of complex coronary disease, diabetes and poor ventricular function [4-7].

The long saphenous vein (LSV) is commonly used as a conduit for CABG in patients with multi-vessel coronary artery disease because it is easy and quick to harvest and it can also provide enough length to bypass multiple diseased coronaries [5,6,8]. The left internal mammary artery (LIMA) to left anterior descending coronary artery (LAD) is the graft of choice for patients receiving a single graft due to its higher long-term patency compared with LSV and improved long-term survival benefit [9,10]. The use of LIMA is, however, restricted to a single graft primarily, though occasionally can be used to sequentially graft another neighbouring coronary vessel as well. Alternatively, patients requiring multiple grafts may receive IMA in conjunction with the radial artery or another arterial graft such as the right internal mammary [11,12]. However, the utilisation of multiple arterial grafting in CABG remains low in general [13,14]. Whereas, for patients with multi-vessel coronary artery disease, at least one or more LSV graft will still be used and the LSV remains an important conduit in CABG. The use of LSV is not without limitations; its longevity continues to be complicated by the development of vascular inflammation, intimal hyperplasia (IH) and accelerated atherosclerosis leading to compromised graft efficacy and ultimately, graft failure [15].

Vein graft failure (VGF) can be separated into two distinct temporal categories; firstly, early graft failure can be defined as that which has been reported within one-year post-implantation and it

occurs in 3-15% of venous grafts. However, early graft failure is most commonly due to technical mismanagement leading to thromboses which may cause the vessel to become occluded. Whereas, late stage graft failure is characterised by the prolonged development of superimposed atherosclerosis on the vessel wall; by contrast, this late stage VGF occurs in approximately 50-60% of grafts within 10 years (Figure 1) ^[16]. Many cellular processes occur within the graft that can trigger the development of IH; these are initiated by both pre- and post-implantation injury of the vessel wall. Initially, migration of vascular smooth muscle cells (VSMCs) from the medial to intimal layer due to phenotypic switching from contractile to synthetic state occurs ^[17,18]. Increased levels and activation of matrix-degrading metalloproteinases (MMPs) facilitate VSMC migration through remodelling of the extracellular matrix (ECM) ^[19]. Once within the intima, VSMCs proliferate, which, when accompanied with increased ECM deposition (collagen and proteoglycans, but not elastin), initiates the narrowing and stiffening of the bypass graft (Figure. 2) ^[20,21].

Surgical advances in CABG have led to the great increases in patency rates of LSV grafts with the introduction of practices such as low-pressure vessel distension and the use of the ‘no-touch’ technique, whereby, during harvest of the LSV, the perivascular fat surrounding the vessel is preserved ^[22]. Of particular growing interest, is the role that preservation of perivascular adipose tissue (PVAT) plays in the pathophysiology of the vein graft. PVAT has long been overlooked in vascular biology, far from being simply an energy source, PVAT releases and receives a number of functional substances known to influence vessel behaviour, with functions as diverse as relaxation to inflammation ^[23]. It is well recognised that the use of the ‘no-touch’ technique results in greater preservation of the endothelium following harvest; however, what is less well documented is the function that remaining PVAT has in maintaining VSMC quiescence and contraction ^[24]. The refinement of LSV surgical harvest techniques has enabled a reduction in conduit damage prior to implantation; however, despite efficacy of surgical advancements, very few pharmacological studies have been able to improve long-term vein graft patency ^[25-28]. A greater understanding of

the disease pathophysiology, in particular, identifying the immediate changes following implantation is necessary to enable improved vein graft patency in the future.

