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SPOTLIGHT REVIEW

Mechanotransduction, immunoregulation, and metabolic functions of CD31 in cardiovascular pathophysiology

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Abstract Biomechanical changes in the heart and vessels drive rapid and dynamic regulation of blood flow, a vital process for meeting the changing metabolic needs of the peripheral tissues at any given point in time. The fluid movement of the blood exerts haemodynamic stress upon the solid elements of the cardiovascular system: the heart, vessels, and cellular components of the blood. Cardiovascular diseases can lead to prolonged mechanical stress, such as cardiac remodelling during heart failure or vascular stiffening in atherosclerosis. This can lead to a significantly reduced or increasingly turbulent blood supply, inducing a shift in cellular metabolism that, amongst other effects, can trigger the release of reactive oxygen species and initiate a self-perpetuating cycle of inflammation and oxidative stress. CD31 is the most abundant constitutive co-signalling receptor glycoprotein on endothelial cells, which line the cardiovascular system and form the first-line of cellular contact with the blood. By associating with most endothelial receptors involved in mechanosensing, CD31 regulates the response to biomechanical stimuli. In addition, by relocating in the lipid rafts of endothelial cells as well as of cells stably interacting with the endothelium, including leucocytes and platelets, CD31–CD31 trans-homophilic engagement guides and restrains platelet and immune cell accumulation and activation and at sites of damage. In this way, CD31 is at the centre of mediating mechanical, metabolic, and immunological changes within the circulation and provides a single target that may have pleiotropic beneficial effects.

Keywords Haemodynamics • Metabolism • Inflammation • Endothelial • Mechanosensing • CD31 • Cross-regulation

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1. Introduction

Haemodynamic stress plays a key role in the determinism and issue of cardiovascular pathologies developing at sites of maximal flow perturbation and involving metabolic and inflammatory pathways, such as atherogenesis[1] or aortic valves calcification.[2] Indeed, the mammalian arterial tree has evolved to work at high pressure in an intricately branched system and haemodynamic stress is the most important denominator in cardiovascular physiology and pathology.[3]

The blood flow rate across the different vascular segments must be continuously regulated in order to match the metabolic need of the cells, which varies across the different peripheral tissues at any given point in time. To this end, the basal level of blood pressure (potential energy) in a given segment must be high enough to allow the modulation of the blood flow (kinetic energy) in selected downstream branches. Accordingly, the 'resistance' of the multiple terminal arteries must be dynamic by constricting to prevent the unnecessary use of oxygenated blood in resting tissues or dilating to allow the reception of large amounts of oxygenated blood in metabolically demanding tissues.

The basal metabolic demand of the cells that compose the cardiovascular system itself, most notably of the heart, is very high. Tissue-specific metabolic pathways concerned with the production of energy undergo significant shifts in pathological haemodynamic conditions. The cardiac muscle preferentially employs fatty acids (and ketones, in the case of prolonged fasting) as its major fuel. If the heart is subject to enhanced and prolonged mechanical stress causing pathologic shape changes, ranging from hypertrophic remodelling through to dilated cardiomyopathy, preceding the state of the failing heart, glucose oxidation increases, and β-oxidation falls.[4] In turn, this metabolic shift could contribute to the progression of heart failure.[5]

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Laminar shear stress regulates the expression of Krüppel-like factor 2 (KLF2) and phosphofructokinase-2/fructose-2,6-bisphosphatase-3 (PFKFB3), both are essential for reducing the rate of glycolysis and maintaining the quiescent metabolic state of endothelial cells.[6]

Dramatic local haemodynamic changes, including low shear stress due to the increased blood viscosity, and increased permeability due to the loss of the endothelial barrier integrity, occur in acute inflammatory conditions.

The metabolism of endothelial vascular cells is profoundly affected by local inflammation not only due to occurrence of energy-dependent cellular and molecular biological processes but also by impaired capillary perfusion that results from the microvascular plugs made of platelet-leucocytes cell aggregates sticking on the inflamed endothelium.[7]

Finally, pathological mechanical stress unbalances the delicate physiological endothelial cell metabolism by triggering the production of reactive oxygen species (ROS) that can trigger pleiotropic effects driven by the activation of transient potential channels[8] or by direct interaction with the membrane lipids of adjacent vascular and cardiac cells, globally impacting upon the cardiovascular system, including the induction of a pro-inflammatory state (recently reviewed in Ref.[9]).

Considering these findings, it is clear that homeostasis of cardiovascular mechanics is tightly coupled with the immunometabolic state of endothelial cells, both in physiology and pathology (*Figure 1*).

2. Importance of mechanotransduction for cardiovascular immunometabolism

The metabolic needs of peripheral organs vary according to their specific activity at any given time. Thus, the haemodynamics must rapidly adapt to increase the flow rate and improve the energy balance of tissues under pathological conditions. Typically, inflammatory processes unavoidably enhance the metabolic needs of the target tissues. The metabolic flux at the single cell level can be impaired by local (reduced supply of nutrients and toxic waste removal) or systemic (heart failure, abrupt blood volume loss) haemodynamic disturbances. This results in widespread cell death that potentiates a vicious circle, further impairing the altered respiratory processes due to redox imbalance, protein modification, and inflammation.[10] Of note, the local mechano–metabolic–inflammatory changes do not only impact on the biology of the endothelial cells but also of the nearby blood elements, such as leucocytes and platelets. The concomitant, unrestricted activation of all these elements in the microcirculation of organs that are subject to ischaemia and reperfusion sequences can trigger dramatic endovascular inflammatory reactions. This contributes to the organ damage associated with the 'no-reflow' phenomenon,[11] including after organ transplantation.[12]

Pathological cardiovascular conditions leading to abrupt variations of the vessel through which the blood flows, such as atherosclerosis and heart valve stenosis, impact on the metabolic needs of the downstream segment because the associated dominant turbulences and vortices require increased kinetic energy to maintain themselves.[13] Myocardial ischaemia can also occur in the absence of significant epicardial coronary atherosclerosis ('microvascular angina'). This condition is linked to a combination of an endothelial abnormal metabolism, pro-inflammatory phenotype, and perturbed flow-dependent regulation of vascular resistance.[14]

Definitely, the cells interacting at the blood/vascular interface must be able to permanently cross-regulate all three types of stimuli: mechanical, immune, and metabolic.

