Clinical Science (2019) **133** 1581–1585
https://doi.org/10.1042/CS20190541

PORTLAND PRESS

Commentary

Un-JAMming atherosclerotic arteries: JAM-L as a target to attenuate plaque development

B Sumayyah H Sokeechand and Bernardo L Trigatti

Department of Biochemistry and Biomedical Sciences and Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Ontario, Canada

Correspondence: Bernardo L. Trigatti (trigatt@mcmaster.ca)

Atherosclerosis is a chronic inflammatory disease and a major driver of heart attack and stroke. Atherosclerosis development is driven by the infiltration of leukocytes, including monocytes and neutrophils, among other inflammatory cells into the artery wall, monocyte differentiation to macrophages and uptake of oxidized low density lipoprotein. Macrophage activation and inflammatory cytokine production are major factors which drive ongoing inflammation and plaque development. Identification of novel pathways driving this on-going inflammatory process may provide new opportunities for therapeutic intervention. In their article published in Clinical Science (2019) (vol 133, 1215–1228), Sun and colleagues demonstrate a novel role for the junction adhesion molecule-like (JAML) protein in driving on-going atherosclerotic plaque inflammation and plaque development. They report that JAML is expressed in macrophages and other cells in atherosclerotic plaques in both humans and mice, and that silencing JAML expression attenuates atherosclerotic plaque progression in mouse models of early and late stage plaque development. They demonstrate that JAML is required for oxidized-low density lipoprotein (OxLDL)-induced up-regulation of inflammatory cytokine production by macrophages, pointing to it as a potential therapeutic target for reducing ongoing plaque inflammation.

Atherosclerosis is a major cause of heart attack and stroke and is a major contributor to death globally [1,2]. Atherosclerosis is a chronic inflammatory disease involving the accumulation of low density lipoprotein (LDL) particles, monocyte-derived macrophages and other inflammatory cells including neutrophils in the arterial wall (see Figure 1). LDL that gains entry and is retained in the artery wall is subject to chemical modification including oxidation, and oxidized-LDL (OxLDL) is among many triggers of endothelial cell activation leading to up-regulation of adhesion molecules that facilitate the interactions of inflammatory/immune cells and their extravasation into the artery wall, driving atherosclerotic plaque growth [3]. Leukocyte recruitment to and migration across endothelial cells in the vascular wall at sites of inflammation is a complex process involving multiple steps and protein–protein interactions between endothelial cell surface molecules and cognate receptors on leukocytes (reviewed in [4,5]). Within the artery wall monocytes differentiate into macrophages and take up OxLDL to form lipid-laden foam cells which drive atherosclerosis progression (reviewed in [6,7]). Neutrophils also participate at all stages of atherosclerosis development in promoting plaque growth. This includes assisting in monocyte recruitment to plaques, secretion of myeoleroxidase leading to oxidation of LDL within plaques, and promotion of inflammation via diverse pathways including release of neutrophil extracellular traps (see [8,9] for recent reviews). These processes take place throughout the development of the atherosclerotic plaque, from early stages, in which it is mainly comprised of monocyte-derived macrophages, and is referred to as a fatty streak, to advanced stages in which it is comprised of a variety of other immune cells, including neutrophils, in addition to macrophages [6,7,9]. In advanced lesions, foam cells undergo apoptosis but are not efficiently cleared, resulting in the deposition of cellular debris, including extracellular cholesterol esters and phospholipids, forming a lipid rich necrotic core [10–12]. OxLDL and other stimuli within plaques trigger cytokine production by plaque macrophages, which drives continued recruitment of immune and

