

# Commentary

## Endothelial Caveolae and Caveolin-1 as Key Regulators of Atherosclerosis

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### *Caveolin-1 and Caveolae*

The term caveolae was defined more than fifty years ago by Yamada as a “vesicle, cave, or recess communicating with the outside of a cell, and extending inward, indenting the cytoplasm and the cell membrane.”<sup>1</sup> A few years earlier, Palade had identified the same structures as plasmalemmal vesicles.<sup>2</sup> Indeed, Palade and collaborators were the first to propose a function for these structures in the transport of macromolecules from the lumen of blood vessels to peripheral tissues.<sup>3,4</sup> These studies suggested a role for caveolae in the development of atherosclerosis.

The main protein component of caveolae was later identified as caveolin-1,<sup>5</sup> which was found to be necessary for caveolae formation. However, until recently, very little work had been performed to examine the importance of caveolin-1 in the development of atherosclerosis. Given the relative cholesterol enrichment of caveolae compared with the rest of the plasma membrane, a few studies have proposed that caveolae and caveolin-1 might be involved in the regulation of intracellular cholesterol homeostasis. Moreover, when caveolin-1 expression was examined in numerous organs and cell types, it was found that caveolin-1 was highly expressed in terminally-differentiated cells, with endothelial cells and adipocytes expressing the highest levels.

Atherosclerosis is a disease of the blood vessel characterized by the presence of lesions formed by the initial buildup of cholesterol-enriched lipoproteins followed by the accumulation of macrophages and smooth muscle cells. Interestingly, endothelial cells, macrophages, and smooth muscle cells, which are involved in the progression of this disease, all express caveolin-1 and display caveolae, as observed by electron microscopy. In a previous study using caveolin-1-deficient mice, we have shown that a complete caveolin-1 deficiency is associated with a major reduction in the development of atherosclerosis.<sup>6</sup> These findings have independently been confirmed by others.<sup>7</sup> However, the role of caveolin-1 in the

development of atherosclerosis is highly dependent on the cell type in which this protein is expressed.<sup>8</sup>

### *Evidence for a Role of Caveolin-1/Caveolae in Atherosclerosis*

Caveolin-1 is a 178-aa plasma membrane protein with a unique conformation. It adopts a hairpin-like structure with both N and C termini in the cytoplasm, and its central domain is included in the plasma membrane. Numerous studies have shown that caveolin-1 can regulate cellular signaling pathways in various cell types. Previous work has identified a domain—termed the caveolin-scaffolding domain—responsible for the protein’s ability to regulate signal transduction. This region of caveolin-1 (residues 82-101) can bind signaling molecules such as EGF-R or other proteins like endothelial nitric oxide synthase (eNOS) and maintain them in an inactive state.<sup>9</sup>

Because caveolin-1 is particularly abundant in endothelial cells, it is believed to have a critical function in this cell type. Initial studies have indicated that endothelial caveolin-1 may act as a proatherogenic protein.<sup>8</sup> The basis for this assertion came from the postulated role of caveolae in the transcytosis of low-density lipoprotein (LDL) particles.<sup>10</sup> In addition, several putative atherogenic proteins colocalize with caveolae in endothelial cells. Some of these proteins are involved in lipoprotein metabolism (SR-BI, CD36, RAGE), cellular proliferation and migration (PDGF-R, VEGF-R), inflammation (TNFR), or the regulation of various signaling pathways (Src, G proteins).<sup>11,12</sup> Taken together, these data suggest that caveolae and caveolin-1 must play an important role in the regulation of endothelial function and in the development of atherosclerosis.

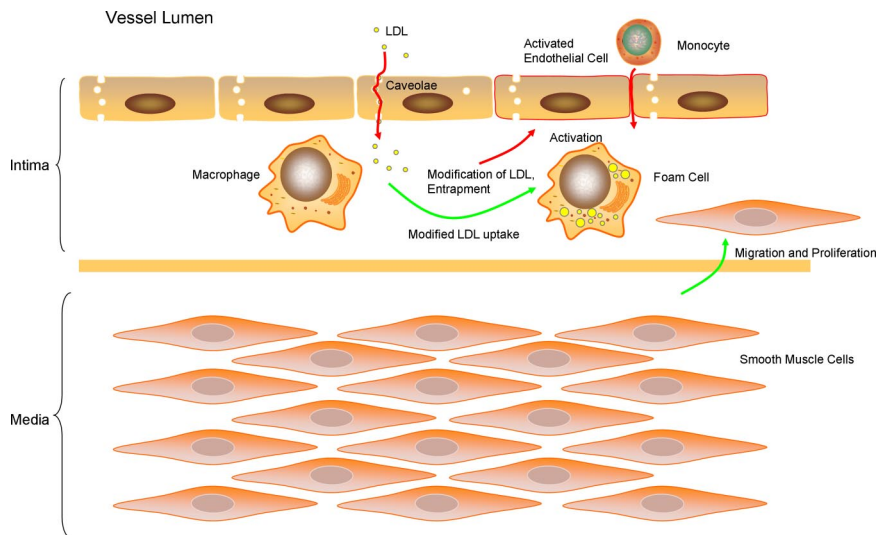
Macrophage involvement in the development of atherosclerosis has been well established. Important studies have demonstrated macrophage uptake of modified lipoproteins at the sites of inflammation. If not properly controlled, mac-