In this setting, CD31 (also known as PECAM-1) may play a crucial and, as yet, neglected role. This transmembrane, highly glycosylated, and trans-homophilic Ig-like immunoreceptor tyrosine-based inhibition motif (ITIM) protein is the most abundant cell surface molecule on endothelial cells.[15] It is also expressed by leucocytes and platelets and is known to function as a co-receptor with a variety of specific mechanical, metabolic, and immune intracellular signalling pathways. The specific regulatory functions of CD31 in platelet or leucocyte signal transduction are reviewed in detail elsewhere.[16,17] The purpose of the present review is to delineate the holistic potential of CD31 in cardiovascular pathology through its cell–cell and multiple pathway cross-regulatory functions.

3. Mechanical and immunometabolic cross-signalling pathways in endothelial cells

Endothelial cells can orchestrate complex mechanical, metabolic, and inflammatory crosstalk in response to each of these stressors, acting either concomitantly or individually. An isolated mechanical stress (e.g. abrupt reduction in hydrostatic pressure due to hypovolaemia in the capillary bed or sudden increase in circumferential tensile stretch of large arteries due to a hypertensive peak) straightforwardly impacts on the metabolic activity of the local endothelial cells as the signalling pathways involved in the adaptive cell phenotype modifications are energy dependent. The outcome of the endothelial response eventually depends on the extent and chronicity of the stimulus and can vary from pro-survival/reparative to pro-inflammatory and even lethal, following the rules of stress-induced hormesis.[18] In turn, an isolated inflammatory trigger (e.g. in the case of infection or an ongoing autoimmune process) inevitably increases the cellular metabolic need, and by driving the release of multiple vasoactive molecules, can also affect the local haemodynamics.

Such extraordinary biological plasticity requires a constant and coordinated regulation of metabolic and biophysical signalling pathways. Indeed, the endothelial cell receptors and co-signalling molecules linking mechanosensing with metabolic and inflammatory pathways must be able to dynamically assemble in supramolecular complexes, at different sites of the polarized endothelial cells, according to the changing nature of the prevalent haemodynamic force. The rapid mobilization and redistribution of signalling proteins all over the endothelial cell surface is orchestrated by flask-shaped structures formed by the plasma membrane lipids enriched with signalling molecules that are sensitive to both biomechanical and metabolic stress, the so-called 'caveolae'.[19] Furthermore, the signalling supramolecular signalling platforms can be effective only within specific plasma membrane microdomains, called 'lipid rafts'.[20] Of note, metabolic disorder can dramatically affect the lipid raft composition and function[21] and can therefore directly impact on the regulation of endothelial cellular biological responses to the dynamic stressors. On the other hand, the use of statins, intended to correct metabolic disorders, exert wider effects in cardiovascular pathophysiology due to their 'pleiotropic' action, including specific anti-inflammatory and even anti-arrhythmic effects.[22] Indeed, by inhibiting the production of isoprenoid cholesterol intermediates, statins prevent the post-translational prenylation of small GTP-binding proteins such as Rho and Rac, and the activity of their downstream effectors such as Rho kinase and nicotinamide

adenine dinucleotide phosphate oxidases.[23] Such indirect effect of statins on mechano- and immune-signalling could also explain their effect on atrial arrhythmias, the occurrence of which has recently been linked to sustained mechanical stress (stretching) causing inflammation around the pulmonary veins in athletes.[24] The specific effects of statins on CD31 expression and function remain however unknown.

The localization of different mechanosensing receptor complexes at the apical or basal pole or at the lateral border of vascular endothelial cells varies according to the specific mechanic force to which they respond.[25,26] Interestingly, the prevalent localization of endothelial CD31 varies with the type of the vessel: in large vessels, where the rapid flow is parallel to the wall, the high shear stress maximally twists the endothelial cell–cell junctions and CD31 molecules are concentrated at the lateral cell borders. Instead, on microvascular endothelial cells, where the speed of the flow is minimal, the prevalent haemodynamic force is normal (perpendicular) to the wall and CD31 is maximally concentrated on the luminal and abluminal endothelial plasma membrane and in submembrane intracellular vacuoles, whereas it is present at very low density at the lateral plasma membrane.[27] These findings suggest that CD31, the most abundant constitutive receptor glycoprotein of the endothelium, can co-clusters with the solicited mechanoreceptors and exert a global cross-regulation of endothelial immunometabolic responses in different haemodynamic conditions (*Figure 2*). Importantly, studies in diabetic patients have highlighted the importance of the cell metabolic homeostasis for the appropriate post-translational modifications of the CD31 molecule, which are mandatory for its physiological expression and signalling properties.[28,29]

4. CD31: a trans-homophilic co-signalling protein at the cross-road between mechanical stress, metabolism and inflammation