Received: 20 June 2019
Revised: 20 June 2019
Accepted: 02 July 2019

Version of Record published:
22 July 2019

Clinical Science (2019) **133** 1581–1585
https://doi.org/10.1042/CS20190541

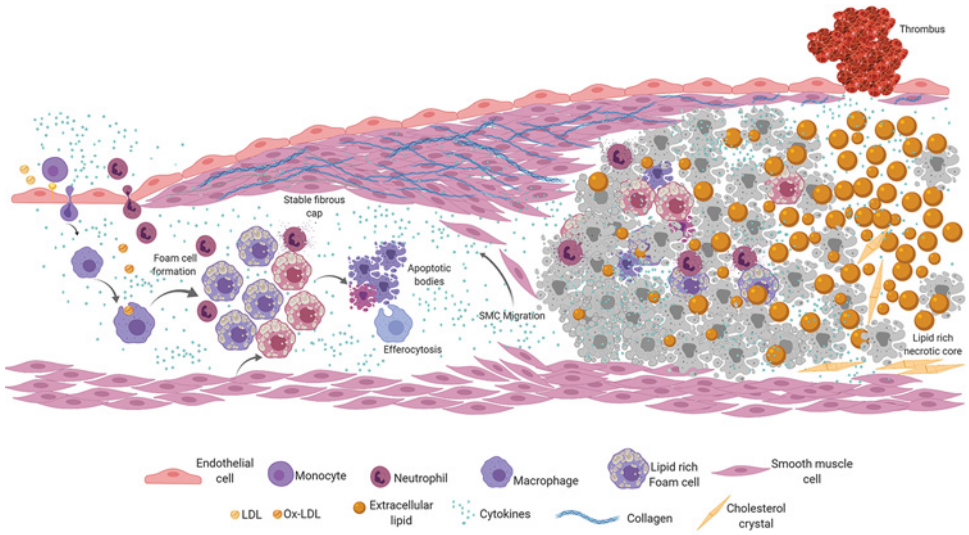

Figure 1. Development and progression of atherosclerotic plaques

Sub-endothelial accumulation and oxidation of LDL particles lead to activation of endothelial cells and recruitment of inflammatory cells including monocytes and neutrophils to the site of lesion. Monocytes differentiate into macrophages and take up OxLDL to form the lipid-rich foam cells (left). OxLDL-driven cytokine production induces smooth muscle cells migration into the intima. Smooth muscle cells secrete collagen forming a stable fibrous cap (middle). In advanced plaques, apoptotic bodies are not cleared effectively leading to the formation of cellular debris, which together with extracellular cholesterol esters, cholesterol crystals and other lipids form a lipid rich necrotic core. Plaques with large necrotic cores, abundant macrophages and thin fibrous caps with little collagen content and collagen degradation are prone to rupture resulting in formation of a thrombus (right). The figure was created using BioRender.com.

inflammatory cells including monocytes and neutrophils, and promote proliferation of smooth muscle cells and their migration into the intima [13]. Smooth muscle cells produce collagen to form a fibrous cap [14,15] while macrophages secrete proteases which degrade collagen, thinning the fibrous cap [16–18]. Atherosclerotic plaques with thin fibrous caps, poor in collagen and smooth muscle cells and rich in inflammatory cells including macrophages and containing large necrotic cores are prone to rupture [12]. Atherosclerotic plaque rupture leads to thrombosis and occlusion of the remaining lumen of the artery, leading to myocardial infarction or stroke [12] (see Figure 1).

Although atherosclerosis has been recognized for over two decades as a chronic inflammatory disease [3], conventional therapeutic interventions to prevent atherosclerosis are largely aimed at alleviating hyperlipidemia. These include statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) and proprotein convertase subtillisin kexin type 9 (PCSK9) inhibitory antibodies, which up-regulate LDL receptor gene expression and reduce LDL receptor protein degradation, respectively [19]. More recent findings, however, have demonstrated the therapeutic benefit of targeting specific inflammatory pathways, such as those mediated by interleukin 1β in humans [20,21]. The identification of additional inflammatory pathways that could be targeted to protect against atherosclerosis development would be beneficial.

