Circulation Research

ATHEROSCLEROSIS COMPENDIUM

CD31 as a Therapeutic Target in Atherosclerosis

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ABSTRACT: The potential of CD31 as a therapeutic target in atherosclerosis has been considered ever since its cloning in the 1990s, but the exact role played by this molecule in the biologic events underlying atherosclerosis has remained controversial, resulting in the stalling of any therapeutic perspective. Due to the supposed cell adhesive properties of CD31, specific monoclonal antibodies and recombinant proteins were regarded as blocking agents because their use prevented the arrival of leukocytes at sites of acute inflammation. However, the observed effect of those compounds likely resulted from the engagement of the immunomodulatory function of CD31 signaling. This was acknowledged only later though, upon the discovery of CD31's 2 intracytoplasmic tyrosine residues called immunoreceptor tyrosine inhibitory motifs. A growing body of evidence currently points at a therapeutic potential for CD31 *agonists* in atherothrombosis. Clinical observations show that CD31 expression is altered at the surface of leukocytes infiltrating unhealed atherothrombotic lesions and that the physiological immunomodulatory functions of CD31 are lost at the surface of blood leukocytes in patients with acute coronary syndromes. On the contrary, translational studies using candidate therapeutic molecules in laboratory animals have provided encouraging results: synthetic peptides administered to atherosclerotic mice as systemic drugs in the acute phases of atherosclerotic complications favor the healing of wounded arteries, whereas the immobilization of CD31 agonist peptides onto coronary stents implanted in farm pigs favors their peaceful integration within the coronary arterial wall.

Key Words: acute coronary syndrome ■ atherosclerosis ■ inflammation ■ leukocytes ■ peptides

CD31 provides a compelling example of the translational undertaking of a molecule first discovered in the fundamental research field. However, CD31's translational route has been complicated since the role attributed to CD31 has been tremendously reconsidered since its discovery 30 years ago. Fundamental research has brought essential insight into CD31's biological and physiological functions making medical progress possible. This review retraces CD31's winding route from pure knowledge to clinical interventions and application for human health.

Importance of CD31 in the Blood-Vessel Interface Homeostasis

CD31, a highly glycosylated Ig-like membrane receptor expressed by leukocytes, platelets, and endothelial cells, is the most abundant membrane glycoprotein constitutively expressed on the vascular endothelium.[1] The full-length isoform of human CD31 is a 130 kDa type I transmembrane glycoprotein comprising 6 Ig-like extracellular domains, a short transmembrane segment, and a cytoplasmic tail of varying length due to alternative splicing.[2] Due to the specificities of its extracellular and intracellular residues, CD31 is regarded as both an adhesive and a signaling protein. Putative adhesion functions were attributed to specific residues distributed on both sides of the β-sheet of the first Ig-like domain. These are able to engage in homophilic interactions as shown by site directed mutagenesis[3,4] and crystal structure models.[5] In arterial segments, CD31 is concentrated at the intercellular endothelial borders where it coclusters with junction molecules. However, CD31 is not directly involved in the formation of either adherens or tight cell-cell endothelial junctions.[6,7] Indeed, the association constant of CD31 protein-protein interactions is in the order of the micromolar,[3,4] which is too weak for stable protein-protein "adhesion."[8] Furthermore, the localization of CD31 molecules over the cell plasma membrane is not fixed but varies dynamically, linked to the rearrangements of the actin

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Nonstandard Abbreviations and Acronyms

ICAM-1	intercellular adhesion molecule 1
ITIM	immunoreceptor tyrosine inhibitory motif
MMP9	matrix metalloproteinase 9
NFκB	nuclear factor κB
PECAM-1	platelet endothelial adhesion molecule 1
SH2	Src homology 2
TNF-α	tumor necrosis factor α
VCAM-1	vascular cell adhesion protein 1

cytoskeleton,[6] and driven by the formation of membrane microdomains upon cell stimulation.[9,10] Evenly distributed in resting cells, the localization of CD31 molecules varies with the type of prevalent mechanic stress exerted on the endothelial cells paving the different vascular segments.[11] The engagement of CD31 at sites of active vascular endothelial cell stimulation is particularly important for maintaining homeostasis in the circulation.[12–14] Thus, the transhomophilic engagement of endothelial CD31 at the intercellular borders in arteries, where the endothelial cells are maximally twisted and stressed, is essential for the maintenance of flow-driven physiological adjustments.[15,16]

At lower density, CD31 is also constitutively expressed by all hematopoietic cells and remains expressed on blood flowing elements,[1] apart from erythrocytes. Indeed, CD31 is expressed also by erythroid progenitors, but the expression cannot be sustained and stops after erythroblasts enucleation.[17]

Due to the transhomophilic nature of its receptor functions, CD31 signaling is not restricted to a particular cell type nor a particular signaling pathway as it can be engaged by different stimuli on different CD31$^+$ positive cells. On platelets and leukocytes, the function of CD31 is important for preventing inappropriate reactions within the circulation, by driving a leave me alone signal, necessary to prevent cell-cell aggregation,[18,19] and by raising the activation threshold of tyrosine/inositol kinase-dependent pathways.[20–22] Interestingly, recent studies have demonstrated that CD31 signaling is involved in the privilege of vascular endothelial cells of being protected against immune attacks.[23]

Thus, in physiological conditions, CD31 is expressed constitutively and exclusively on cells interacting at the blood-vessel interface and its cross-cell transhomophilic function is required for maintaining homeostasis in the circulation (Figure 1).