CD31 is a highly glycosylated single-pass type I membrane Ig-like signalling protein constitutively and exclusively expressed by cells interacting at the blood-vessel interface. Its highest expression density is found on endothelial cells ($1*10^6$ copies/cell), followed by myelo-monocytes ($1*10^5$ copies/cell), lymphocytes ($1*10^4$ copies/cell), and platelets ($5*10^3$ copies/cell).[30] The interest for CD31 as a signalling molecule has been growing ever since the discovery of the two specific phosphorylatable tyrosine motifs comprised in its cytoplasmic tail.[31,32]

Of note, the physical interaction of CD31 molecules is not enough *per se* to drive CD31 signalling because its intracellular ITIMs are not able to auto-phosphorylate and endothelial CD31 are not phosphorylated in normal flow conditions. Upon active cell stimulation, however, the trans-homophilic engagement of CD31 on interacting cells triggers the translocation of the CD31 molecules within lipid rafts.[29] Here, the intracellular CD31 ITIMs can be phosphorylated by a variety of Src tyrosine kinases engaged by the co-clustered and stimulated signalling receptors (reviewed in Ref.33). Hence, CD31 signalling is not restricted to a particular cell type nor a particular signalling pathway as it can be engaged by different stimuli on different $CD31^+$ positive cells.

Once phosphorylated, the CD31 ITIMs can recruit a variety of protein and lipid Src Homology 2 (SH2) phosphatases[34,35] and can act on both protein and lipid tyrosine kinase pathways. For example, the tyrosine phosphatase SHP-1 and the inositol phosphatase SHIP-2 can both be recruited by endothelial CD31 to counteract phosphoinositide 3-kinase (PI3K)-dependent endothelial superoxide formation[36] and glucose metabolism-dependent oxidative stress.[37] Recently, it has been shown that CD31 signalling function can underlie unsuspected cell biological processes such as statin-driven regulation of platelet activity[38] or the protection of vascular endothelial cells against alloimmune responses.[39] Due to this peculiar mode of action and its trans-homophilic nature, CD31 acts as an '*esperanto*' language at the surface of the $CD31^+$ cells permanently interacting in the circulation, allowing for a coordinated cell–cell crosstalk at the blood/vessel interface.

The cell signalling modulatory functions of CD31 have mostly been explored in the field of platelet biology (reviewed in Ref.16) and cellular immunology (reviewed in Ref.17).

In physiologic conditions, CD31 engagement drives mutual cell–cell detachment signals and raises the activation threshold of $CD31^+$ cells, globally acting as a negative co-signalling receptor on platelet and leucocytes.[33] In the absence of concomitant stimulation, the CD31–CD31 cell–cell interaction in between resting leucocytes and/or platelets is only transient and does not engage the receptor.

The case is different for the endothelial cells, which adhere on their basal membrane and establish permanent CD31–CD31 interactions at their lateral borders. The permanent trans-homophilic interaction of CD31 molecules engaged at the cell–cell junctions of the adherent endothelial cells[40] and their basal serine phosphorylation may account for the prevalent positive effect on endothelial cell survival and physiologic functions.[41] The combination with the tyrosine kinase-dependent ITIM phosphorylation at cardiovascular sites, mainly triggered by the stimulation of mechanosensing receptors and hence depending upon specific haemodynamic conditions, may explain why multiple, and occasionally conflicting, functions have been attributed to endothelial CD31.[42]

The fact that CD31 trans-homophilic engagement drives a mutually active detaching signal[43] together with the notion that endothelial CD31 ITIMs are not phosphorylated in resting conditions[31] argues against a role of CD31 in the structure of the endothelial junctions. Instead, since the endothelial cells are 'enchained' to each other to pave the luminal face of blood vessels, CD31–CD31 regulatory signalling at the endothelial lateral border might be crucial for ensuring endothelial homeostatic adaptive responses against endoluminal mechanical, metabolic, and immune stressors.[15]

In all cases, the specific co-inhibitory or co-stimulatory effect of CD31 signalling in the cross-regulation of endothelial biological processes depends upon the concomitant signalling that has allowed CD31 phosphorylation and the function of the recruited SH2 phosphatase in the different endothelial signalling pathways (*Figure 3*). Importantly, not only protein tyrosine phosphatases but also lipid phosphatases (SHIP-1/2) can be recruited by CD31. Similar to the described action of phosphatase and tensin homolog (PTEN) (reviewed in Ref.44) the lipid phosphatases recruited by CD31 can counter-regulate endothelial PI3K-mediated signalling pathways, commonly engaged by a number of inflammatory, metabolic, and mechanical stressors.

The association of CD31 molecules with the endothelial caveolae, focal adhesion kinases, and junctional endothelial signalling proteins allows it to regulate the global signalling response of the endothelium, including its specific immunometabolism, to all types of mechanical stress to which the solid cardiovascular structures (vessel wall, valves, and heart muscle),