In a recent article published in Clinical Science, Sun et al. [22] provide preclinical data that implicates one such target. They report that silencing the expression of junctional adhesion molecule-like (JAML) protein protects against atherosclerosis development. JAML shares structural features with junctional adhesion molecule (JAM) family members, which are immunoglobulin superfamily proteins that are expressed at cell junctions on epithelial and endothelial cells and in leukocytes and platelets [23,24]. They have been associated with leukocyte adhesion to and transmigration across the endothelium [23,24]. Two other members of this family, JAM-A and JAM-C have been associated with monocyte infiltration and acceleration of lesion formation in atherosclerosis-prone mice [25,26]. JAML was reported to be present in both monocytes and neutrophils, to be highly expressed under inflammatory conditions and to play crucial roles in monocyte and neutrophil adhesion and trans-endothelial migration [23,27–30]. Furthermore, the gene encoding JAML (adhesion molecule, interacts with CXADR antigen 1 [AMICA1]) has been implicated as part of a microRNA regulated network, up-regulated in blood cells from patients with myocardial infarction [31]. Despite this, the role of JAML in atherosclerosis had not previously been examined.

In their study, reported in *Clinical Science*, Sun et al. [22] first demonstrated that JAML is expressed in atherosclerotic plaques both from humans and from apolipoprotein E (apoE) deficient mice, in the latter case with increased expression upon high fat diet feeding. In human atherosclerotic plaques, JAML protein was present in macrophages, smooth muscle and endothelial cells, and in cultured cells, JAML protein levels were highest in murine macrophages and increased upon incubation with OxLDL [22].

To examine the role of JAML in atherosclerosis, Sun et al. [22] treated apoE deficient mice with a JAML-targeting, short hairpin ribonucleic acid (shRNA), which they showed reduced JAML protein levels in the aorta and carotid arteries of treated mice [22]. They analyzed two models of atherosclerosis to examine effects of JAML silencing on early and late stages of plaque development. To examine relatively early stages of plaque development, Sun et al. fed apoE deficient mice a high fat diet for 6 weeks before using a lentivirus to deliver either a JAML-silencing shRNA or a control shRNA that left JAML expression intact. Mice were maintained on the high fat diet for a further 4 weeks before analysis. Importantly, Sun et al. [22] also analyzed a cohort of mice fed the high fat diet for 6 weeks to establish the average baseline level of atherosclerosis corresponding to the time their intervention began. While atherosclerotic plaques more than doubled in average size between 6 and 10 weeks of high fat diet feeding in the control treated mice, average atherosclerotic plaque growth was held to a 40–50% increase in the mice in which JAML expression was silenced [22]. To determine the effects of JAML silencing on advanced atherosclerotic plaques, Sun et al. [22] used a model system in which apoE-/- mice fed a high fat diet underwent surgery to have a restrictive collar placed around their right common carotid artery (RCCA) [22]. This narrows the artery, triggering hemodynamic stress which, together with the high fat diet, trigger atherosclerotic plaque development in the affected carotid artery [32,33]. 10 weeks into the high fat diet feeding, the restrictive collars were removed and JAML shRNA or control scrambled shRNA were infused into the RCCA [22]. Mice were maintained on the high fat diet for another 4 weeks prior to analysis of atherosclerotic plaques in the RCCA. As for their early atherosclerosis study, the present study included a group of mice harvested and analyzed at a time corresponding to when the JAML shRNA treatment was initiated to establish a baseline level of atherosclerosis to better determine the effects of JAML silencing. In this advanced atherosclerosis model, the plaques that developed in the RCCA virtually completely occluded the artery and did not appear to be different in size. However, Sun and co-workers found that necrotic cores grew from approximately 7.5 to 23% of the plaque area in the control mice treated with scrambled shRNA whereas necrotic cores grew from 7.5% only to approximately12% of the total plaque area in the mice that received the JAML silencing shRNA. Furthermore in both the early and advanced atherosclerosis studies, JAML silencing led to reduced plaque lipid and macrophage content and increased content of smooth muscle cells and collagen, considered to be markers of plaque stabilization from rupture. Therefore, in both the early and advanced atherosclerosis models, JAML silencing dramatically attenuated plaque development [22].

Sun and colleagues also demonstrated that aortic plaques from JAML-treated apoE-/- mice exhibited lower abundance of neutrophils in addition to the reduced macrophage content, and that there were no effects of JAML silencing on macrophage proliferation either in culture or in aortic atherosclerotic plaques in apoE-/- mice suggesting that JAML silencing may have reduced monocyte and possibly neutrophil recruitment into plaques [22], consistent with JAML's known role mediating leukocyte trans-endothelial migration [23,27–30]; however, they did not test this directly.