CD31 Gene Expression and Regulation

The human gene encoding CD31 is located in the long arm of chromosome 17 (17q23.3).[24] Similar to other constitutive proteins, the rapid rate of biosynthesis[25] and steady expression of CD31 in basal conditions is allowed by the presence of a promoter that appears as disperse, meaning that transcription can be initiated at several closely spaced sites, distributed over 204 bp nearby the translational start site.[26] Of all CD31$^+$ cells, only lymphocytes are able to modulate the level of CD31 transcription. CD31 mRNA levels are reduced shortly after antigen-specific activation in both CD4$^+$ and CD8$^+$ cells but it significantly differs thereafter in the memory lymphocyte sub-populations[27] possibly due to cell-specific epigenetic regulation[28] across their long life. On memory CD4$^+$ T cells, the reactivation of which is restricted to lymphoid organs and regulated by multiple immune check-points, it remains low[29] whereas the effector memory CD8$^+$ T cells,[27] which can easily be reactivated by the presentation of the antigen in the major histocompatibility complex class I of any cell in the organism, reacquire a maximal expression of CD31. The reacquisition of this particular immune check point might be required to prevent inappropriate and potentially life-threatening systemic immune responses within the circulation.[30]

In addition to transcriptional regulation, the cell-specific function of CD31 can vary in different cells at given time points due to the occurrence of various forms of alternative exon splicing, mainly involving the intracytoplasmic tail[31] but also the extracellular domain 5[32] and the transmembrane segment encoding exon, the latter yielding a soluble full-length isoform detectable in the circulating blood.[25]

Finally, a number of single nucleotide variants have been described, the most frequent polymorphisms within the white population forming 4 different alleles: LSRa, LSRg, VNGa, and VNGg, with frequencies of 0.14, 0.28, 0.27, and 0.31, respectively.[33] The association between the different polymorphisms and cardiovascular risk clinical variables/end points is somehow conflicting, with both positive and negative trends described in atherothrombotic conditions.[34–37]

CD31 Signaling

To conceive the most appropriate therapeutic agents, it is important to determine the key elements involved in CD31 functions. Antibodies targeting the first Ig-like domains of CD31 as well as soluble CD31 proteins comprising its transhomophilic sequence can effectively engage the receptor and serve for pharmacological studies.[38] Yet, although the CD31 protein sequence is well conserved across species, human proteins are not appropriate for use in rodent preclinical studies. Indeed, the different type of glycosylation and the difference of certain amino acids comprised in the transhomophilic region are sufficient to invalidate the binding of conformational monoclonal antibodies as well as the occurrence of cross-species transhomophilic engagement of CD31.[39]