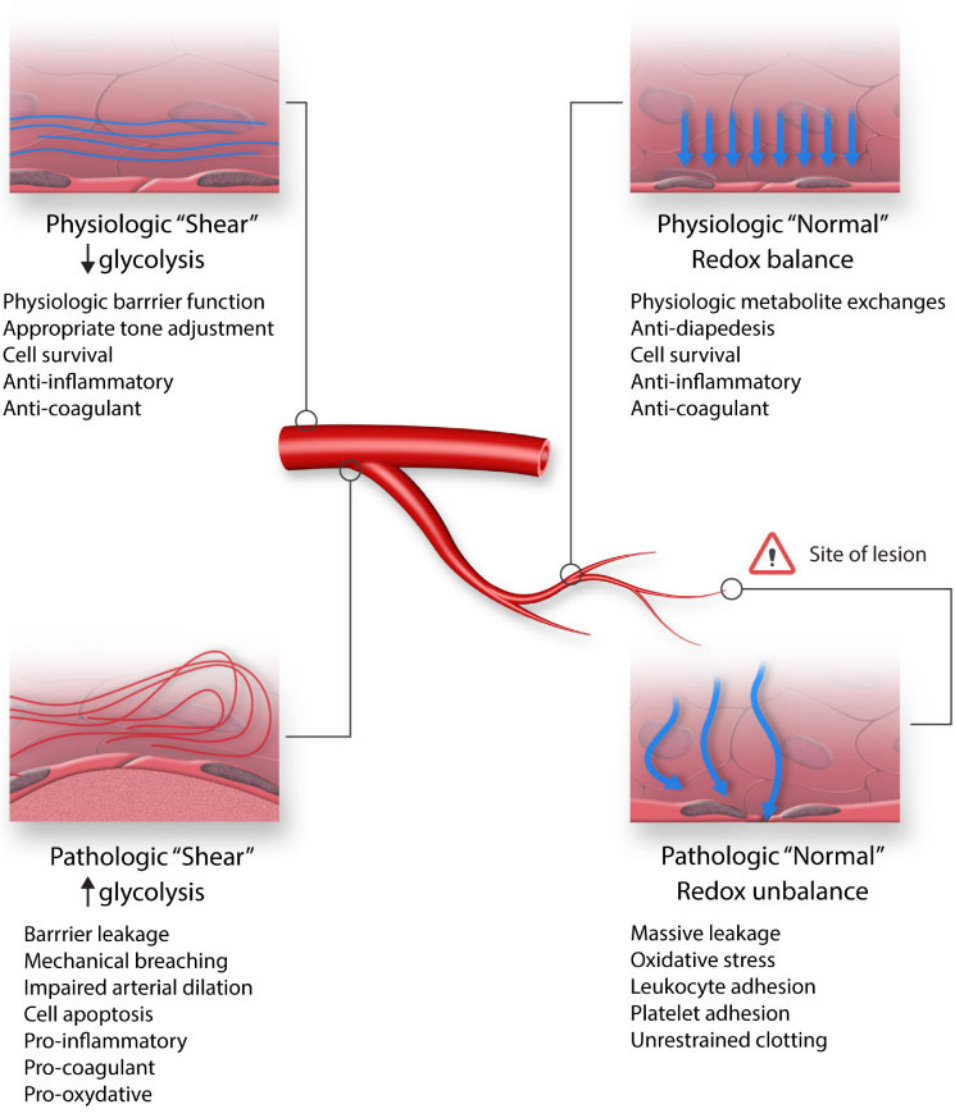

Figure 1 Impact of physiologic or pathologic 'Shear' and 'Normal' stresses on endothelial metabolism and phenotype at different vascular sites. Endothelial cell metabolism is highly sophisticated[97] and can vary considerably from a site to another and as a function of the local mechanical stress, already in physiologic conditions. The occurrence of pathologic Shear or Normal stress considerably warps the endothelial metabolic pathways and contributes to the eventual local damage.[6]

as well as the solid circulating blood elements, are subject in the circulation.

5. CD31-mediated regulation of endothelial cell immunometabolic responses at sites of 'normal' stress

Normal and *shear* stress are the two main forces exerted by the flowing blood onto the cardiovascular structure (*Figure 1*).[3] The 'normal' stress is perpendicular to the surface and corresponds to the compression exerted by the hydrostatic pressure onto it. The importance of normal stress in cardiovascular pathophysiology has received very little attention as compared to the 'shear' stress counterpart. In healthy hearts and elastic arteries, the *normal* stress is minimal, and its impact is limited by the normal 'strain'. The normal strain is the ability to extend (elasticity) of the wall which increases the surface onto which the stress is exerted. This resulting surface is in the denominator of the force unit (Dynes/cm^2). Remarkably, in the case of pathological remodelling (thickness, fibrosis, reduced elastic capital) that reduces the deformability of the cardiovascular surfaces, the normal stress exceeds the adaptive function of the wall strain, exaggerating the mechanic stimulation of the biological surface.

The degree of normal stress provided by the hydrostatic pressure is particularly important when the blood flow is close to zero. This situation is apparent in the heart cavities when the four valves are closed or in the capillary bed where the blood flow velocity drops abruptly at the arteriolar capillary end. The increased hydrostatic pressure at the