Intriguingly, Sun and co-workers, also detected lower levels of the inflammatory cytokines tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) in plaques from mice in which JAML was silenced [22]. To examine the mechanism behind this, they examined the effects of JAML silencing on OxLDL-induced cytokine production by mouse macrophages in culture. They showed that JAML silencing impaired OxLDL-mediated TNFα and IL-6 production by macrophages by reducing the phosphorylation of the p65 subunit of the NF-κB transcription factor, inactivating this pathway. They further demonstrated that JAML silencing reduced OxLDL-induction of the mitogen activated protein (MAP) kinase, extracellular signal regulated kinase (ERK)1/2, but not the OxLDL-induction of the MAP kinases p38 or Jun N-terminal kinase (JNK). Prior studies have reported OxLDL signaling mediated by a variety of receptors, of which the scavenger receptor cluster of differentiation 36 (CD36) is among the most well-studied [34–37]. OxLDL signaling via CD36 has been reported to trigger p38 MAP kinase and JNK but not ERK1/2 phosphorylation [34]. The finding that JAML silencing impaired OxLDL-mediated ERK1/2 but not p38 or JNK phosphorylation, suggests that CD36-mediated OxLDL signaling was likely not affected by JAML silencing. Other cell surface molecules reportedly involved in OxLDL signaling include the lectin-like oxidized LDL receptor-1 (LOX-1) and the focal adhesion kinase (FAK) [35–37]. OxLDL-mediated signaling via LOX-1 and FAK has been reported to involve both ERK1/2 and NF-κB activation [35–37] and both LOX-1 and FAK are reportedly expressed in macrophages [38,39], raising the possibility that JAML could be involved in OxLDL signaling via these pathways. If and how JAML silencing may impair OxLDL signaling via these or other receptors remains to be determined. One possible mechanism may include a role for JAML

Clinical Science (2019) **133** 1581–1585
https://doi.org/10.1042/CS20190541

in inducing or maintaining expression of the key receptors (ruled out by Sun et al. [22] for CD36, but not tested for LOX-1 or FAK). Another possibility is a more direct role for JAML in the signaling pathway; for example, JAML is known to form complexes with other cell surface receptors such as the coxsackie and adenovirus receptor (CAR) and to directly participate in signaling by recruitment of phosphatidyl-inositol 3 kinase (PI3K) to its cytoplasmic domain [40]. PI3K initiates signaling via protein kinase B (AKT) and in some cell types cross-talk between the PI3K/AKT pathway and the ERK1/2 pathway has been reported [41]. This raises the intriguing possibility that JAML could form part of the OxLDL signaling complex. This, however, remains to be tested directly.

A number of open questions remain. What, if any, is the contribution of reduced monocyte and/or neutrophil recruitment to the attenuated plaque development as a result of JAML silencing? What are the mechanisms by which JAML silencing reduces OxLDL-mediated signaling leading to cytokine production? Can inhibition of JAML function recapitulate the effects of JAML silencing in attenuating atherosclerosis progression and will the same effects be seen in human disease?

Regardless of the mechanism involved, the study by Sun et al. [22] demonstrates a heretofore unappreciated role of JAML in regulating OxLDL-mediated signaling in macrophages leading to NF-κB activation and inflammatory cytokine production and secretion. This, together with the previously identified role for JAML in mediating recruitment of monocytes and neutrophils, suggests that JAML controls a number of key inflammatory processes driving plaque progression. The findings of Sun and co-workers provide important proof of principle in a pre-clinical model system that JAML may represent an attractive therapeutic target for slowing the development and promoting the stabilization of atherosclerotic plaques.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AKT, protein kinase B; apoE, apolipoprotein E; CD36, cluster of differentiation 36; ERK, extracellular signal regulated kinase; FAK, focal adhesion kinase; IL-6, interleukin 6; JAM, junctional adhesion molecule; JAML, junction adhesion molecule-like; JNK, Jun N-terminal Kinase; LDL, low-density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; MAP, mitogen activated protein; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cell; OxLDL, oxidized LDL; PI3K, phosphatidyl-inositol 3 kinase; RCCA, right common carotid artery; RNA, ribonucleic acid; shRNA, short hairpin RNA; TNFα, tumor necrosis factor α.