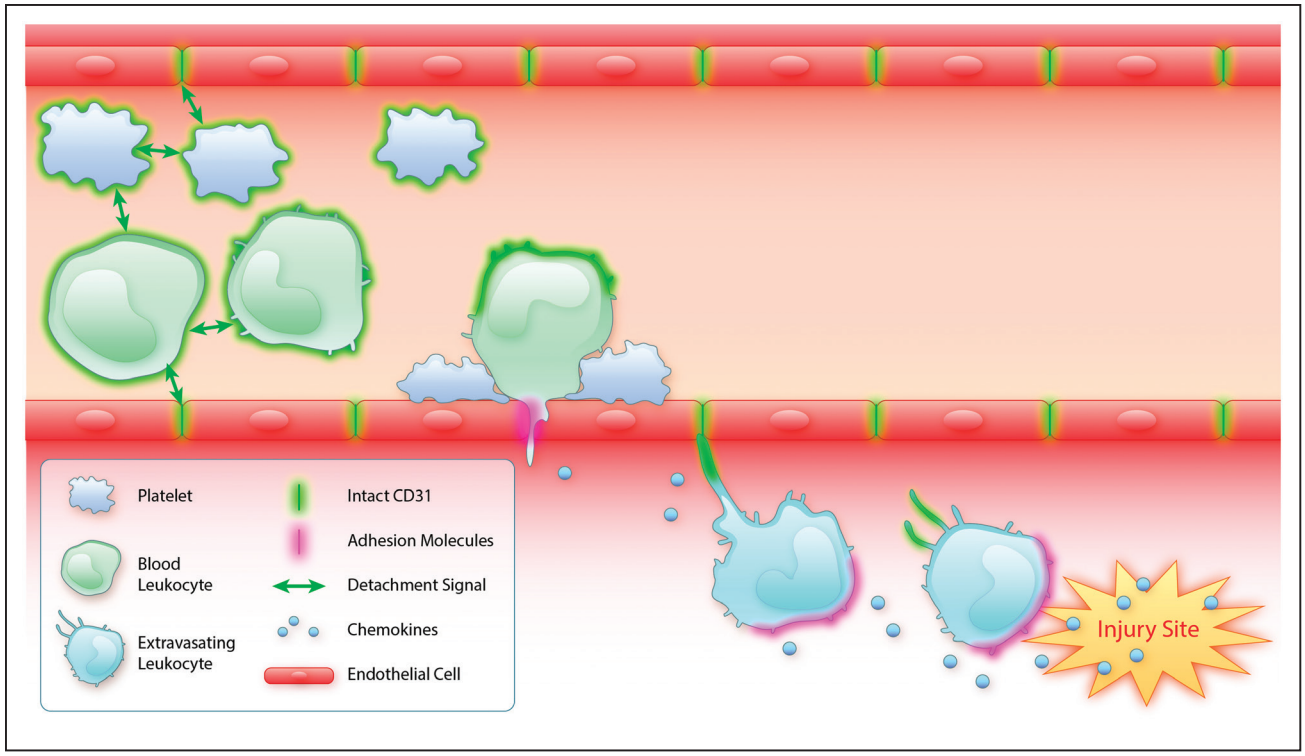

Figure 1. Distribution and function of CD31 molecules on cells at the blood-vessel interface.
CD31 molecules are evenly distributed over the plasma membrane of blood flowing leukocytes and platelets and are concentrated at the intercellular endothelial junctions at sites of high-shear stress. At the surface of interacting CD31$^+$ cells, the transhomophilic engagement of CD31 drives a mutual detachment signal which is essential to prevent their aggregation within the vessels to maintain a fluid circulation in physiological conditions. In case of injury, the cytokines and chemokines released by damaged sites reach the microcirculation and induce a phenotypic change of the stimulated platelets, leukocytes and endothelial cells, restricted to the microvessels of the injured tissue. At these sites, CD31 molecules of all interacting cells redistribute away from the cell-cell contact points to allow the adherence of responsive platelets and leukocytes to the inflamed endothelium. Once the leukocytes have passed the intercellular endothelial junction and reached the basal membrane, the concentration of CD31 molecules at their rear may actively promote integrin closure and allow leukocyte uropod detachment from the extracellular matrix, which is necessary for the movement of leukocytes along the chemokine gradient towards the site of inflammation. (Illustration Credit: Ben Smith).

The signaling properties of the CD31 cytoplasmic tail rely on its inducible and reversible detachment from the plasma membrane, allowing the sequential phosphorylation of its serine and tyrosine residues comprised in an α-helical conformed sequence.[40] The phosphorylation of serine in position 702, in close contact with the plasma membrane, is required for tail detachment from the membrane which allows the phosphorylation of the membrane-interacting C-terminal immunoreceptor tyrosine inhibitory motif (ITIM). The latter is necessary for allowing the phosphorylation of the N-terminal ITIM, which is excluded from the lipid-interacting segment.[40] In the absence of concomitant stimulation, neither antibodies nor transhomophilic proteins can drive the phosphorylation of CD31 S702 and of its 2 ITIMs because CD31 is not endowed with autophosphorylation ability. CD31 signaling can only occur, and be targeted, in the lipid rafts where it can get phosphorylated by other signaling receptors at the time of cell stimulation.[20]

Once phosphorylated, the CD31 ITIMs recruit SH2 (Src homology 2) tyrosine/inositol phosphatases[41,42] resulting in both inhibitory and activating signaling actions, depending on the net effect of these phosphatases in the different signaling pathways concomitantly engaged on CD31$^+$ cells.

In physiological conditions, CD31 signaling is not engaged in resting blood leukocytes and platelets.[43] The eventual recruitment and engagement of CD31 on these elements at sites of low shear where they may establish cell-cell interactions mainly results in a coinhibitory signal,[19] because most platelet and leukocyte activation pathways are tyrosine/inositol kinase dependent.[12] Instead, the arterial endothelial cells establish permanent CD31-CD31 interactions at their lateral borders and CD31 ITIM phosphorylation occurs in basal conditions, triggered by several mechanoreceptors sensitive to high flow speed. Here, the cosignaling functions of CD31 are essential for the survival[20] and physiological response of the vascular endothelium to the multiple types of mechanic, immune, and metabolic stresses (recently reviewed by Caligiuri).[12]

CD31 as a Therapeutic Target in Atherosclerosis

The potential use of CD31 as a therapeutic target for atherosclerosis has been considered ever since its cloning, but the role played by the molecule remains controversial and the therapeutic strategy that could apply stands elusive. Because CD31-targeting antibodies block leukocyte transmigration both in vitro[44] and in vivo,[45] one could envisage the use of CD31 antagonists to combat the inflammatory processes of atherosclerosis. Yet, mechanistic studies have in fact shown that CD31 ligation by a specific antibody engages/sustains CD31 signaling in both leukocytes and endothelial cells.[46] It is, therefore, plausible that the anti-inflammatory action observed in those experiments rather resulted from an agonist effect of the CD31-binding antibodies. Similarly, the immunomodulatory effect obtained using soluble CD31 proteins in vitro[47] and in vivo[48] can be explained by intracellular signaling engagement through a transhomophilic interaction with the N-terminal domain of cell-bound CD31.[5] In apolipoprotein E knockout mice, the overexpression of a bivalent CD31 soluble construction consistently resulted in a reduced immune response mirrored by an enriched regulatory T-cell population and a reduction in the extent of atherogenesis, in vivo.[49] The immunoregulatory effect of strategies targeting the transhomophilic domains of CD31, by either antibody ligation or with the use of recombinant soluble proteins, can however work only for a limited time because the transhomophilic portion of CD31 is lost by a proteolytic shedding invalidating the target[50] (Figure 3).

Immune Responses in Atherosclerotic Diseases: Role of CD31?