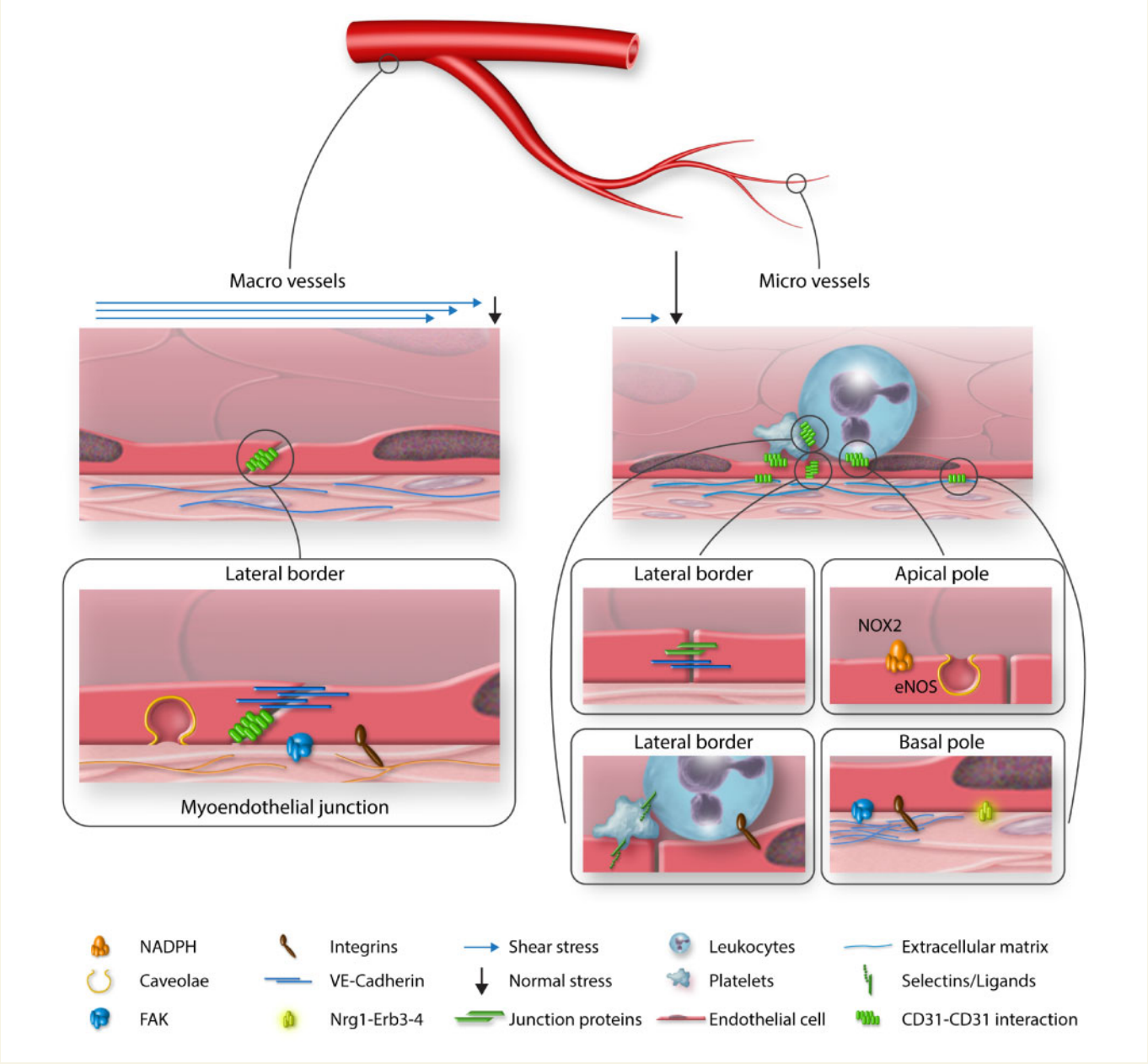

Figure 2 CD31 can modulate several receptors, at different sites, on endothelial cells. CD31–CD31 interaction follows that of the solicited membrane receptors at the surface of the polarized endothelial cells, mainly depending upon the prevalent type of mechanical force. The shear stress (horizontal grey arrows), prevalent mechanical force in resistive and macro vessels, maximally solicits the twisted the cell–cell junctions at the lateral border and the myo-endothelial junction at the basal pole. There, CD31 can modulate the phosphorylation of the tyrosine/inositol-dependent mechanoreceptors involved in endothelial cell–cell and cell–matrix physiologic cross-signalling.[98,99] The prevalent normal mechanic stress (vertical black arrow) exerted on microvascular endothelial, and the ensuing endothelial stretching, cells solicits several different mechanoreceptors, distributed all over the cell surface. The venular end of capillary vessels, the co-signalling functions of CD31 can impact on the biology of the endothelium also indirectly, through its action on the blood elements. Indeed, the cell–cell interaction between endothelial cells, platelets, and leucocytes is maximal at these sites of the circulation, where the speed of the flow (and the shear) is close to zero.

capillary terminus allows the transport of oxygen and nutrients from the vessel lumen to the extravascular space in the peripheral organs. In the absence of immune and/or metabolic pathological conditions, the hydrostatic stress is buffered by the endothelium which exerts a physiologic barrier function. Only small molecules in solution can be exchanged with the extravascular tissue, whereas large molecules and cells are retained in the lumen. To this end, adequate sensing and response to the hydrostatic pressure is required both at the luminal (apical pole) and at the abluminal (basement) of the endothelial epithelium. The centripetal compression exerted on the endothelial cell membrane by the hydrostatic pressure generates a tensile stretch which can be buffered by a rapid elongation of the cell plasma membrane due to an actin- and adenosine tri-phosphate (ATP)-independent disassembly of the caveolae.[45] However, in blood stasis and vascular congestion, either due to the

