Emerging regulators of vascular smooth muscle cell function in the development and progression of atherosclerosis

Jason Lee Johnson*

Laboratory of Cardiovascular Pathology, School of Clinical Sciences, University of Bristol, Research Floor Level Seven, Bristol Royal Infirmary, Bristol BS2 8HW, UK

Received 13 January 2014; revised 1 July 2014; accepted 14 July 2014; online publish-ahead-of-print 22 July 2014

After a period of relative senescence in the field of vascular smooth muscle cell (VSMC) research with particular regards to atherosclerosis, the last few years has witnessed a resurgence, with extensive research re-assessing potential molecular mechanisms and pathways that modulate VSMC behaviour within the atherosclerotic-prone vessel wall and the atherosclerotic plaque itself. Attention has focussed on the pathological contribution of VSMC in plaque calcification; systemic and local mediators such as inflammatory molecules and lipoproteins; autocrine and paracrine regulators which affect cell–cell and cell to matrix contacts alongside cytoskeletal changes. In this brief focused review, recent insights that have been gained into how a myriad of recently identified factors can influence the pathological behaviour of VSMC and their subsequent contribution to atherosclerotic plaque development and progression has been discussed. An overriding theme is the mechanisms involved in the alterations of VSMC function during atherosclerosis.

Keywords
Atherosclerosis • Vascular smooth muscle cells • Migration • Foam cell formation • Calcification • Matrix metalloproteinases

1. The duality of vascular smooth muscle cells in atherosclerosis

It is considered that the presence of VSMCs within advanced atherosclerotic plaques is beneficial and protective, due to their pivotal role in the formation and sustenance of the fibrous cap. It is the VSMC-rich fibrous cap which guards advanced lesions from plaque rupture and subsequent thrombosis. Conversely, the genesis of atherosclerotic lesions also involves the preponderance of VSMCs. Atherosclerotic plaques develop at a multitude of pre-defined sites within the arterial vasculature—these locations are termed ‘athero-susceptible’ and appear to be shared between man and animal species used to model in vivo atherosclerosis. Such predilection is considered to be a response of endothelial cells to alterations in blood flow including low shear stress, turbulence, and oscillating flow. When endothelial cells are exposed to these haemodynamic alterations they are rendered dysfunctional and are characterized by impaired expression of endothelial nitric oxide synthase (eNOS). Decreased nitric oxide (NO) bioavailability facilitates a multitude of pro-atherogenic effects including increased platelet adherence and aggregation, monocyte adhesion/infiltration, oxidative modification of accumulated lipoproteins, and vasoconstriction. Additionally, a reduction in nitric oxide levels can result in the increased expression of matrix metalloproteinases (MMPs) and exert pro-proliferative and pro-migratory effects on VSMCs. Saliently, while in mouse models, monocyte/macrophage accumulation is the first visible sign of atherogenesis, in man, the fore-runner to nascent atherosclerotic plaques is adaptive intimal thickening. This is a normal developmental process characterized by the intimal accumulation of VSMCs embedded within a proteoglycan-rich extracellular matrix, as alluded to, commonly at sites of altered haemodynamic blood flow. The transition into the earliest recognized form of an atherosclerotic lesion, termed a pathological intimal thickening, is associated with morphological and biochemical alterations to the VSMCs and their extracellular matrix. This evolution includes the increased retention and modification of lipoproteins, VSMC uptake of modified lipoproteins, VSMC apoptosis, infiltration of macrophages, presence of micro-calculiations, and the formation of a lipid core. Although the precise sequence of mechanisms is yet to be resolved, a better understanding of these initial pathological processes may clarify how lipid/necrotic cores form and expand into advanced unstable plaques. Interestingly, the pathological advancement of early atherogenesis in man cannot be definitely modelled in animal models, as they regularly lack both adaptive and pathological intimal thickenings. Nonetheless, insights can be gained from in vivo models of atherosclerosis to elucidate the contribution of VSMC to plaque advancement but always require validation in human cells and tissues.