2. The role of ECs in vascular homeostasis

ECs play a pivotal role in regulating vascular homeostasis, with the quiescent endothelium protecting arteries through the promotion of an anti-coagulant and anti-inflammatory environment, as well as regulating lipoprotein permeability ^[29]. Redox balance is extremely important in the maintenance of normal functionality in the endothelium ^[30]. The bioavailability of endothelium derived relaxing factors, nitric oxide (NO) and prostaglandin (PGI₂), is fundamental to the maintenance of vascular tone, as well as the regulation of EC quiescence and VSMC proliferation, migration and contractility (Figure. 3a) ^[31]. The dysregulation of NO balance has profound effects for the vascular wall, particularly in the context of the vein graft, with the tipping of this balance to reduced NO availability and increased ROS production, in particular, superoxide (O₂^{•-}) generation, primarily through eNOS uncoupling by combined actions of NADPH oxidase (NOX) and arginase enzymes ^[30,32-34]. Increased superoxide generation is common in a number of vascular pathologies, functioning to scavenge NO (together form peroxynitrite (ONOO⁻) and directly regulating VSMC proliferation, which begins the development of a highly atherogenic environment initiated by endothelial dysfunction ^[35]. Similarly, the two transcription factors, lung Kruppel-like factor 2 (KLF2) and Nuclear factor erythroid 2-related factor (Nrf2), which target a myriad of genes involved in the antioxidant and cellular defence responses, in particular, eNOS and heme-oxygenase-1 (HO-1), are of paramount importance in maintenance of redox balance ^[36].

Once activated, ECs initiate complex networks of adhesion molecules, cytokines and recruitment of inflammatory cells, which act to disseminate physiological changes within the endothelium itself and the vascular wall through interactions with VSMCs and ECM ^[37]. Vasospasm, surgical trauma, and ischaemia during the harvesting of the conduit typically result in EC activation ^[38,39]. It has

been reported that the surgical trauma during vein harvest can lead to the induction of pro-inflammatory signalling pathways and EC activation and loss, which can be greatly prevented by the utilization of minimum or no touch techniques during the harvest process ^[40-42]. ECs are further activated by the sudden exposure to new mechanical forces in the arterial circulation, including distension and increased shear stress ^[43,44].

EC activation results in the triggering of the 'adhesion cascade' characterised by the rapid expression of chemokines such as monocyte chemotactic protein 1 (MCP-1)) and the expression of adhesion molecules, including those of the Selectin family (E-Selectin and P-Selectin) and the cellular adhesion molecule family, intercellular adhesion molecule 1 (ICAM1), platelet endothelial cell adhesion molecule 1 (PECAM1) and vascular cell adhesion molecule 1 (VCAM1) ^[45,46]. Initial activation of the endothelium rapidly leads to leukocyte recruitment, which is in part mediated through changes in calcium (Ca^{2+}) homeostasis and the Ras homologue (RHO) pathway (Figure. 3b) ^[47,48]. Activation of the RHO pathway through ligand binding to G-Protein coupled receptors (GPCRs) functions to loosen tight junctions between endothelial cells and activate GTPases, whereas increases in intracellular Ca^{2+} facilitates the movement of P-Selectin to the luminal surface ^[49]. Together, these rapid actions of GPCRs and Ca^{2+} help to facilitate leukocyte trans-endothelial migration ^[50]. However, the actions of transduction through RHO and Ca^{2+} are fleeting and GPCRs quickly become desensitised (approximately 10 minutes post-stimulus); as such, this phase of acute inflammation is commonly known as type I ^[51]. Prolonged inflammatory activation of ECs requires the sustained action of physiologic stress- or cytokine-mediated signalling through the involvement of mitogen activated protein kinases (MAPKs) and pro-inflammatory transcription factors nuclear factor kappa B (NF- κ B) and activating protein 1 (AP-1) ^[52-54]. Typically, NF- κ B and MAPK mediated inflammatory responses involve the comparable, but not exclusive, expression of many genes associated with the adhesion cascade, cytokines, chemokines and MMPs in a time-dependent and evolving manner between 10 minutes and 24 hours after activation (Figure 3c) ^[55,56]. Having

such integral roles in the propagation of inflammatory responses makes these pathways exciting therapeutic targets for the treatment of many vascular pathologies and other systemic disorders involving inflammation.