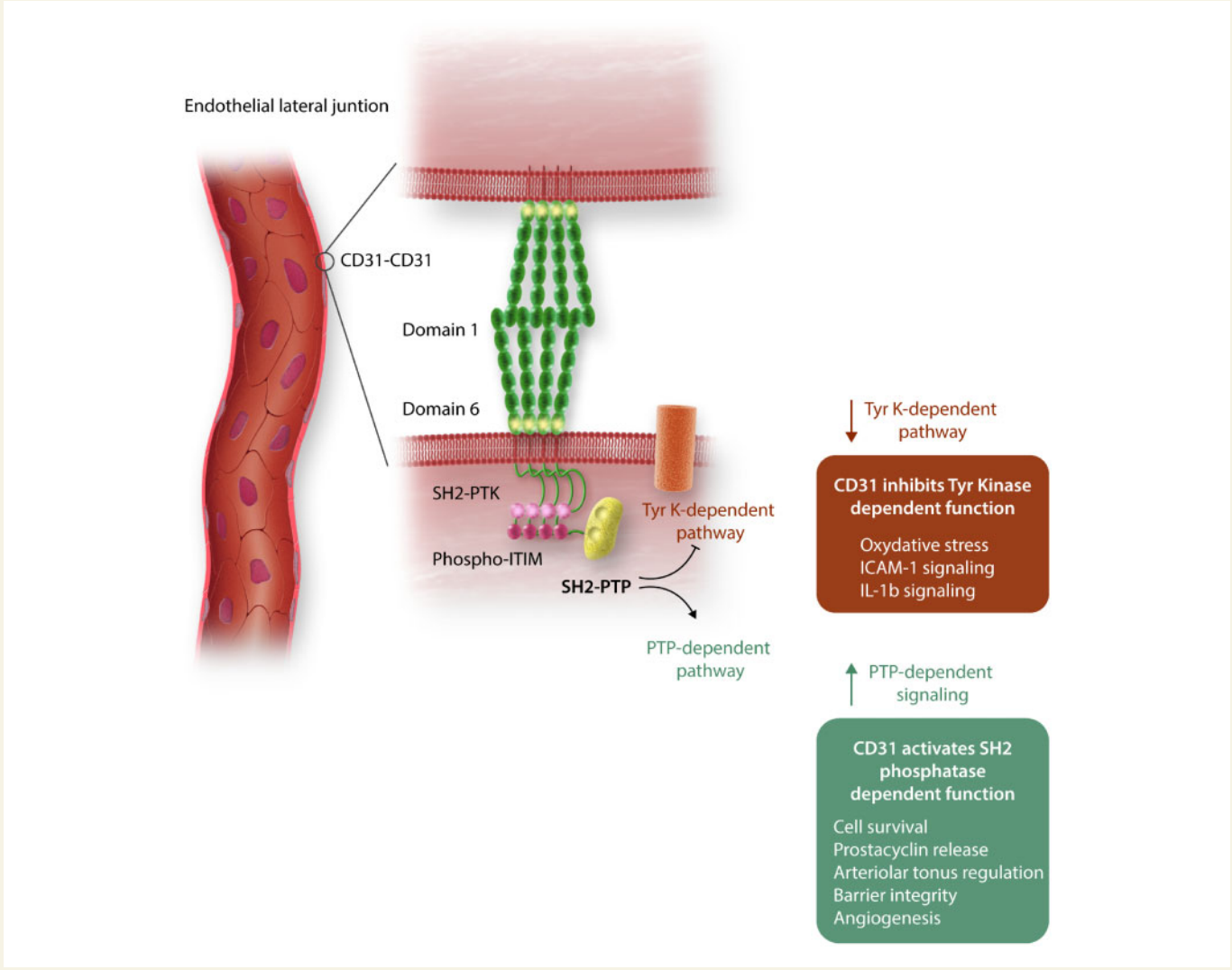

Figure 3 CD31 exerts different cardiovascular protective functions in endothelial cells. At the endothelial lateral cell–cell border, CD31 is constantly engaged in trans-homophilic interaction, ready to be ITIM-phosphorylated by co-clustered and concomitantly acting activating receptors able to drive tyrosine-kinase (Tyr K)-dependent pathways. The Phospho-ITIM can then recruit and activate SH2 domain-containing phosphatases which eventually mediate CD31 signalling functions. The net effect of the latter is inhibitory for Tyr Kinase dependent cell functions and activatory for SH2 phosphatase dependent functions. Thus endothelial CD31 can inhibit the formation of reactive oxygen species,[100,101] ICAM-1,[102] and IL-1β[93] inflammatory signalling whereas, at opposite, it is required for cell survival,[41] prostacyclin release,[72] arteriolar tonus regulation,[76,79] barrier integrity,[103] and angiogenesis.[104]

failure of the heart pump or to a physical obstruction to the recirculation of the blood (vascular or valve thrombosis), the hydrostatic pressure increases beyond the limits of these basic physiologic regulatory mechanisms.

The association of CD31 with the endothelial caveolae appears to be important for 'sensing' the pathological increase in *hydrostatic pressure* due to a vascular occlusion and guiding NADPH oxidase 2 (NOX2)-mediated angiogenesis to restore blood supply.[46] By modulating the phosphorylation of the associated vascular epithelium (VE)-cadherin, such stretch-induced engagement of CD31 at the endothelial cell–cell border could explain how the maintenance of physiologic hydrostatic pressures can exert a beneficial effect on the endothelial barrier function, in the presence of pro-thrombotic or pro-inflammatory stimuli.[47]

Vice versa, when the barrier function of the endothelium becomes leaky at sites of thrombosis and/or inflammation, the intravascular hydrostatic pressure drops. In these conditions, CD31 co-signalling, via the associated SHP2 phosphatase, plays an important role in regulating the prothrombotic phenotype of endothelial cells by modulating tissue factor expression[48] and in the recovery of disrupted endothelial cell–cell junctions by dephosphorylating VE-cadherin-associated β-catenin and promoting the mobility of VE-cadherin at the plasma membrane.[49] This will eventually contribute toward restoring the physiologic intravascular hydrostatic pressure.