Immune cells abundantly infiltrate atherosclerotic lesions supporting a role for immune responses in atherosclerotic diseases.[51–54] Upregulation of endothelial adhesion molecules at atherosclerotic-prone sites plays an important role in the sequence of events leading to the accumulation of blood-borne leukocytes within the arterial wall, a critical step in the initiation and progression of atherosclerotic plaques.[55] Due to its abundant localization on the vascular endothelium and its structural similarity with Ig-like membrane adhesion molecules, CD31 was initially classified among adhesion molecules (this is why it was called PECAM-1 [platelet endothelial adhesion molecule 1]).[56] Furthermore, the presence of 2 putative consensus NFκB (nuclear factor κ B) binding sites in its promoter[57] and the concentration of CD31 clusters at sites of perturbed stress on arterial endothelial cells, where the NFκB pathway is activated[58] suggested a proinflammatory mediator role for endothelial CD31 by favoring the extravasation of leukocytes at sites of perturbed shear stress. Light microscopy morphometric studies on cross-sections of selected arterial sites in CD31 knockout hyperlipidemic mice supported the hypothesis of a NFκB-dependent proatherosclerotic role for endothelial cell CD31,[59] although no data indicated that NFκB indeed regulates CD31 expression. In fact, functional studies have shown that the transhomophilic engagement of endothelial CD31 by transmigrating inflammatory cells actually drives a negative feedback on the proinflammatory NFκB activity in endothelial cells.[60] Furthermore, an exhaustive evaluation of the spatially complex atherosclerotic plaque distributed along the arterial tree by micro-CT 3D imaging rather points at a protective role for CD31 in experimental atherosclerosis.[61] Indeed, at variance with the expression of the adhesion molecules VCAM-1 (vascular cell adhesion protein 1) and ICAM-1 (intercellular adhesion molecule 1) which are upregulated, CD31 expression is constitutive and maximal in physiological conditions and does not increase at atherosclerosis-prone sites.[62,63] Instead, endothelial cells at sites of inflammation[64] including the neovessels in human atherosclerotic plaques,[65] typically appear as CD31 negative suggesting that its loss, rather than its presence, may be involved in atherosclerosis.

CD31 Cosignaling Regulates Leukocyte Migration

CD31 might be involved in the accumulation of leukocytes within atherosclerotic plaques, but the exact role of this molecule remains debated.

Due to the supposed cell adhesive properties of CD31, specific monoclonal antibodies and recombinant proteins were regarded as blocking agents in the early 1990s because their use prevented the passage of leukocytes across TNF-α (tumor necrosis factor-α) stimulated human endothelial monolayers in vitro.[44] However, it is plausible that CD31-targeting antibodies and proteins prevent leukocyte transmigration because they engage the immunomodulatory feature of CD31 signaling. This was acknowledged only later, upon the discovery of its 2 intracytoplasmic ITIMs.[66] Furthermore, CD31's immunomodulatory role was supported by the notion that in the presence of CD31-targeting agents leukocytes do not progress through the intercellular junction and stop their migration at the apical surface of endothelial cells.[44] Finally, transendothelial migration is not hampered in CD31 knockout mice,[67,68] definitively arguing against a role for CD31 in triggering leukocyte transendothelial transmigration and plaque initiation.

The ITIM cosignalling function of CD31 may instead be essential for conducting the stream of migrating leukocytes towards inflammatory sites by regulating the tyrosine kinase-dependent opening of leukocyte integrins[69] that promotes cell adhesion. The activity of phosphatases recruited by CD31 ITIMs might play a key role in cell integrin reacquisition of a closed/bent conformation which is essential to allow dissociation of the cell membrane at the cell uropod and cell progression along the chemokine gradient.[70] This hypothesis