References

1 National Institutes of Health (2012) *Morbidity & Mortality: 2012 Chart Book on Cardiovascular, Lung, and Blood Diseases*, p. 116, National Institutes of Health, Bethesda
2 World Health Organization ((2017)) Cardiovascular diseases (CVDs). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
3 Ross, R. (1999) Atherosclerosis – an inflammatory disease. *N. Engl. J. Med.* **340**, 115–126, https://doi.org/10.1056/NEJM199901143400207
4 Ley, K., Laudanna, C., Cybulsky, M.I. and Nourshargh, S. (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* **7**, 678–689, https://doi.org/10.1038/nri2156
5 Schnoor, M., Alcaide, P., Voisin, M.-B. and van Buul, J.D. (2015) Crossing the vascular wall: common and unique mechanisms exploited by different leukocyte subsets during extravasation. *Mediators Inflamm.* **2015**, 946509, https://doi.org/10.1155/2015/946509
6 Moore, K.J., Sheedy, F.J. and Fisher, E.A. (2013) Macrophages in atherosclerosis: a dynamic balance. *Nat. Rev. Immunol.* **13**, 709–721, https://doi.org/10.1038/nri3520
7 Cybulsky, M.I., Cheong, C. and Robbins, C.S. (2016) Macrophages and dendritic cells. *Circ. Res.* **118**, 637–652, https://doi.org/10.1161/CIRCRESAHA.115.306542
8 Döring, Y., Soehnlein, O. and Weber, C. (2017) Neutrophil extracellular traps in atherosclerosis and atherothrombosis. *Circ. Res.* **120**, 736–743, https://doi.org/10.1161/CIRCRESAHA.116.309692
9 Döring, Y., Drechsler, M., Soehnlein, O. and Weber, C. (2015) Neutrophils in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **35**, 288–295, https://doi.org/10.1161/ATVBAHA.114.303564
10 Andrés, V., Pello, O.M. and Silvestre-Roig, C. (2012) Macrophage proliferation and apoptosis in atherosclerosis. *Curr. Opin. Lipidol.* **23**, 429–438, https://doi.org/10.1097/MOL.0b013e328357a379
11 Tabas, I. (2007) Apoptosis and efferocytosis in mouse models of atherosclerosis. *Curr. Drug Targets* **8**, 1288–1296, https://doi.org/10.2174/138945007783220623
12 Gonzalez, L. and Trigatti, B.L. (2017) Macrophage apoptosis and necrotic core development in atherosclerosis: a rapidly advancing field with clinical relevance to imaging and therapy. *Can. J. Cardiol.* **33**, 303–312, https://doi.org/10.1016/j.cjca.2016.12.010
13 Loppnow, H., Werdan, K. and Buerke, M. (2008) Invited review: vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. *Innate. Immun.* **14**, 63–87, https://doi.org/10.1177/1753425908091246
14 Raines, E.W. and Ferri, N. (2005) Thematic review series: the immune system and atherogenesis. Cytokines affecting endothelial and smooth muscle cells in vascular disease. *J. Lipid Res.* **46**, 1081–1092, https://doi.org/10.1194/jlr.R500004-JLR200