* Corresponding author. Tel: +44 117 342 3190. Email: jason.l.johnson@bris.ac.uk
Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2014. For permissions please email: journals.permissions@oup.com.
2. Modulators of VSMC migration

The proliferation and migration of VSMCs facilitate early lesion development but are equally important for maintaining plaque stability through the maintenance of a protective fibrous cap overlying the thrombogenic lipid core of advanced lesions. Thus understanding key regulators of VSMC growth within different stages of atherosclerosis will facilitate and inform future strategies to modulate the disease process. MMPs have been inexorably linked to VSMC growth and atherosclerosis.27 In particular, MMP-9 has been demonstrated to play a fundamental role in VSMC migration and although may promote early lesion development appears to protect against advanced plaque progression.13 – 17 A recognized mechanism of action through which MMPs may modulate VSMC behaviour is the processing of cell–cell and cell–matrix interactions. N-cadherin represents a major cell–cell adhesion molecule in VSMCs and has been demonstrated to be cleaved by several MMPs (MMP-7, -9, and -12), affecting VSMC behaviour including migration, proliferation, and apoptosis.18,19 Moreover, administration to atherosclerotic mice of a soluble form of N-cadherin, acting as a mimetic, increased VSMC migratory leading edge of the cell, including small Rho GTPase members of new focal adhesions and associated lamellipodia protrusions from the actin cytoskeleton.30 Cysteine-rich proteins (CRP) are a family of LIM domain proteins including CRP1, CRP2, and CRP4, which facilitate protein–protein interaction along the actin-based cytoskeleton.30 Additionally, CRPs can also localize to the nucleus where they interact with transcription factors to mediate VSMC gene expression. Accordingly, CRPs have been implicated in regulating VSMC differentiation, proliferation, migration, and survival.31 – 33 Interestingly, mice deficient for either CRP1 or CRP2 or both are viable and fertile, while neointimal formation is affected after arterial injury.32,34 implying differential regulatory mechanisms modulate VSMC CRP expression during embryonic development and in response to vascular injury.35 Transforming growth factor beta (TGF-β) signalling may represent one such mechanism, as two recent independent studies have demonstrated that TGF-β stimulates CRP2 expression in VSMC.36,37 However, the responses to arterial injury in mice lacking CRPs are divergent; neointimal formation was attenuated in CRP1 global-deficient mice, increased in CRP2 whole-body deficient animals, and unaltered in mice lacking both CRPs systemically.32,34 These results intimate that the two CRPs are involved in different cellular responses to arterial injury and may exert antagonistic effects on each other. Thus understanding the molecular and cellular mechanisms involved in CRP regulation of VSMC behaviour may elucidate their pathophysiological role and reveal potential therapeutic approaches.

More recently, oxidative stress has been proposed to regulate MMP-directed shedding of N-cadherin from VSMCs and promote their migration. Jagadeessa et al.21 revealed that thrombin utilizes Nox1 to trans-activate the epidermal growth factor receptor (EGFR), which in turn induces MMP-9 up-regulation/activity and subsequent shedding of N-cadherin, resulting in augmented VSMC migratory capacity. Similarly, a further study has demonstrated that Toll-like receptor (TLR)2 signalling also employs Nox1 to regulate MMP-induced VSMC migration. Lee et al.22 revealed that the synthetic TLR2 ligand Pam3CSK4, induced interaction of TLR2 with Nox1 and subsequent reactive oxygen species generation, which in turn increased MMP-2 activity and subsequent VSMC migration. Furthermore, it was shown that VSMC release of pro-inflammatory molecules involved in monocyte recruitment were also augmented. Together these findings imply that oxidative stress within VSMCs, as a consequence of injurious insults such as thrombosis, bacterial infection, or modified lipoproteins, may facilitate the transition and development of early atherosclerotic lesions.