3. CABG conduit comparison

LSV and IMA differ vastly in structure and functional capability. The IMA is structurally a very elastic artery with a medial layer formed of distinct sheets of interspersed wave-like collagen and VSMCs arranged circumferentially with very little variability under arterial pressure ^[57]. Interestingly, the IMA shares a great deal of structural homology with the LAD, the primary differences being that it possesses a smaller VSMC medial area and more elastin ^[58]. Whereas the LSV is considerably more varied in size and structure, with a much narrower medial layer composed of primarily collagen, fenestrated elastic laminae, less elastin and fewer VSMCs ^[57]. Thus, the LSV suffers from ‘compliance mismatch’ under higher physiologic pressures in the arterial circulation than is typical in the venous circulation, whereby the vessel is unable to passively expand to accommodate the higher pressure due to its inherently thinner, less dense medial layer and its highly aligned collagen ^[59]. Furthermore, arterial and venous vascular beds emanate from molecularly distinct foundations and exhibit early differences in signalling pathways including ephrin B2/EphB4, VEGF-A and Notch which define vessel identity before initiation of circulatory flow ^[60].

Within mature blood vessels, the endothelium is typically continuous and non-fenestrated, with each cell within the monolayer being attached through intercellular tight or adherens junctions ^[61]. Arterial ECs (aECs) exhibit many more tight junctions per cell than venous ECs (vECs), owing to the considerably higher pressure within the arterial system ^[62]. Greater tight junction distribution in aECs function to prevent para-cellular transport, thus reducing endothelial permeability to solutes and lipids ^[63]. Moreover, different haemodynamic patterns between the arterial and venous tree

result in aECs alignment with the direction of flow and they become elongated and flattened, whereas vECs do not align with flow and are morphologically shorter and wider ^[64] (Figure. 3). Interestingly; such morphological patterns can be reversible both *in vivo* and *in vitro* ^[65] suggesting that EC phenotypes maintain a degree of plasticity. This adaptability is particularly evident *in vitro*, with the loss and gain of numerous expressed genes characteristic of EC type (or location) once cells have been passaged, clearly showing the impact of cellular microenvironment on EC phenotype ^[66].

When comparing the impact of altered haemodynamics (mainly shear stress), we noted that vECs respond to acute changes in shear stress by inducing a pro-inflammatory profile mediated by the activation of MAPK signalling pathway, while aECs remained resistant to such stimuli ^[67]. This differential response is related the fact that aECs react to shear stress by upregulating anti-inflammatory mediators such as MKP-1 and NRF-2, and this response appears to be innately reduced in vEC ^[68,69].

4. Intimal hyperplasia and the endothelium

IH is the result of chronic structural changes occurring in vein grafts due to abnormal migration and proliferation of VSMCs accompanied by an increase in the amount of ECM ^[18,38,70]. The role of ECs in the development of IH has not yet been fully elucidated; previous studies have suggested that ECs may not be important as they are lost following surgery ^[71]. Although, most of these studies observed late time points and did not address the immediate changes in ECs. Moreover, it has not been determined whether EC denudation and loss is purely mechanical or is driven by EC activation and apoptosis in response to acute changes in unconditioned ECs. It is widely accepted that harvesting and implantation of LSV impacts on EC integrity to some degree ^[72], though recent studies using improved surgical resection suggest that the endothelium is better preserved following