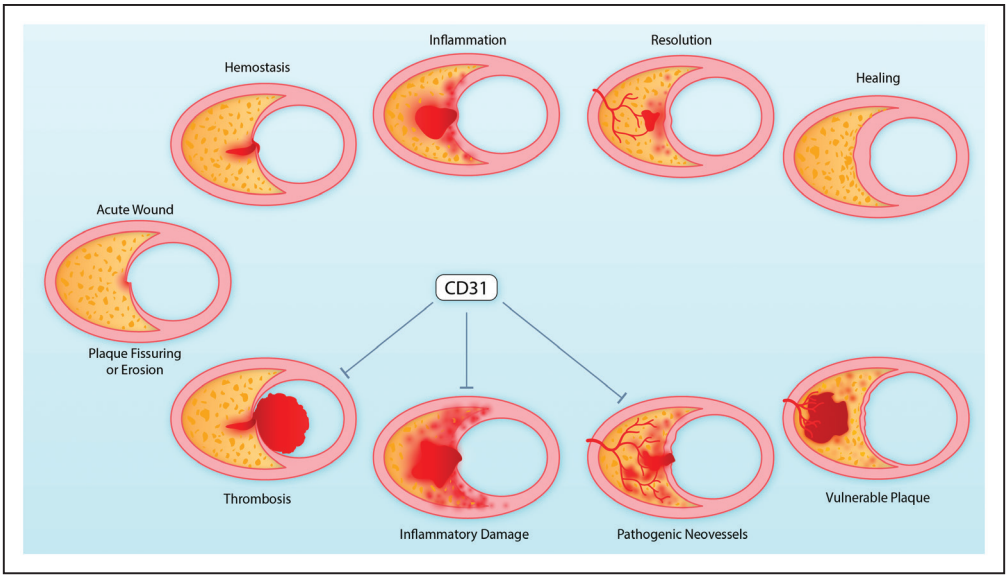

Figure 2. Putative role of CD31 in healing after acute atherosclerotic complication.
Atherosclerotic complications are linked to the occurrence of an acute wound of the intimal arterial layer covering an atherosclerotic plaque (plaque fissuring or erosion) which triggers a finely orchestrated and efficient cascade of biologic events conducting to healing. Similar to the healing cascade occurring at sites of dermal wounds, the first step is hemostasis activation by the exposure of extracellular matrix and tissue matter to the flowing blood, which allows the immediate closure of the wound by a platelet/fibrin scaffold. Blood leukocytes are attracted by the chemokine gradient and activated by locally released cytokines. The ensuing inflammation is necessary to clear the damaged tissue and plays a key role in wound healing. Its resolution should, however, occur as soon as possible to allow the phenotypic switch of leukocytes towards a reparative phenotype and avoid collateral damage while promoting the arrival of oxygen and nutrients through the neovessels. The completion of each step of the sequence is essential for the building of new tissue and the formation of a solid scar. The latter accounts for the formation of a thick fibrous cap which signs the healing of the arterial sites and stabilization of the culprit atherosclerotic plaque. Recurrent wounding and ineffective healing resulting in excessive activation of the hemostatic system, the lack of regulation and persistence of inflammation and the occurrence of intraplaque hemorrhage forming intraplaque neovessels, could underlie the occurrence of clinical complications. A poor (if any) healing at sites of plaque wound may indeed account for the large necrotic core (failure of dead cells and matrix debris clearance) and the thin cap (crumbly scar) which are characteristic of vulnerable plaques. Fortunately, most often the healing cascade is well orchestrated and effective, and the occurrence of clinical atherothrombotic events is relatively rare compared with the frequency of plaque fissuring and erosion. CD31 might play a crucial role for the efficient development of the healing cascade at sites of atherothrombosis. By regulating the activation threshold of platelets, it might reduce the extent of the thrombosis occurring at the site of plaque fissure or erosion. By guiding the stream of transmigrating leukocytes and modulating their activation, CD31 can limit the unwanted collateral inflammatory damage. By ensuring the barrier function, CD31 can reduce the leakage and intraplaque hemorrhage from plaque neovessels. Illustration Credit: Ben Smith.

is supported by experimental data showing that leukocyte arrival at the inflammatory site is delayed in the absence of CD31. This is due to migratory machinery polarization defects, preventing leukocytes to follow the direction of the chemokine gradient.[71] Moreover, this is combined with an increased leukocyte halt at the level of the perivascular membrane[68] likely due to uropod detachment impairment.[70] Ultrastructural analysis of reactive lymph nodes had demonstrated that CD31 molecules are concentrated at the ending of extended membrane protrusions formed by extravasating leukocytes at the interface with the microvascular endothelium.[72] Additionally, time-lapse videomicroscopy has shown that CD31 molecules rapidly disappear from the leading edge to converge with the β-integrins localized in a large cluster at the tip of the uropod of migrating leukocytes.[73] Here, CD31 cosignaling may be pivotal for permitting β-integrins refolding in closed bent conformation, allowing the atraumatic detachment of the leukocyte uropod[70] (Figure 1).

The Immunoregulatory Functions of CD31 on T Cells Are Impaired in Atherothrombosis

The cosignaling functions of CD31 exert a key role during T-cell development in the thymus[74] as well as in the regulation of extrathymic T-cell threshold activation (reviewed by Marelli-Berg et al[21] and Newman et al[46]). Remarkably, the T cells accumulated within human atherothrombotic plaques appear to have lost the expression of CD31.[75] Consistently, the frequency of such CD31 negative T cells in the blood is associated with both a hyper-reactive (easily activated) phenotype and the occurrence of thrombotic complications of atherosclerosis in both patients[75] and hypercholesterolemic mice.[76] The CD31 protein expressed by activated T cells can be reduced due to a transcriptional downregulation in memory T cells.[27] However, CD31 exposure is likely underestimated on activated lymphocytes because the epitopes of the monoclonal antibodies commonly used for its immunodetection are comprised in the N-terminal Ig-Like domains which are lost due to proteolytic cleavage