Further consequences of alterations in cell–cell and cell–matrix interactions are cytoskeletal changes, which have fundamental effects on cell motility.23 Pro-migratory cytoskeletal re-arrangement requires the orchestration of numerous protein complexes to facilitate the generation of new focal adhesions and associated lamellipodia protrusions from the migratory leading edge of the cell, including small Rho GTPase members such as Rac1.24 Scaffolding proteins are also necessary at the adhesion-actin cytoskeleton interface to expedite migratory cues, such as p130Cas.25 Indeed, PDGF-stimulated VSMC migration has been shown to be mediated via p130Cas activation/phosphorylation.26 Moreover, in parallel with the earlier study indicating that co-operation between oxidative stress and EGFR can modulate VSMC migration, via MMP-dependent N-cadherin shedding.21 it has been shown that this signalling cascade also augments p130Cas phosphorylation,27 further promoting the migratory capacity of cells. Additionally, upon phosphorylation, p130Cas can complex with focal adhesion kinase (Fak) at the leading edge of migratory cells to activate and localize MMP-dependent motility.28,29 Taken together, these studies suggest that p130Cas activation plays a pivotal role in dictating the migratory potential of VSMC, and that modulating its activity or expression may abrogate its pro-migratory effects.

Cysteine-rich proteins (CRP) are a family of LIM domain proteins including CRP1, CRP2, and CRP4, which facilitate protein–protein interaction along the actin-based cytoskeleton.20 Additionally, CRPs can also localize to the nucleus where they interact with transcription factors to mediate VSMC gene expression. Accordingly, CRPs have been implicated in regulating VSMC differentiation, proliferation, migration, and survival.31 – 33 Interestingly, mice deficient for either CRP1 or CRP2 or both are viable and fertile, while neointimal formation is affected after arterial injury.32,34 implying differential regulatory mechanisms modulate VSMC CRP expression during embryonic development and in response to vascular injury.35 Transforming growth factor beta (TGF-β) signalling may represent one such mechanism, as two recent independent studies have demonstrated that TGF-β stimulates CRP2 expression in VSMC.36,37 However, the responses to arterial injury in mice lacking CRPs are divergent; neointimal formation was attenuated in CRP1 global-deficient mice, increased in CRP2 whole-body deficient animals, and unaltered in mice lacking both CRPs systemically.32,34 These results intimate that the two CRPs are involved in different cellular responses to arterial injury and may exert antagonistic effects on each other. Thus understanding the molecular and cellular mechanisms involved in CRP regulation of VSMC behaviour may elucidate their pathophysiological role and reveal potential therapeutic approaches.

More recently, it has been proposed that CRPs may regulate VSMC motility through interactions with p130Cas signalling complexes, afforded to them through their LIM domains. Also, CRP2 cellular localization is regulated by alterations in the VSMC actin cytoskeleton,38 similar to p130Cas. Accordingly, in a recent issue of *Cardiovascular Research*, Chen et al.39 examined the role of CRP2 in p130Cas-induced VSMC migration. Expectedly, PDGF-BB stimulated migration was increased in CRP2 deficient VSMCs, independent of effects on adhesion or spreading, but associated with increased lamellipodia formation. Using wild-type VSMC and through add-back experiments, they observed that CRP2 localized with stress fibres of the cytoskeleton in a LIM1-dependent manner, which is necessary for CRP2 to retard lamellipodia formation and decrease motility. Importantly, CRP2 via its LIM1 domain directly interacted with the substrate domain of p130Cas at the site of focal adhesions, reducing lamellipodia and VSMC migration. Moreover, the sequestering of p130Cas at focal adhesions by CRP2 abrogated its phosphorylation, intimating a critical role of p130Cas phosphorylation in VSMC migration and neointimal formation. Supportingly, high levels of phospho-p130Cas were observed in medial and neointimal VSMC of arteries after carotid ligation.39 Also, pharmacological inhibition of p130Cas phosphorylation or siRNA knockdown both reduced neointimal formation. However, it is also plausible that other regulators of p130Cas phosphorylation may play a role, particularly as phospho-p130Cas levels were elevated within the ligated artery of these studies suggest that p130Cas activation plays a pivotal role in dictating the migratory potential of VSMC, and that modulating its activity or expression may abrogate its pro-migratory effects.20,40 Other MMP levels and activity41 – 43 modulate VSMC behaviour, neointimal formation, and atherosclerosis. Thus endorsing similar studies evaluating the therapeutic potential of modulating CRP2 and p130Cas function in atherosclerosis.
3. Vascular smooth muscle foam cell formation: contribution to pathological intimal thickening