15 Bennett, M.R., Sinha, S. and Owens, G.K. (2016) Vascular smooth muscle cells in atherosclerosis. *Circ. Res.* **118**, 692–702, https://doi.org/10.1161/CIRCRESAHA.115.306361
16 Wu, H., Du, Q., Dai, Q., Ge, J. and Cheng, X. (2018) Cysteine protease cathepsins in atherosclerotic cardiovascular diseases. *J. Atheroscler. Thromb.* **25**, 111–123, https://doi.org/10.5551/jat.RV17016
17 Miyazaki, T. and Miyazaki, A. (2018) Dysregulation of calpain proteolytic systems underlies degenerative vascular disorders. *J. Atheroscler. Thromb.* **25**, 1–15, https://doi.org/10.5551/jat.RV17008
18 Newby, A.C. (2015) Metalloproteinases promote plaque rupture and myocardial infarction: a persuasive concept waiting for clinical translation. *Matrix Biol.* **44–46**, 157–166, https://doi.org/10.1016/j.matbio.2015.01.015
19 Gupta, K.K., Ali, S. and Sanghera, R.S. (2019) Pharmacological options in atherosclerosis: a review of the existing evidence. *Cardiol. Ther.* **8**, 5–20, https://doi.org/10.1007/s40119-018-0123-0
20 Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C. et al. (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.* **377**, 1119–1131, https://doi.org/10.1056/NEJMoa1707914
21 Ridker, P.M. (2019) Anti-inflammatory therapy for atherosclerosis: interpreting divergent results from the CANTOS and CIRT clinical trials. *J. Intern. Med.* **285**, 503–509, https://doi.org/10.1111/joim.12862
22 Sun, Y., Guan, J., Hou, Y., Xue, F., Huang, W., Zhang, W. et al. (2019) Silencing of junctional adhesion molecule-like protein attenuates atherogenesis and enhances plaque stability in ApoE-/- mice. *Clin. Sci.* **133**, 1215–1228, https://doi.org/10.1042/CS20180561
23 Moog-Lutz, C., Cavé-Riant, F., Guibal, F.C., Breau, M.A., Di Gioia, Y., Couraud, P.O. et al. (2003) JAML, a novel protein with characteristics of a junctional adhesion molecule, is induced during differentiation of myeloid leukemia cells. *Blood* **102**, 3371–3378, https://doi.org/10.1182/blood-2002-11-3462
24 Mandell, K.J. and Parkos, C.A. (2005) The JAM family of proteins. *Adv. Drug Deliv. Rev.* **57**, 857–867, https://doi.org/10.1016/j.addr.2005.01.005
25 Zernecke, A., Liehn, E.A., Fraemohs, L., von Hundelshausen, P., Koenen, R.R., Corada, M. et al. (2006) Importance of junctional adhesion molecule-A for neointimal lesion formation and infiltration in atherosclerosis-prone mice. *Arterioscler. Thromb. Vasc. Biol.* **26**, e10–e13, https://doi.org/10.1161/01.ATV.0000197852.24529.4f
26 Shagdarsuren, E., Djalali-Talab, Y., Aurrand-Lions, M., Bidzhekov, K., Liehn, E.A., Imhof, B.A. et al. (2009) Importance of junctional adhesion molecule-C for neointimal hyperplasia and monocyte recruitment in atherosclerosis-prone mice-brief report. *Arterioscler. Thromb. Vasc. Biol.* **29**, 1161–1163, https://doi.org/10.1161/ATVBAHA.109.187898
27 Zen, K., Liu, Y., McCall, I.C., Wu, T., Lee, W., Babbin, B.A. et al. (2005) Neutrophil migration across tight junctions is mediated by adhesive interactions between epithelial coxsackie and adenovirus receptor and a junctional adhesion molecule-like protein on neutrophils. *Mol. Biol. Cell* **16**, 2694–2703, https://doi.org/10.1091/mbc.e05-01-0036
28 Guo, Y.-L., Bai, R., Chen, C.X.-J., Liu, D.-Q., Liu, Y., Zhang, C.-Y. et al. (2009) Role of junctional adhesion molecule-like protein in mediating monocyte transendothelial migration. *Arterioscler. Thromb. Vasc. Biol.* **29**, 75–83, https://doi.org/10.1161/ATVBAHA.108.177717