6. CD31 regulation of 'stretch'-driven endothelial cell responses

During systole, the endothelial layer of the elastic arteries is maximally stretched. The pulsatility of the heart pumping circulation adds a temporal dimension to the mechanical stretching endured by the arterial systems, referred to as cyclic stretching. Cyclic stretching induces several

signalling pathways that could be sensitive to the activity of the SH2-phosphatases recruited by CD31, including phosphorylation of focal adhesion kinases (FAK) and activation of mitogen-activated protein kinases (MAPK).[50] The signals generated by such cyclic deformations are critically involved in the biological responses and metabolism of the endothelial cells, particularly at sites of disturbed flow[51] and inflammation.[52] This is evident for understanding the determinism of atherogenesis at the site of arterial branching.[53]

The endothelium of the endocardium is also stretched in response to the normal stress represented by the intraventricular pressure. This differs from the mere hydrostatic pressure and is a sort of *active normal* stress because, on top of the volume load, it depends on the contracting and relaxing properties of the cardiac muscle. When all four valves are closed, the endothelium of the heart ventricles and the endothelium of the subendocardial microvasculature are iteratively compressed by the blood pressurized in the heart ventricles.

An aberrant cyclic mechanical stress exerted on the microvascular endothelium, within the heart muscle, triggers a prompt paracrine release of neuregulin-1β which plays a crucial role in driving appropriate cardiac remodelling in response to increased mechanical overload.[54] This stimulus can be physiological such as during pregnancy[55] or pathological such as following myocardial infarction.[56]

In conditions linked to a dilation of the left ventricle (myocardial infarction, cardiomyopathy, valve disease), enhanced cyclic stretching is a main trigger for the expression/production of neuregulin by the heart microvascular endothelium.[57] The latter drives the hypertrophic, hyperkinetic response of the live myocardium, which is mandatory for preventing further dilation and heart failure.[58] Baseline cardiac evaluation in CD31 deficient mice suggests that CD31 could play a critical role in this setting.[59] Further work is needed to assess the dynamic behaviour of endothelial CD31 and neuregulin signalling in the absence of genetic modification and in conditions of enhanced myocardial stretching.

In conditions where the cardiovascular system is over-stretched (volume overload, vessel/cardiac chamber dilation), CD31 co-signalling with the integrin-RhoA pathway at the basal pole of the endothelial cells that line the endocardium may be important for avoiding their detachment from the basement membrane, by driving focal adhesion growth and adaptive cellular stiffening.[60] However, the strength of the pathological stretch stimuli can overcome the regulatory function of CD31 eventually leading to cell activation-driven juxta-membrane cleavage and shedding of several membrane receptors, including CD31,[61] the loss of which may favour the development of cardiac dilation and heart failure.[59] Interestingly, single nucleotide polymorphisms of the CD31 gene, and in particular the Asn563Ser (targeting the juxta-membrane extracellular portion), appears to be associated with higher plasma levels of soluble CD31, likely due to an abnormal cleavage of the membrane receptor, and higher risk of myocardial and cerebral infarction.[62,63]

7. CD31 endothelial function at sites of high 'shear' stress

In flow conditions, the main type of stress experienced by all cardiovascular structures is 'shear' stress. Shear stress is generated by the friction of the blood flowing over the surface; the vector of this force is parallel to the surface. The endothelial cell response to dynamic shear stress has been shown to depend upon the interaction with platelets, through CD31[64] and experimental work in hypercholesterolaemic mice suggests that endothelial CD31 rather plays an atheroprotective role at sites of in high shear stress.[65]

Indeed, endothelial CD31 molecules can be directly solicited by flow[66] as they are linked to endothelial catenins.[67] The Src kinases activated by mechanical stretch can also phosphorylate CD31 which in turn drives activation of the extracellular signal-related kinase 1/2 (ERK1/2) signalling cascade via P21ras and Raf-1.[68]

At variance with static conditions, the tension across CD31 molecules increases through the action of vimentin and actomyosin, whereas the force applied onto junctional VE-cadherin molecules rapidly decreases in flow conditions.[66] The engagement of CD31 in shear stress can impact on integrins (PI3K) and small GTPase RhoA endothelial cell signalling. This eventually drives adaptive cytoskeletal architecture, including growth of focal adhesions and adaptive cytoskeletal stiffening.[60] Furthermore, in the presence of high shear stress CD31 co-signalling regulates the activation of RhoA, actin polymerization, and the formation of the stress fibres.[69] This underlies the polarization of endothelial cells in the direction of the flow.[70]

A high shear stress implies a high metabolic demand and elicits a specific arginine substrate/nitric oxide metabolic pathway in endothelial cells.[71] The production of nitric oxide (NO) by the endothelial cells subject to high velocity flow drives the relaxation of the adjacent vascular smooth muscle cells by the production of cyclic guanosine monophosphate (cGMP). The consequent vasodilatation directly leads to a reduction of the blood flow velocity and hence of the initial high shear trigger.

CD31 has been reported to colocalize with endothelial nitri oxide synthase (eNOS)[73] where it may play a critical role in regulating the shear stress-induced Gab1 tyrosine phosphorylation[74] and Akt and eNOS dependent vasodilatory response.[75] In normal flow conditions, the intensity of the stimulus (high shear) overcomes the regulatory threshold of CD31. The mechanosensory co-signalling function of CD31 however becomes important in the case of substantial and/or sudden flow changes.[76] Low or reversed shear stress (as in case of arterial occlusion) drive cytoskeletal remodelling and NOX-mediated oxidation,[77] a process potentially controlled by CD31 via the recruitment of SHIP-2.[37] In addition to enhancing microvascular thrombotic occlusion,[78] the absence of CD31 results in vasoconstriction due to the hyperphosphorylation of endothelial eNOS and local release of multiple ROS (ONOO-, O2-°, and H2O2).[79] Similar vaso-occlusive pathological processes may explain the 'redness' of microvessels at inflammatory sites in CD31 knockout mice.[80] Another key CD31-dependent regulatory mechanism, by which blood flow sustains the homeostasis in the circulation underlies the anti-thrombotic/anti-inflammatory phenotype of the endothelium through a activation of cyclooxygenase-2 (COX-2) and prostaglandin I2 (PGI2) release in endothelial cells.[72] Altogether, these findings indicate that CD31 plays a critical role for the maintenance of the arterial vessel caliper and the regulation of the downstream blood flow.