As mentioned previously, VSMC foam cell formation may represent a pivotal step in the transformation of intimal thickenings into nascent atherosclerotic lesions. As recently reviewed,44 VSMCs harbour all the machinery necessary to accumulate modified lipoproteins and transform into foam cells. Moreover, VSMC foam cells are prevalent in both early and advanced human atherosclerotic plaques, however, due to the differences in atherogenesis within animal models; their incidence in plaques from hypercholesterolaemic animals is reduced and limited to advanced lesions. Consequently, there are inherent difficulties in assessing manipulations in animal models due to the different disease aetiologies. Nonetheless, the mechanism underlying VSMC foam cell formation is of obvious importance.

The VSMC-derived extracellular matrix within adaptive intimal thickenings at athero-susceptible sites facilitates the retention of lipoproteins, increasing the susceptibility of their modification.3,45 Moreover, pheno-typically altered VSMCs which populate adaptive intimal thickenings, harbour a myriad of specific receptors which modulate the endocytosis of modified lipoproteins, these include; SRA-I, SRA-II, CD36, lectin-type oxidized LDL receptor 1 (LOX-1), and low-density lipoprotein receptor-related protein 1 (LRP1).44,46 Concomitantly, ATP-binding cassette transporter A1 (ABCA1) and apolipoprotein A1 (ApoA1), which work in concert to mediate the efflux of cholesterol to form nascent HDL particles, the primary canonical process of reverse cholesterol transport, are down-regulated in intimal VSMCs.47 Interestingly, medial VSMCs show no such deficiencies,47 suggesting that increased cholesterol influx alongside decreased efflux contributes to VSMC foam cell formation in early atherosclerotic lesions. Diabetic patients have an increased risk of atherosclerosis-related disorders which can even be predicted in patients with pre-diabetes, as detected by abnormal glucose tolerance tests.48 Concurring, high glucose levels can induce VSMC foam cell formation due to an imbalance in lipid uptake and efflux, as observed by increased CD36 expression and a concomitant abrogation of the expression and function of ATP-binding cassette transporter G1 (ABCG1), in cultured human VSMCs.49

The switching of VSMC from a contractile phenotype to a synthetic one is considered a pre-requisite during the formation of the neointima, as occurs during adaptive intimal thickening, and is associated with increased migratory and proliferative capacity (as reviewed by Lacolley et al.46). Recently VSMC lipid accumulation and foam cell formation has been related with VSMC proliferation. Ma et al.50 demonstrated that inflammatory stimuli (LPS) modulated low-density lipoprotein receptor (LDLR) expression through activation of the mammalian target of rapamycin (mTOR) pathway and Rb phosphorylation, facilitating Sterol Regulatory Element-Binding Protein (SREBP)-2 induction of LDLR expression and ensuing cholesterol uptake. These data imply that mTOR inhibition, especially mTORC1, may provide useful for retarding early plaque progression.