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**Figure 1.** Identification of the role of caveolin-1/caveolae in atherosclerosis. In this figure, the beneficial roles of caveolin-1 in atherosclerosis have been highlighted with **green arrows**. They are primarily limited to functions affecting macrophages (cholesterol metabolism and inflammation) and smooth muscle cells (inhibition of migration and proliferation). While caveolin-1 may have beneficial functions in these cell types, its effect is detrimental to endothelial cell function during atherogenesis (**red arrows**). Endothelial caveolin-1 may promote the transcytosis of LDL into the arterial intima, the activation of endothelial cells, and the subsequent migration of monocytes into the subendothelial space.

rophages are thought to further amplify the inflammation process and therefore aggravate the atherosclerotic development of the plaque. Recent studies have indicated that caveolin-1 could regulate the inflammation process in macrophages.<sup>13</sup> Moreover, its function in the regulation of cellular cholesterol homeostasis may be important to limit foam cell formation.<sup>14</sup> The proper regulation of this pathway is especially important during cholesterol accumulation in macrophages because it escalates the inflammatory response in atherosclerotic lesions.

Caveolin-1 has also been shown to control vascular smooth muscle function. In particular, it has been shown that elimination of vascular smooth muscle caveolin-1 is associated with increased cellular migration and proliferation.<sup>15</sup> *In vivo* studies have confirmed these findings using a mouse model of arterial restenosis. In these cases, it has been shown that the absence of caveolin-1 can increase neointimal formation after carotid artery blood-flow cessation.<sup>15</sup> Consistent with these findings, previous investigations have shown that caveolin-1 expression is decreased in neointimal smooth muscle cells present in atherosclerotic lesions.<sup>16</sup>

Taken together, these data suggest that while endothelial caveolin-1 may be proatherogenic, macrophage and smooth muscle cell caveolin-1 may play an inhibitory role in the development of atherosclerosis (Figure 1).

### **An Essential Role for Endothelial Caveolin-1 in the Development of Atherosclerosis**

In their study published in the current issue of *The American Journal of Pathology*, Fernández-Hernando et al<sup>17</sup> have demonstrated the important if not essential function of caveolin-1 in the development of atherosclerosis. Their data confirmed previous findings they had obtained using caveolin-1-deficient mice that were bred with mice overexpressing caveolin-1 specifically in endothelial cells. However, the current study extends these findings in the *apoE*<sup>-/-</sup> mouse background.

Fernández-Hernando et al show that increased caveolin-1 expression in endothelial cells alone is associated with increased atherosclerosis in this mouse model.

Two important roles for caveolin-1 and caveolae have been proposed during the development of atherosclerosis: inflammation and LDL transfer into the intima. These findings clearly highlight the importance of the endothelium in the development of atherosclerosis. As previously suggested, caveolae/caveolin-1 may be necessary for the transfer of LDL in the intima, where lipoproteins are retained and modified.<sup>8</sup> Caveolin-1 may also control the modification of lipoproteins, because the production of reactive oxygen subspecies has already been shown to be regulated by caveolin-1.<sup>18</sup> However, at this point, we can only speculate as to whether caveolin-1 is involved in the retention of lipoproteins in the subendothelial space.

During the inflammatory process, endothelial vascular cell adhesion molecule 1 (VCAM-1) is thought to allow the adhesion of monocytes to the activated endothelium. After adhesion to the endothelium, monocytes migrate into the subendothelial space where they eventually differentiate into macrophages. As previously suggested,<sup>6,7</sup> Fernández-Hernando et al also show that increased caveolin-1 is responsible for an increased expression of VCAM-1, which may be a direct consequence of the reduced NO production observed in endothelial cells overexpressing caveolin-1. Caveolin-1 expression in endothelial cells may also regulate inflammation in the vasculature via other proinflammatory molecules, such as TNF- $\alpha$ , by regulating their receptor expression.<sup>19,20</sup>

Finally, Fernández-Hernando et al also show reduced proliferative and migrating properties of caveolin-1 overexpressing endothelial cells. The latter findings may be critical for a better understanding of the disease. Caveolin-1 overexpression may reduce the capacity of endothelial cells to regenerate an intact lining of the vessel wall. Therefore, the proliferative and migrating ability of endothelial cells may limit lipid and lipoprotein accumulation at lesion sites.

## Conclusions

Importantly, this study may allow for the investigation of new drugs that specifically target caveolin-1 in endothelial cells. In this regard, Brouet et al<sup>21</sup> have shown that endothelial cell exposure to atorvastatin can decrease caveolin-1 expression in macrovascular but not microvascular cells. Conversely, exposure of macrophages to atorvastatin is associated with increased caveolin-1 expression.<sup>22</sup> However, these effects may need to be confirmed in whole organisms. Moreover, results obtained in animal models may not be reproducible in humans. For example, recent studies by Patel et al<sup>23</sup> have indicated that idiopathic hypertension (IPAH) in mice and humans may have different etiologies and that caveolin-1 expression levels may have different implications in these two systems. In this disease, pulmonary artery smooth muscle cells have been shown to play an essential role in the development of this illness. In their study, Patel et al have shown that increased smooth muscle caveolin-1 expression is observed in pulmonary arteries from patients with IPAH. However, very different observations have been made in caveolin-1-deficient mice and in a rat model for pulmonary hypertension, where it has been shown that caveolin-1 is protective against the development of the disease.<sup>24,25</sup>

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