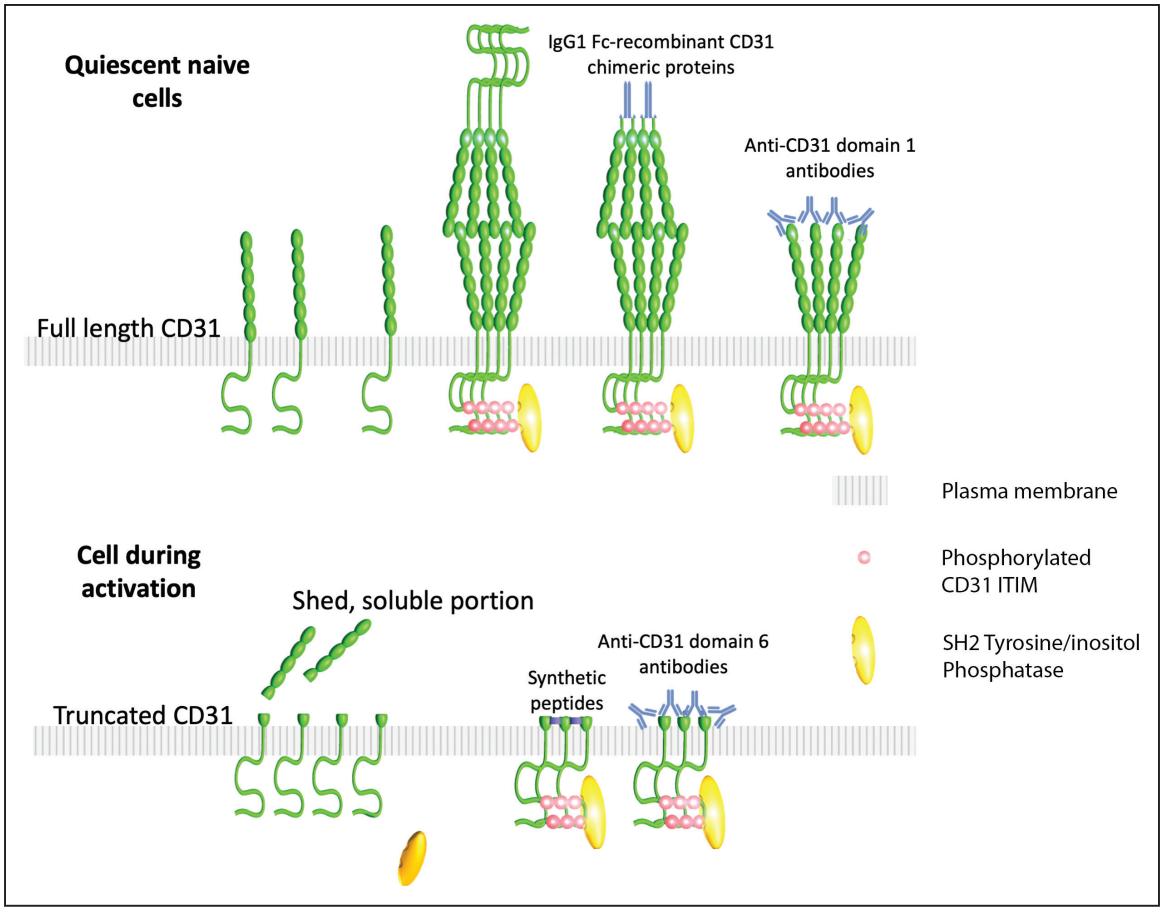

Figure 3. Potential CD31 targets for agonist drug candidates.
An agonistic effect of CD31-targeting molecules has been described for specific antibodies, synthetic peptides, and recombinant proteins. The mechanism of action, and hence the clinical indication, varies according to the portion of the protein that is targeted by the therapeutic candidate. On quiescent CD31$^+$ cells the CD31 molecules are dispersed, and their targeting would not be followed by a signaling effect because CD31 is not able to auto-phopshorylate. The clustering of CD31 molecules in lipid rafts allows their cosignaling activity via the phosphorylation of their intracellular ITIMs by coclustered tyrosine kinase receptors. There, the use of bivalent proteins (made by the fusion between the extracellular portion of CD31 and the Fc portion of IgGs), or of monoclonal antibodies targeting CD31 domain 1, can engage the transhomophilic portion of the native protein and sustain its physiological signaling essentially raising the cell activation threshold. These compounds are, however, useless in pathological situation because the membrane-distal portion of the CD31 molecule is lost due to proteolytic shedding triggered by cell activation. In this case, candidate agonists should be able to bypass the first, transhomophilic step of CD31 receptor engagement. Indeed, experimental work has shown that the lingering fragment of extracellular CD31 that remains exposed at the surface of CD31^{shed} cells can be targeted by homotypic peptides or by antibodies specifically directed to the juxta-membrane sequence of the Ig-like domain 6. Preclinical studies in apolipoprotein E knockout mice suggest a preventive potential (chronic treatment) for gene therapy leading to the production of soluble chimeric proteins made of extracellular CD31 fused with the Fc portion of IgG1.[49] Whereas the use of synthetic peptides homotypic of the juxta-membrane sequence is promising for the management of situations where endogenous CD31 may have already been cleaved, such as acute atherothrombotic events.[96,97]

and shedding.[50] The absence of this portion of the protein invalidates the transhomophilic engagement of the receptor.[5] The resulting loss of CD31 signaling function coincides with the retention of CD31^{shed} activated CD4$^+$ T cells, detectable by antibodies targeting the cytoplasmic tail or membrane-proximal epitopes at sites of ongoing inflammation.[38] Immunoregulatory functions exerted by CD31 have been described in CD4$^+$,[77] CD8$^+$,[78] and B lymphocytes,[30] as well as in innate immune cells such as monocyte-macrophages[79] and dendritic cells.[80] Further evidence for an activation-driven cleavage of CD31 has been repeatedly reported in inflammatory conditions.[81–83]

An inappropriate or excessive shedding of CD31 may contribute to the dysregulations of the immune response associated with atherothrombosis, as suggested by the higher frequency of circulating CD31^{shed} monocytes and CD4$^+$ T cells in acute coronary syndromes.[84,85] Interestingly, the increased expression of MMP9 (matrix metalloproteinase 9) repeatedly observed in acute coronary syndromes has recently been linked to the shedding of CD31 documented on activated T cells in patients.[84]

The causal link between these 2 hallmarks of atherothrombosis can go in both directions: enhanced MMP9 expression by cells interacting at sites of inflammation can contribute to CD31 shedding and, vice versa, CD31^{shed} cells can more easily activate and produce active MMP9. Furthermore, both events are engendered by ongoing inflammatory processes which,

in turn, are part of the body response to noxious stimuli or tissue injury.

Role of CD31 in the Healing of Atherothrombotic Arteries

Immune mechanisms contribute undeniably to atherosclerosis initiation, progression, and complications, but their causal role cannot be established by studies performed after the onset of clinical symptoms.[86] In atherothrombosis, the inflammatory components concur with the thrombotic process, as highlighted >150 years ago by Virchow.[87] The activation of the hemostatic and immune systems occur sequentially at sites of tissue injury and play a critical role in initiating the process of wound healing. In the earliest phases, platelet thrombi serve to immediately close the wound and neutrophil-mediated efferocytosis is necessary to prepare the stage for scar formation. The local activation of platelets and neutrophils must, however, be tightly regulated to avoid damage-aggravating activity and must resolve as soon as possible to allow the progression towards the subsequent proliferative phase which achieves the wound closure by the formation of a fibrotic scar (Figure 2). The activity of the neutrophils abundantly infiltrating the culprit lesions[88] might eventually turn deleterious if they start forming extracellular traps, a process executed by neutrophils unable to digest the noxious stimuli[89] called *NETosis*. Netosis is associated with all forms of plaque complications at sites of coronary atherothrombosis[90] likely including those triggered by intimal erosion rather than fissuring, as suggested by recent experimental data.[91] Interestingly, the deleterious action of neutrophils is critical also in the earliest stages of atherogenesis. There again, this neutrophil action occurs as a consequence of the arterial injury represented by intimal breaches the formation of which is driven by the particular hemodynamic stress at atheroprone arterial sites.[92] The activity of proinflammatory monocyte-derived and resident macrophages is important at later stages, for clearing dead neutrophils and other cell and matrix debris but it must be constrained and short lasting to allow the progression towards the reparative steps. Inflammatory phase resolution initiates with a switch from a proinflammatory to a pro-reparative phenotype of wound infiltrating macrophages and is essential for the formation of a solid scar and the outcome of tissue healing (Figure 2).