Atherogenic lipoproteins have also been shown to induce VSMC proliferation through alterations in calcium (Ca²⁺) signalling/handling, primarily by modulating the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA).51 Moreover, impaired calcium handling is exhibited by

---

**Figure 1** Anti- and pro-migratory effects of CRP2 and p130Cas. (1) Cell-surface N-cadherin expression on adjacent VSMC maintains cell–cell contacts and sustains an anti-migratory phenotype. CRP2 can also retard VSMC migration via its LIM1 domain, directly interacting with the substrate domain of p130Cas, a pro-migratory molecule associated with lamellapodia. (2) Multiple MMPs are capable of shedding N-cadherin resulting in disruption of cell–cell contacts while also triggering intracellular β-catenin signalling and favouring a pro-migratory phenotype. Moreover, MMPs can be directed and co-localize to the lamellapodia of VSMC with phospho-p130Cas, permitting VSMC migration.
VSMCs from athero-susceptible mice and also associated with altered SERCA expression and concomitant changes in cell function. Furthermore, alterations in Ca2+ response can regulate the phenotypic switching of in vitro human coronary artery VSMCs. A central role for SERCA in controlling VSMC behaviour has been further demonstrated by in vivo studies. Oxidative modification of SERCA through TGF-β1 up-regulation of Nox4 (nicotinamide adenine dinucleotide phosphate-oxidase 4) promoted VSMC migration from obese Zucker rats. In these rats, knockdown of Nox4 retarded SERCA oxidation and reduced neointima formation. Additionally, Lipskaia et al. demonstrated that SERCA2 inhibits VSMC proliferation and subsequent balloon injury-induced neointima formation in rats, via the transcription factor nuclear factor of activated T-cells (NFAT). The same authors revealed SERCA2a (in synergy with protein phosphatase inhibitor-1; I-1) regulated Ca2+ handling repressed VSMC phenotypic switching to a synthetic state. Expectedly, VSMC proliferation and neointima formation were increased in I-1 knockout mice, while overexpression of I-1 in the rat angioplasty model retarded VSMC phenotypic switching and neointimal thickening. Therefore, SERCA2a (and I-1) overexpression maybe considered therapeutic strategies during specific stages of atherosclerosis.

It has been demonstrated that VSMC exhibit plasticity within early intimal thickenings which may give rise to a considerable diversity of VSMC responses to prolonged exposure of modified lipoproteins. Aargmann et al. demonstrated divergent sub-populations of VSMCs in primary culture from the same human artery which differently responded to LDL exposure implying that a number of VSMCs from the same arterial site can be reprogrammed in response to modified lipoproteins. It has also been suggested that VSMC harbour the ability to trans-differentiate to a macrophage-like state on cholesterol loading, including the expression of genes considered macrophage-specific. These intriguing mechanisms, devised largely from in vitro studies and in mouse cells, provide a major challenge for the field to further define and validate the mechanisms of VSMC plasticity in response to modified lipoproteins. Notably, recent evidence has shown that cholesterol-loaded lysosomes can be transported from foam cell macrophages to VSMCs in vitro, inducing VSMC foam cell formation. This novel pathway may in part account for the prevalence of VSMC foam cells in early atherosclerotic lesions, which are admixed with macrophage foam cells in pathological intimal thickenings.

The down-stream effects of VSMC foam cell formation on plaque progression are numerous. For instance, perpetual cholesterol accumulation can induce cell death of the lipid-laden VSMC, promoting the migration and proliferation of adjacent VSMCs within the intima. VSMC apoptosis and cell death can also instigate inflammation through the monocyte chemo-attractant protein-1 (MCP-1)-mediated recruitment of circulating monocytes and the release of other pro-inflammatory mediators. Moreover, both MCP-1 and chemokine (C-C motif) ligand 19 (CCL19) can directly modulate VSMC phenotype, growth, and their production of MMPs. Furthermore, continual apoptosis of VSMCs promotes calcification. All of the above would be expected to precipitate the early stages of atherosclerosis formation, recently coined pathological intimal thickening. The above findings are summarized in Figure 2.