surgery ^[73,74]. However, these studies fail to address the degree of dysfunction in the preserved endothelium. In fact, studies using an *ex vivo* perfusion model of LSV demonstrated that vECs are preserved but activated in response to acute shear stress ^[35,37]. We have corroborated these findings by demonstrating that acute exposure of LSV to high shear stress is associated with minimum denudation and significant pro-inflammatory response in vECs ^[75,76]. Our understanding of the role of inflammatory pathways, in particular MAPKs and NF- κ B, in relation to vein graft disease and their mode of regulation remains limited. In a canine model of external jugular vein interposition in the carotid artery, it was suggested that MAPK pathway activation is bimodal, as p38 activation was noted rapidly (between 30 min and 3 h) after surgery and later (4 days), which, at a later time-point, was associated with VSMC activation ^[77]. Topical MAPK inhibitors significantly suppressed graft disease in a rabbit inter-positional model of vein grafting ^[78], although not cell-specific, this provides further evidence for the role of MAPK in vein graft failure. Moreover, pharmacological vasorelaxation suppressed p38 activity and restricted the activation of proliferation and cell cycle progression in the porcine carotid jugular interposition graft ^[79]. Further evidence for the inhibition of inflammatory processes in vein graft disease is scarce. However, in a rabbit model for IH, site-specific delivery of an NF- κ B decoy was shown to significantly suppress neointimal formation ^[80,81]. Taken together, these studies suggest a potentially unexplored role for the therapeutic use of anti-inflammatory compounds in the treatment of VGF.

4.1. Shear stress and IH

Blood vessels are influenced by several forces relating to fluid dynamics (shear stress and hydrostatic pressure) and wall mechanics (traction, and distension) ^[82,83]. Shear stress, which is the tangential frictional force acting longitudinally on the vessel wall imposed by fluid motion on a solid boundary parallel to the fluid direction ^[36], is one of the most important forces that directly regulates EC physiology and vascular inflammation.

There is a significant increase in wall shear stress immediately after implanting LSV into arterial circulation ^[84] leading to rapid cytoskeletal remodelling and activation of multiple signalling cascades in ECs ^[85]. The exposure of ECs to high shear stress can result in the rapid activation of Ras (>1 min) which is followed by an increase in the activities of extracellular-signal regulated kinase (ERK) (10 min) and c-Jun N-terminal kinase (JNK) (30 min) ^[86]. ERK activation may lead to c-fos gene expression and JNK activation may induce c-Jun phosphorylation which acts to increase AP-1/TPA response element (TRE) transcriptional activity ^[87]. Furthermore, acute sudden increases in shear stress result in the early development of inflammation, demonstrated by the expression of MCP-1 and infiltration of monocytes; thus seeding the environment for atherogenesis ^[88]. Inflammatory cells and adhesion cascades are in fact so inextricably linked to vein graft intimal hyperplasia development that depletion of macrophages or knock-down of ICAM-1 has been shown to significantly reduce rates of vein graft disease ^[89,90]. *In vitro* studies using human umbilical vein ECs (HUVECs) showed that acute exposure to high shear stress can lead to early activation of ERK1/2 and p38 ^[91,92]. Furthermore, we have demonstrated that *in vitro* and *ex vivo* exposure of vECs to acute shear stress is associated with the activation of p38; this was in turn associated with increased MCP-1 production. Suppression of p38 activity can also lead to significant reduction in pro-inflammatory responses to acute shear stress ^[71].

Acute shear stress appears to activate NF- κ B in an integrin dependent manner. Once integrins are activated, they are able to begin inducing signals which include activation of small GTPase, RhoA and Rac1 ^[93]. In turn, Rac1 induces NADPH oxidase to produce ROS, functioning to stimulate the critical NF- κ B kinases, I-kappa B kinase beta (IKK β) and NF- κ B inducing kinase (NIK), which together trigger classical activation of the NF- κ B pathway ^[94,95]. Activation of the NF- κ B pathway by shear stress is most likely not as singular or simple as has been suggested, however. There are other elements involved for ultimate transcriptional activation, many of which perhaps remain unidentified. For example, focal adhesion kinase (FAK), a crucial member of the integrin-

dependent shear stress induced EC activation mechanism, does not explicitly stimulate NF- κ B translocation but rather, is responsible for phosphorylation of the transcription factor, which is intimately linked to the transcriptional activity of NF- κ B [96]. Whereas p21-activated kinase (PAK), which is flow dependent, also functions as a crucial element in the oxidant dependent activation NF- κ B through NIK, though, unlike Rac1, has absolutely no effect on ROS production [93].