At sites of perturbed shear stress, where the NFkB pathway is activated and atherosclerotic lesions develop, endothelial CD31 clusters are particularly evident[81] and its genetic deletion results in reduced RhoA-dependent fibronectin assembly[82] and NFkB/AkT-dependent vascular remodelling.[83] Although these finding may suggest that CD31 and inflammation trigger a pathologic response in endothelial cells submitted to perturbed shear stress, it is important to note that the activation of the NFkB pathway at these site is indeed vital for driving cytoprotective and anti-inflammatory effects,[84] eventually protecting against atherosclerosis.[65]

8. Role of CD31-mediated regulation of endothelial biology face to 'osmotic' stress

Mammalian cells can regulate and preserve different osmolarity across their plasma membrane and the ability to maintain distinct intracellular and extracellular solute microenvironments is essential for maintaining fluid homeostasis. Variations within the range of the human serum (285–295 mOsm/Kg) are considered as 'isotonic' and do not cause any harm; whereas, an osmotic stress is exerted by extracellular solutions with osmolarities above (hypertonic) or below (hypotonic) this range.

Endothelial CD31 is ITIM phosphorylated upon hyperosmotic stress.[85] Importantly, the hyperosmotic stress caused by high glucose concentration reduces the expression of CD31[86] and plays a role in pathological diabetic vascular remodelling.[87] The underlying mechanism could be related to the deformation of the cell shape (swelling or shrinking in case of hypotonic or hypertonic pressure, respectively), which increases the energy expenditure and drives pro-inflammatory endothelial processes.[87] The iterative switch between hypoosmotic and hyperosmotic stress, such as during glycaemia fluctuations in diabetic patients, adds to the stress by driving oxidative processes, endothelial dysfunction and even chromatin remodelling.[88]

9. CD31 regulation of endothelial-leucocyte cross-talk during inflammation

In inflammatory conditions, endothelial CD31 functions are important for guiding and restraining leucocyte accumulation in the target tissue (reviewed in Ref.17).

CD31 was initially thought to play the role of adhesion molecule but experimental studies have consistently shown that adhesion is not an issue in the absence of CD31. Instead, the leucocytes remain trapped at the level of the endothelial basal membrane and fail to reach the inflammatory site in CD31 KO mice.[89] Indeed, the leucocyte extravasation is coordinated by sequential activation of selectins, adhesion molecules and integrin-driven ITAM signals, all of which are potentially sensitive to ITIM receptor regulation[90] and the observed trapping of leucocyte at the basal pole of endothelial cells may possibly due to a defective closure of the integrins at the leucocyte uropod, a process that requires the action of phosphatases.[91]

Being a co-signalling molecule, the behaviour of CD31 on the interactive leucocytes, platelets, and endothelial cells of the microvessels allowing leucocyte transmigration at inflammatory site depends on its relocation and phosphorylation state at the time of cell stimulation and the use of genetically deficient mice of receptor blockade might not be the best suited to assess the multiple signalling functions of CD31.

Indeed, the engagement of intercellular adhesion molecule 1 (ICAM-1) on endothelial cell by the β2 integrins of transmigrating leucocyte triggers the dephosphorylation of the CD31 ITIMs,[92] allowing the stable contact between the squeezed leucocytes with the endothelial cells to occur. However, the trans-homophilic engagement of endothelial CD31 by emigrating leucocytes drives a negative feedback on NFκB activity of the interacting endothelial cell.[93] This blunts the pro-inflammatory endothelial cell phenotype and limits successive leucocyte extravasation.

Following leucocyte diapedesis, CD31 can rapidly reappear at the surface of endothelial cells because it is stored within their lateral recycling compartment.[42] Complete restoration of surface CD31 molecules is completed within 3 h because the synthesis of CD31 is constitutively maintained at a very high rate of production within these cells.[94] Thus, CD31 can rapidly go back to exert its gate-keeper role on the endothelial barrier to prevent excessive extravasation of blood leucocytes.[49]

In addition to its role in limiting leucocyte extravasation space, CD31 is also important for driving the cell phenotype switches required for promoting wound healing upon completion of the inflammatory phase.[95] Furthermore, endothelial CD31 is required for guiding the direction of cell migration that is necessary in effective vascular healing. In the absence of CD31, endothelial cells display increased cell motility but lack coordination and fail to close the wound. Experimental work suggest that the trans-homophilic engagement, allowing endothelial CD31 ITIM phosphorylation,[96] favours the activation of the Gα/Sphingosine-1-Phosphotase/RhoGTP signalling required for the spatially oriented migration of endothelial cells.

10. Conclusions

Biomechanical forces in the heart and vessels profoundly influence cellular metabolism that, amongst other effects, can trigger the release of ROS and initiate a self-perpetuating cycle of inflammation and oxidative stress. Due to its unique trans-homophilic and receptor co-signalling properties, CD31 can orchestrate cross-responses to biomechanical, metabolic and inflammatory stimuli with cell specific effects. In this way, CD31 is at the centre of mediating mechanical, metabolic and immunological changes and provides a single target that may have pleiotropic beneficial effects in the cardiovascular system.

Conflict of interest: none declared.

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