A further pro-atherosclerotic consequence of VSMC foam cell formation and subsequent apoptosis, arises due to their impaired clearance. Particularly within a hyperlipidemic setting, the reduced phagocytosis/efferocytosis of apoptotic VSMCs can result in secondary necrosis, which triggers the release of pro-inflammatory cytokines including MCP-1, IL-1α, and IL-1β from the dying cells and surrounding viable VSMCs. Interestingly, IL-1β can induce VSMC growth, phenotypic switching, MMP production, and facilitate intimal thickening. Accordingly, atherogenesis is reduced in mice with a global deficiency in IL-1β or when treated with a IL-1β neutralizing antibody. These studies would support targeting IL-1β to inhibit the development and progression of atherosclerosis. Indeed, a phase Iib randomized, placebo-controlled trial entitled CANTOS is currently underway to assess the anti-inflammatory and anti-atherosclerotic effects of a monoclonal anti-human IL-1β antibody (canakinumab) in stable but high-risk cardiovascular patients. However, it must be noted that despite its proven anti-inflammatory effects in man, it is possible that blocking VSMC IL-1β signalling in advanced atherosclerotic plaques may exert deleterious effects on fibrous cap stability, such as reduced VSMC growth and phenotype-specific matrix remodelling.

As mentioned earlier, the transition of adaptive intimal thickenings into the earliest stage of atherosclerosis involves the accumulation of modified lipoproteins by intimal VSMCs and their subsequent transition into foam cells, upon which they take on macrophage-like properties and aid disease progression. Accordingly, targeting lipid-laden VSMCs at this early stage represents a therapeutic opportunity to halt disease development and the later formation of advanced clinically relevant plaques. More knowledge is obviously required on the underlying mechanisms of intimal VSMC plasticity and the need for lineage-specific markers when assessing the contribution of foam cells within all stages of atherosclerosis. Indeed from numerous in vitro and in vivo animal studies, the current dogma postulates that resident VSMC that undergo phenotypic switching participate in early atherosclerotic formation and the development of advanced lesions. However, recent cell lineage studies have challenged this tenet, highlighting that in mouse models of atherosclerosis, blood-born cells foster the ability to express markers considered smooth muscle cell-specific, while VSMCs themselves can display proteins associated with macrophages at the expense of their smooth muscle differentiation markers. As such, uncertainty remains as to which cells within atherosclerotic plaques are actually of VSMC origin. Moreover, Tang et al. have suggested that a population of ‘multi-potential vascular stem cells’ propagate the media of the blood vessel wall and harbour the ability to reconstitute both medial cells and foster neointima formation after vascular injury, although this paradigm has been robustly challenged. Taken together, there is a need for refinement and accepted definitive characterization markers of VSMC lineage alongside compelling tracing studies in an array of in vivo cardiovascular models. In the absence of definitive evidence that allows the delineation of VSMCs from other VSMC-like cells within the blood vessel wall and the atherosclerotic plaque, caution should be heeded when interpreting both in vitro and in vivo studies.