Given the nature of our highly pressurized and branched arterial tree, the mechanic injury is likely iterative and does not necessarily result in clinically manifestations as demonstrated by ex vivo analysis of coronary plaques[93] as well as in vivo, by optical coherence tomography.[94] The outcome of the healing process triggered by plaque injury appears critical for the fate of atherothrombosis: disproportioned inflammation and its persistence may lead to the thinner fibrous cap of vulnerable plaques, reflecting a poor scar formation, whereas, at the opposite, inappropriate production of growth factors may explain the observed accelerated post-rupture plaque progression, due to exaggerated stromal cell proliferation and production of extracellular matrix.[95] Thus, suggesting strategies aimed at ensuring a proper orchestration of the processes required for arterial wall healing at sites of atherothrombosis, rather than prevention of plaque fissuring, may be the target of future therapies in atherosclerosis.

In this setting, a defective expression of CD31 by the macrophages infiltrating atherothrombotic wounds is associated with their delayed healing[96] and several lines of evidence point at CD31 homotypic recombinant proteins or peptides as very interesting drug candidates to prevent and treat atherothrombotic events[49,97] (Figure 3). The activation threshold regulation exerted by CD31 in platelets[22] may be important to limit the hemostatic phase and avoid the formation of a rapid and stable occlusive thrombus[18] following plaque fissuring/erosion.

By inhibiting the production of isoprenoid cholesterol intermediates, statins may limit the palmitoylation of the C-terminal ending of CD31's intracellular tail hence favoring CD31 signaling by granting the access of kinases to its phosphorylatable residues which would otherwise be hidden in the tail loop hooked to the plasma membrane. Such an effect might explain why the observed potentiation of the antiplatelet function exerted by statins depends upon the presence of CD31.[98]

The immunoregulatory effects of CD31 might be beneficial in acute ischemic events for guiding the infiltration of the culprit lesions by blood neutrophils and restricting their activation sequence as recently shown in experimental mesenteric ischemia-reperfusion.[99] Finally, CD31 agonists may accelerate the resolution of the inflammatory phase, which is critical for the outcome of wound healing, by favoring the phenotypic switch of infiltrated macrophages from proinflammatory to pro-reparative.[96]

Potential Benefits of a CD31 Mimicking Coating on Coronary Stents

The physiological functions of CD31 on the vascular endothelium are ensured by a very dense presence of this molecule at its surface. Here, it drives the cosignaling necessary for the cytoprotection and barrier function of adjacent endothelial cells and for raising the activation threshold of blood flowing platelets and leukocytes, by engaging in transhomophilic interactions with their respective CD31 molecules.

Endovascular prostheses (stents) provide a mechanical support to coronary arteries subjected to percutaneous angioplasty and are thus systematically implanted at the end of virtually all revascularization procedures. Their metallic nature, however, hampers the success of the procedure at medium-long term, because the cells

entering in contact with the stent struts perceive them as a foreign body and are hence activated locally to reject it. As a consequence, a chronic inflammatory process ensues, eventually resulting in a new, in-stent, stenosis. The use of immunosuppressive drugs, eluted by active stents, effectively reduces the inflammatory reaction but, in the long-term, the risk of in stent stenosis and potentially catastrophic late stent thrombosis is important for all types of stent, despite protracted administration of dual antiplatelet therapy.[100]