Taken together, these studies of the flow induced activation of the endothelium describe a relatively confusing picture, particularly in the context of the vein graft. Nevertheless, focal inhibition of acute shear stress mediated induction of NF- κ B and MAPK pathways represent an exciting therapeutic direction in the treatment of vein graft disease.

5. Current clinical pharmacological strategies

Developments of pharmacologic interventions for the treatment of VGF continue to endeavour in pursuit of a suitable therapeutic target for both cellular specificity and efficacy. Secondary prevention of the symptoms of late VGF pharmacologically provides equally demanding questions relating to treatment strategy. Addressing maintenance of EC functionality after surgical trauma is of paramount importance both pre- and peri-operatively for SVGs; this requires preservation of endothelial coverage, as well as preservation of the vasomotor actions of ECs (reviewed in detail elsewhere [97]). Recent developments have led to the renewed interest in the pleiotropic effects of statins for preservation of EC function, due to their role in the conservation of redox balance in the endothelium and vascular wall [98]. In addition to the widely understood lipid-lowering effects of statins, it has been reported that they are able to prevent activation of pro-oxidant systems whilst boosting cellular defence mechanisms. Pre-treatment of CABG patients with Atorvastatin improved redox balance in patients after surgery, as well as in an *ex vivo* SVG model, shown by reduced plasma levels of monodialdehyde, a marker of oxidative stress, and inhibition of NOX activity respectively [99]. Antoniadou and colleagues further showed that Atorvastatin ameliorates vascular

redox state through eNOS coupling in the presence of its co-factor, BH₄, ultimately leading to reduced vascular superoxide production and improved vessel function ^[100]. Furthermore, it is well understood that Aspirin has a profound impact on reduction of early graft failure by prevention of thrombotic events due to its antiplatelet activity; however, Aspirin alone does not appear to have much impact on longer-term graft patency ^[72].

In addition to more well-established treatments, the potential for gene therapy in VGF offers an interesting therapeutic direction. Intraoperatively, the saphenous vein is easily accessible, allowing in situ delivery of gene therapies directly to the tissue ^[72,76]. However, to date, only one randomised controlled trial has assessed gene therapy; the PREVENT-IV study ^[27]. The trial involved the use of a decoy oligonucleotide, edifoligide, which binds to the transcription factor, E2F, known to have a role in the initiation of IH. Edifoligide appeared to have no benefit in preventing VGF at between 12 and 18 months post-surgery and the trial failed at stage IV ^[27]. Despite certain advances in the development of pharmacologic agents for the acute treatment of VGF, the longer-term benefits of many of these studies are yet to be demonstrated in their extension of vein graft viability.

6. Conclusions

The use of LSV as conduit in surgery is complicated by the development of vein graft disease leading to vein stenosis. The role of vECs in the development of IH and accelerated atherosclerosis is not yet fully elucidated. The implantation of LSV into arterial circulation can lead to the activation of vECs in response to acute exposure to high shear stress and the activation of different pro-inflammatory cascades. However, the limited amount of available data suggests that acute increases shear stress in the vein graft may be associated with the activation of MAPK and NF- κ B signalling pathways, which may result in vEC apoptosis and loss (Figure 4). Suppression of acute activation of the venous endothelium represents a largely unexplored possibility in the treatment of

vein graft disease and may provide the potential to improve graft patency both in the short and longer term.

Figure. 1. Early vs Late stage vein graft failure. Early graft failure primarily occurs due to technical problems during surgery causing significant damage to the endothelium which ultimately result in thrombosis. Late stage graft failure is a much slower process leading to development of superimposed atherosclerosis on the vein graft wall. EC: endothelial cell; ROS: reactive oxygen species; ECM: extracellular matrix; VSMC: vascular smooth muscle cell; SS: shear stress; 10m: 10 minutes; 2wks: 2 weeks; 1 yr: 1 year.