4. VSMC and calcification: role of Wnt/β-catenin pathway and galectin-3

Calcification of atherosclerotic plaques is common and further increases with age, and is associated with plaque burden rather than directly with vulnerability. In general, plaque calcification does not correlate with symptomatic plaques, but contrastingly confers stability to plaques. Nonetheless, plaque calcification can represent as two major forms: spotty (also referred to as micro-calcification) and dense (also termed macro- or lamellar calcification), and it has been recently identified that spotty calcification typifies culprit plaques as opposed to stable.
Accordingly, the pattern rather than the total amount of plaque calcification is more informative for plaque risk prediction. Interestingly, spotty calcification is also a hallmark of early atherosclerosis in man, associated with the formation of pathological intimal thickenings. Moreover, a recent fate-mapping study in a mouse atherosclerotic model demonstrated that VSMCs represent the largest number of calcific cells in atherosclerotic lesions. Furthermore and as mentioned earlier, chronic VSMC apoptosis promotes atherosclerotic plaque calcification and progression. Supportingly, insulin-like growth factor-1 (IGF1) enhances VSMC survival and blocks calcification, in part through moderate calcium induction of osteoprotegerin. Smooth muscle foam cell formation can induce apoptosis as alluded to earlier, and has also been associated with VSMC calcification. Goettsch et al. identified an osteogenic role of the nuclear factor of activated T cells (NFAT) signalling pathway in VSMCs exposed to oxLDL, in part through up-regulation of Runx-related transcription factor 2 (RUNX2), which is known to promote VSMC-specific vascular calcification. Intriguingly, NFAT signalling is in part inactivated by Glycogen synthase kinase 3 beta (GSK3β) which has been recently shown to retard VSMC osteoblast differentiation and mineralization. Furthermore, members of the Wnt signalling pathway can modulate NFAT and GSK3β activity, and accordingly Wnt5a promotes VSMC calcification. Additionally, MMPs can modulate Wnt/β-catenin signalling through the cleavage of VSMC cadherins and numerous MMPs have been linked to atherosclerotic plaque calcification. It is therefore conceivable that MMPs, through cadherin-mediated Wnt/β-catenin signalling, induce VSMC calcification and plaque progression, highlighting culpable MMPs and pivotal members of the Wnt/β-catenin signalling pathway as viable therapeutic targets.

Galectin-3, a carbohydrate-binding protein belonging to the galectin gene family [also called by synonyms such as Mac-2, and carbohydrate-binding protein 35 (CBP-35)], mediates cell–cell and cell–matrix interactions and modulates a myriad of biological functions. Galectin-3 cleavage has been proposed as a surrogate marker of MMP activity and its role in atherosclerosis has received abundant attention of late, providing conflicting results. Human and mouse atherosclerotic plaques studies have intimated that galectin-3 promotes plaque progression, primarily through amplification of inflammation and increased foam cell formation. Conversely, it was shown that galectin-3 can also retard plaque progression through down-regulation of pro-inflammatory pathways and removal of modified lipoproteins, partly through decreased expression of the receptor for advanced glycation end-products (RAGE). Interestingly, galectin-3 can also directly act as a receptor and therefore mediate uptake of advanced glycation end-products (AGE); however, in the presence of modified lipoproteins, galectin-3 mediated endocytosis of AGE is reduced due to ligand
competition with modified lipoproteins.\textsuperscript{96} Furthermore, Galectin-3-mediated uptake of AGE enhances VSMC proliferation.\textsuperscript{97} With regard to vascular calcification, Menini \textit{et al.}\textsuperscript{98} have recently demonstrated divergent effects of AGE on the type of calcification formed in atherosclerotic plaques, and therefore plaque phenotype, dependent on ligation of galectin-3 or RAGE. VSMC content and galectin-3 expression was associated with stable lesions exhibiting sheet-like/lamelated macro-calcification, whereas RAGE expression prevailed in unstable plaque with abundant spotty/granular micro-calcification.\textsuperscript{98} The divergent effects on plaque calcification are in part explained by a regulatory role of galectin-3 on RAGE expression (as alluded to above\textsuperscript{99}) and further strengthened by the demonstration that RAGE expression is increased in galectin-3 deficient VSMCs and modulates osteogenic differentiation.\textsuperscript{98} In fact, this novel galectin-3/RAGE dyad in VSMC-mediated atherosclerosis calcification may be mediated by the Wnt/\(\beta\text{-catenin}\) signalling pathway, which we have discussed can control the calcification process, but is also regulated by galectin-3,\textsuperscript{100,101} in part through modulation of GSK-3\(\beta\) activity.\textsuperscript{102} Accordingly, it has been proposed that VSMC galectin-3 ligation (i.e. modified lipoproteins) augments Wnt/\(\beta\text{-catenin}\)-regulated osteoblastogenesis and macro-calcification. In opposition, RAGE ligation (i.e. advanced glycation end-products) favours vascular osteoclastogenesis and micro-calcification, promoting an unstable plaque phenotype.\textsuperscript{98} The above findings are summarized in Figure 3.