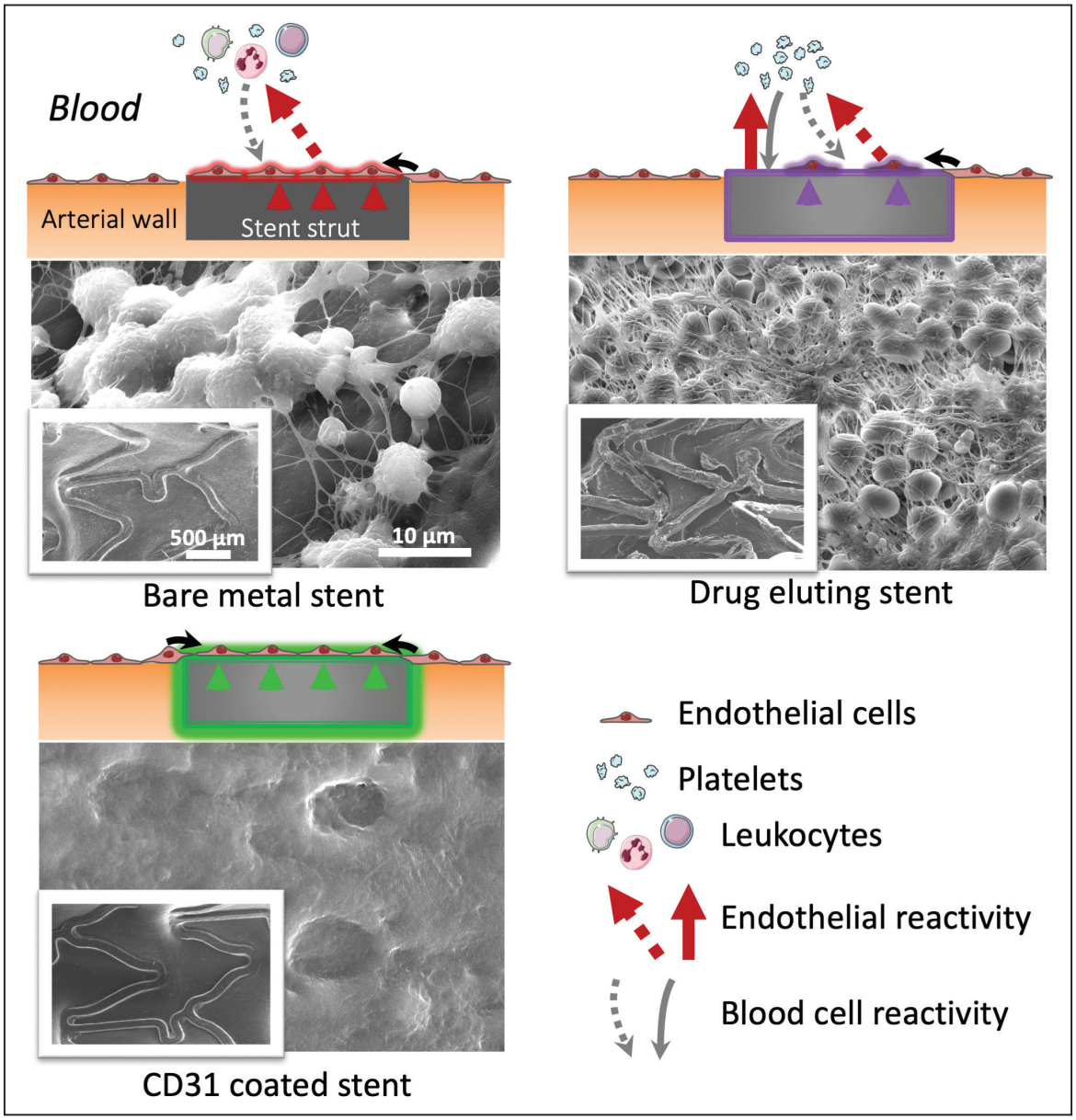

Figure 4. The immobilization of a CD31 homotypic peptide on coronary stents improves the integration of the metallic device in the arterial wall.

Stent implantation implies the penetration of metallic stent struts into the arterial wall. A rapid endothelialization is observed over bare metal stents, but the phenotype of the endothelial cells covering the stent struts is altered (red arrow heads) resulting in a prothrombotic and proinflammatory phenotype which eventually triggers an adverse reaction of blood leukocytes and platelets against the device. The immunosuppressive drugs eluted by drug eluting stents can prevent the activation of leukocytes but not that of platelets and eventually affect also the viability of the endothelial cells covering the stent struts (violet arrow heads=dead endothelial cells), which results in an enhanced thrombotic risk. The use of a CD31-mimetic peptide covalently immobilized on bare metal stents can mimic the presence of an intact endothelium by providing a CD31 engaging signal to endothelial cells, platelets and leukocytes. Preclinical studies in pigs show that CD31-coated stents can combine the beneficial features of bare metal stents (complete endothelialization as assessed by electron scanning microscopy 7 d after implantation in pig coronary arteries) and of drug eluting stents (reduced thrombotic/inflammatory reaction) without their respective drawbacks (adherence and activation of platelets and leukocytes on bare metal stents; loose endothelial coverage resulting in persistence of platelet activation 7 d after implantation in pig coronary arteries). The use of CD31 biomimetic coating may, therefore, serve to foster a more physiological integration of coronary stents within the arterial wall.[103]

Autopsy studies have suggested that endothelial coverage completeness of the stent struts is critical for the positive outcome of the procedure. Coating strategies aimed at actively favoring the attachment of circulating progenitor endothelial cells onto the stent have recently emerged. However, considering the low frequency and functional defects of endothelial progenitors in atherosclerotic patients, it is not surprising that their performance in clinical trials is disappointing.[101] The use of stents coated with synthetic CD31 molecules able to engage in transhomophilic interaction with the endogenous receptor on the cells of the blood-vessel interface might be an interesting alternative, as suggested by in vitro experiments[102] and preliminary preclinical studies in pigs[103] (Figure 4).

CONCLUSIONS

CD31 has been considered as a target for atherosclerosis ever since its discovery. Initially regarded as a proinflammatory and hence proatherosclerotic molecule, blocking agents were envisaged. The concomitant accumulation of evidence in favor of protective signaling in vascular biology has, however, clouded the rationale behind this perspective and delayed the development of candidate therapeutic strategies. The most recent data show that the physiological, atheroprotective functions of CD31 are lost in patients. Preclinical studies consistently point at a therapeutic potential for CD31 agonists for managing individuals with atherosclerotic disease manifestations. The administration of soluble agonists may promote arterial healing and are an option in the management of patients early after the occurrence of acute atherothrombotic manifestations. On the contrary, CD31 signaling properties could also be beneficial in a medical device development perspective. The immobilization of molecules able to drive CD31 signalization on stent surfaces may be envisaged as an endothelial-mimetic coating for masking the foreignness of the metal struts and improving the integration of arterial stents.

ARTICLE INFORMATION

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Disclosures

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