Figure 2. Mechanism of intimal hyperplasia. Temporal development of late stage vein graft failure beginning from surgical trauma and altered haemodynamics, ultimately leading to intimal thickening and vascular remodelling through the combined actions of multiple vascular cell types and extracellular matrix interactions. EC: endothelial cell; ECM: extracellular matrix; VSMC: vascular smooth muscle cell; IEL: internal elastic lamina; EEL: external elastic lamina.

Figure. 3. Endothelial cell inflammatory response. The endothelium is the first point of contact for systemic sources of inflammation; as such, it's response is hugely important in propagation of inflammatory stimuli through the vessel wall and beyond. (A) Within the quiescent venous endothelium (i.e. in veins with normal flow without venous reflux, venous insufficiency or stasis), ECs upregulate levels of KLF2 and Nrf2 dependent genes, including eNOS and HO-1, and secrete gasotransmitters NO and PGI₂. (B) Upon insult of acute high shear stress following immediate implantation into the arterial circulation, vECs activate the Rho-GTPase pathway which affects downstream effectors and the cytoskeleton. Within the first 10 minutes of acute high flow there is also an increase in intracellular calcium leading to movement of P-Selectin to the luminal surface. Together these actions constitute type I (acute) inflammation. (C) Following this, there is activation of a more prolonged (type II) inflammatory response, which results in the activation of MAPK mediated and NF- κ B pro-inflammatory pathways; resulting in expression of adhesion molecules, cytokines, chemokines and increases in intracellular ROS. The interplay between ECs and VSMCs acutely in the vein graft remains unclear with the extent of intercellular communication a relatively unexplored area. PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; Akt: Protein kinase B; PKA: Protein kinase A; AMPK: adenosine monophosphate-activated protein kinase; ERK5: Extracellular-signal-regulated kinase 5; HMOX-1: heme-oxygenase 1; NQO-1: NADPH-dehydrogenase 1; GCLM: Glutamate-cysteine ligase regulatory subunit; Nrf2: Nuclear factor erythroid 2-related factor 2; NOS3: endothelial nitric oxide synthase; LSS/PSS: Laminar or pulsatile shear stress; ASS1: Argininosuccinate synthetase; CNP: C-natriuretic peptide; KLF2: lung-Kruppel-like factor 2; NO: Nitric oxide; PGI₂: Prostaglandin; EC: endothelial cell; SREBP: Sterol regulatory element-binding protein; MAPK: Mitogen activated protein kinase; Ca²⁺: Calcium; VSMC: Vascular smooth muscle cell; JNK: c-Jun N-terminal kinases; p38: P38 mitogen-activated

protein kinases; PKC ζ : Protein kinase C, zeta; AHSS: Acute high shear stress; AP-1: activating protein 1; ROS: Reactive oxygen species; IKK: I κ B kinase complex; I κ B α : Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; p50/p65: Nuclear factor kappa-light-chain-enhancer of activated B cells (50 and 65kDa heterodimeric subunits); MCP-1: Monocyte chemotactic protein 1; IL-8: Interleukin 8; VCAM-1: Vascular cell adhesion protein 1; MMPs: Matrix metalloproteinases; PDGF-BB: Platelet-derived growth factor subunit B homodimer; TGF- β : Transforming growth factor beta; KLF4: Kruppel-like factor 4; COXII: Cyclooxygenase 2.

Quiescent/Native endothelium

EC activation (0-10 mins) Type I acute inflammation

EC activation (10 mins-24 hrs) Type II inflammation

Figure. 4. Proposed mechanism of the impact of acute high shear on vECs. Acute high shear leads to activation of signalling pathways such as NF- κ B and MAPK in vECs, leading to up-regulation of pro-inflammatory genes and resultant phenotype changes in VSMC which cause the development of IH. NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; p38: P38 mitogen-activated protein kinases; EC: Endothelial cell; SMC: Vascular smooth muscle cell.

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