There are numerous other regulatory processes which can propagate VSMC-driven calcification within the atherosclerotic plaque, including developmental factors such as bone morphogenetic proteins (BMPs), matrix Gla protein, and the osteoprotegerin/RANKL axis; inflammatory factors including TNF-\(\alpha\), Gas6, Fetuin A, and TGF-\(\beta\); and metabolic factors incorporating oxidant stress and oxidized lipids, hyperphosphataemia, and vitamin D.\textsuperscript{103} However, there remains a paucity of therapeutic approaches to reverse atherosclerotic plaque calcification and, accordingly, this area of cardiovascular disease has received heightened attention. HMG-CoA reductase inhibitors (statins) are currently under investigation, primarily due to their pleiotropic effects, including many of the aforementioned regulatory factors of vascular calcification. Supportingly, simvastatin was demonstrated to reduce intra-plaque calcification within advanced lesions of Apoe-deficient mice.\textsuperscript{104} However, a recent coronary computed tomographic angiography (CCTA) study revealed an association of statin use with an increased prevalence and burden of calcification in patients with coronary artery atherosclerosis.\textsuperscript{105} There is also a body of evidence suggesting an association between MMPs and vascular calcification.\textsuperscript{106} Two independent studies have demonstrated that Apoe-knockout mice which are also deficient for MMP-2\textsuperscript{89} or MMP-12\textsuperscript{42} exhibit reduced atherosclerotic plaque calcification compared with their relevant wild-type controls. Disappointingly, a prospective, randomized, double-blind trial determining the effect of the non-specific MMP inhibitor doxycycline on MMP expression within atherosclerotic carotid plaques, failed to decrease the presence of calcification despite reducing MMP-1 expression.\textsuperscript{107} These findings may imply that a more targeted approach is necessary. Consistent with this, administration of an MMP-12 specific inhibitor to Apoe-deficient mice suppressed atherosclerotic plaque calcification and was associated with promoting a stable plaque phenotype.\textsuperscript{42} As stated previously, MMPs can modulate cadherin mediated cell–cell interactions and also cleave

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{VSMC and calcification—role of galectin-3 and \(\beta\text{-catenin}\). Similar to RAGE, galectin-3 can mediate uptake of advanced glycation end-products (1); however, in the presence of modified lipoproteins, galectin-3-mediated endocytosis of AGE is reduced due to ligand competition with modified lipoprotein (2). Furthermore, galectin-3 endocytosis down-regulates RAGE expression (3). Moreover, VSMC galectin-3 ligation (i.e. modified lipoproteins) augments Wnt/\(\beta\text{-catenin}\) regulated osteoblastogenesis and macro-calcification, which is associated with stable atherosclerotic plaques (4). Conversely, RAGE ligation (i.e. advanced glycation end-products) favours vascular osteoclastogenesis and micro-calcification, promoting an unstable plaque phenotype (5). MMPs such as MMP-12 may indirectly induce micro-calcification through the cleavage of galectin-3 and removing its inhibitory action on RAGE.}
\end{figure}
Consequently, elucidating the underlying regulators of these divergent growth, and transformation into foam cells, may drive atherogenesis and retaining fibrous cap stability. Conversely their phenotypic modulation, regards to maintaining plaque stability they are fundamental through particularly mechanisms driven by VSMCs. The galectin-3/AGE axis provide an esoteric approach to stabilize atherosclerotic lesions, approach to reduce atherosclerotic plaque calcification. Therefore selective MMP inhibition may also serve as salient therapeutic strategies targeting VSMC with regards to preventing atherosclerotic plaque progression and its clinical sequelae.

Conflict of interest: none declared.

References
Emerging regulators of VSMCs in atherosclerosis

459


Wnt signalling in smooth muscle cells and its role in cardiovascular disorders.