29 Weber, D.A., Sumagin, R., McCall, I.C., Leoni, G., Neumann, P.A., Andargachew, R. et al. (2014) Neutrophil-derived JAML inhibits repair of intestinal epithelial injury during acute inflammation. *Mucosal. Immunol.* **7**, 1221–1232, https://doi.org/10.1038/mi.2014.12
30 Alvarez, J.I., Kébir, H., Cheslow, L., Chabarati, M., Larochelle, C. and Prat, A. (2015) JAML mediates monocyte and CD8 T cell migration across the brain endothelium. *Ann. Clin. Transl. Neurol.* **2**, 1032–1037, https://doi.org/10.1002/acn3.255
31 Wu, K., Zhao, Q., Li, Z., Li, N., Xiao, Q., Li, X. et al. (2018) Bioinformatic screening for key miRNAs and genes associated with myocardial infarction. *FEBS Open Bio* **8**, 897–913, https://doi.org/10.1002/2211-5463.12423
32 von der Thüsen, J.H., van Berkel, T.J. and Biessen, E.A. (2001) Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. *Circulation* **103**, 1164–1170, https://doi.org/10.1161/01.CIR.103.8.1164
33 Zabelaara, A., Thusen, J., Mboesten, L., Hoeben, R., Kockx, M., Versnel, M. et al. (2005) Increased vulnerability of pre-existing atherosclerosis in ApoE-deficient mice following adenovirus-mediated Fas ligand gene transfer. *Atherosclerosis* **183**, 244–250, https://doi.org/10.1016/j.atherosclerosis.2005.03.044
34 Rahaman, S.O., Lennon, D.J., Febbraio, M., Podrez, E.A., Hazen, S.L. and Silverstein, R.L. (2006) A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. *Cell Metab.* **4**, 211–221, https://doi.org/10.1016/j.cmet.2006.06.007
35 Yang, T.-C., Chang, P.-Y. and Lu, S.-C. (2017) L5-LDL from ST-elevation myocardial infarction patients induces IL-1β production via LOX-1 and NLRP3 inflammasome activation in macrophages. *Am. J. Physiol. Heart Circ. Physiol.* **312**, H265–H274, https://doi.org/10.1152/ajpheart.00509.2016
36 Yang, T.-C., Chang, P.-Y., Kuo, T.-L. and Lu, S.-C. (2017) Electronegative L5-LDL induces the production of G-CSF and GM-CSF in human macrophages through LOX-1 involving NF-κB and ERK2 activation. *Atherosclerosis* **267**, 1–9, https://doi.org/10.1016/j.atherosclerosis.2017.10.016
37 Yurdagul, A., Sulzmaier, F.J., Chen, X.L., Pattillo, C.B., Schlaepfer, D.D. and Orr, A.W. (2016) Oxidized LDL induces FAK-dependent RSK signaling to drive NF-κB activation and VCAM-1 expression. *J. Cell Sci.* **129**, 1580–1591, https://doi.org/10.1242/jcs.182097
38 Kume, A., Nishiura, H., Suda, J. and Suda, T. (1997) Focal adhesion kinase upregulated by granulocyte-macrophage colony-stimulating factor but not by interleukin-3 in differentiating myeloid cells. *Blood* **89**, 3434–3442
39 Kume, N., Moriwaki, H., Kataoka, H., Minami, M., Murase, T., Sawamura, T. et al. (2006) Inducible expression of LOX-1, a novel receptor for oxidized LDL, in macrophages and vascular smooth muscle cells. *Ann. N.Y. Acad. Sci.* **902**, 323–327, https://doi.org/10.1111/j.1749-6632.2000.tb06332.x
40 Verdino, P., Witherden, D.A., Havran, W.L. and Wilson, I.A. (2010) The molecular interaction of CAR and JAML recruits the central cell signal transducer PI3K. *Science* **329**, 1210–1214, https://doi.org/10.1126/science.1187996
41 Niba, E.T.E., Nagaya, H., Kanno, T., Tsuchiya, A., Gotoh, A., Tabata, C. et al. (2013) Crosstalk between PI3 kinase/PDK1/Akt/Rac1 and Ras/Raf/MEK/ERK pathways downstream PDGF receptor. *Cell. Physiol. Biochem.* **31**, 905–913, https://doi.org/10.1159/000350108