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Cavin-1: caveolae-dependent signalling and cardiovascular disease

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Keywords: Cavin-1, caveolae, inflammation, signal transduction, cyclic AMP

Abbreviations: MCD: methyl- β -cyclodextrin, STAT: signal transducers and activators of transcription, SOCS: suppressor of cytokine signalling.

Abstract

Caveolae are curved lipid raft regions rich in cholesterol and sphingolipids found abundantly in vascular endothelial cells, adipocytes, smooth muscle cells, and fibroblasts. They are multifunctional organelles with roles in clathrin-independent endocytosis, cholesterol transport, mechanosensing, and signal transduction. Caveolae provide an environment where multiple receptor signalling components are sequestered, clustered, and compartmentalised for efficient signal transduction. Many of these receptors, including cytokine signal transducer gp130, are mediators of chronic inflammation during atherogenesis. Subsequently, disruption of these organelles is associated with a broad-range of disease states including cardiovascular disease and cancer. Cavin-1 is an essential peripheral component of caveolae that stabilises caveolin-1, the main structural/integral membrane protein of caveolae. Caveolin-1 is an essential regulator of endothelial nitric oxide synthase (eNOS) and its disruption leads to endothelial dysfunction which initiates a range of cardiovascular and pulmonary disorders. While dysfunctional cytokine signalling is also a hallmark of cardiovascular disease, knowledge of caveolae-dependent cytokine signalling is lacking as is the role of cavin-1 independent of caveolae. This review will introduce caveolae, its structural components, the caveolins and cavins, their regulation by cAMP, and their potential role in cardiovascular disease.

Caveolae

Initially described in 1953 [1] using electron microscopy, caveolae are 50-100nm cup/omega-shaped [2] stable lipid raft regions found abundantly in vascular endothelial cells, adipocytes, smooth muscle cells, and fibroblasts. Caveolae account for 50% of the surface area of adipocytes and around 20% of the surface area of continuous microvascular endothelium [3]. These multifunctional organelles have roles in endocytosis, cholesterol homeostasis, mechanosensing, and signal transduction, as reviewed by Razani et al [4]. Caveolae provide a cholesterol- and sphingolipid-rich environment that sequesters and compartmentalises multiple receptor and non-receptor signalling components such as cytokine receptors e.g. gp130 and endothelial nitric oxide synthase (eNOS) [5] (Figure 1A). Caveolae formation and function are dependent on the integral membrane proteins caveolin-1-3 and the peripheral proteins cavin-1-4. Membrane curvature is critical for the regulation of signal transduction, for example, non-caveolae caveolin-1 is unable to inhibit eNOS [6]. Loss of caveolae results in a broad-range of disease states such as lipodystrophy [7], muscular dystrophy [7], cardiovascular disease [8], and cancer [9–12], possibly due to the plethora of signalling components which it regulates.

The biogenesis of caveolae has been described elsewhere [13]. In brief, homo-oligomers of caveolin-1 and hetero-oligomers of caveolin-1/2 are formed in cholesterol, sphingolipid-rich regions within the endoplasmic reticulum (ER) while larger oligomeric complexes are formed within the Golgi. Maturation continues following budding, trafficking, and fusing with the plasma membrane, the latter of which involves syntaxin-6 [14]. Palmitoylation anchors caveolin oligomers at the plasma membrane. Cavins co-localise with mature caveolins only at the plasma membrane, where they regulate membrane curvature, depth of caveolae, and endocytosis [13]. Cavins do not directly interact with caveolins but potentially requires several adaptor proteins such as filamin [15], syndapin (pacsin)II [16], Eps15 homology domain-containing 2 [17], and dynamin [16]. Once formed, caveolae exist as stable structures that are disrupted by endocytic events or disease states. Cholesterol is essential for caveolae biogenesis and as such cholesterol-depleting agents such as methyl- β -cyclodextrin (MCD), filipin, and statins (nystatin, lovastatin) prevent the formation of caveolae.

Caveolae, cAMP, and inflammatory signalling

Caveolae sequester multiple receptor signalling complexes such as the gp130 receptor [18], a mediator of inflammation during atherogenesis [19]. The IL6-stimulated gp130 receptor is negatively regulated by the cAMP-inducible suppressor of cytokine signalling (SOCS) 3 [20]. SOCS3 is present at low basal levels but is rapidly elevated in response to activation of adenylyl cyclase (AC). SOCS3 inhibits IL6 signalling by directly binding gp130 and constitutively attached Janus kinase (JAKs) and by forming an elongin-cullin-SOCS (ECS) E3 ubiquitin ligase and thus targeting substrates for K48-polyubiquitination and proteasomal degradation. Cyclic-AMP-modulating signalling components also localise to caveolae such as AC isoforms 3, 5, and 6, PDE3B, and GPCRs [21] while loss of caveolae results in the redistribution AC but with increased isoproterenol-stimulated cAMP production [22]. Thus compartmentalising modulators of cAMP and cAMP-regulated signalling components suggests a link between caveolae and cAMP-mediated regulation of pro-inflammatory responses.

Caveolae are themselves cAMP-regulated signalling organelles, for example, caveolin-1 is down-regulated in response to cAMP in rat cardiac myoblasts (H9C2 cells) and smooth muscle cells (RASMC) [22]. In addition, caveolin-1 mRNA has been reported to be up-regulated in response to several cytokines including TNF α [23]. Caveolae have been demonstrated to be essential for IL6 signalling in multiple myeloma cells positive for caveolin-1 [18]. Cholesterol depletion by MCD as well as inhibition of caveolin-1 phosphorylation was enough to block IL6 signalling as well as signalling via the Akt-1 pathway implicating both membrane curvature and caveolin-1-dependent regulation. Furthermore, signal transducers and activators of transcription (STATs), the downstream effectors of IL6 signalling, have been detected in DRM isolated from human hepatoma Hep3B cells along with gp130 and caveolin-1 [24]. STAT activation was found to be reduced following MCD

treatment indicating the importance of caveolae in IL6 mediated events [24]. Interestingly, caveolin-1 has been shown to have SOCS functionality via its ability to inhibit prolactin-induced STAT5 signalling. All caveolin family members share sequence similarities with SOCS1/3 within the kinase inhibitory region (KIR) while all JAKs contain a caveolin-binding domain (CBD) [23]. Thus, caveolins might directly bind JAKs via a conserved KIR [25]. As such, caveolae/caveolins might be significant regulators of cytokine signalling.

SOCS3 is induced by several stimuli including cytokines (IL6), chemo-attractants (IL8), and also by bacterial components (LPS, CpG DNA) via activation of toll-like receptors (TLRs). Interestingly, TLR3-dependent polyinosinic-polycytidylic acid-induced gene expression of SOCS3 is down-regulated in mandarin fish (*Siniperca chuatsi*) following expression of the scaffold domain of a caveolin-1 homologue [26]. As such, structural components of caveolae might be involved in the regulation of cytokine signalling at several levels.

In summary, these data suggest an intimate link between caveolae, cAMP, cytokine signalling, and the inflammatory response highlighting their potential as therapeutic drug targets.

Caveolae: the building blocks

Caveolins

Caveolins are a family of three integral membrane proteins (caveolin-1-3) which serve as the main structural components of caveolae. While forming hairpin-shaped proteins, they do not penetrate the outer leaflet of the plasma membrane and have both N- and C-terminal domains facing the cytoplasm. Caveolin-1/2 are co-expressed in most cell types with highest levels found in endothelial cells and adipocytes, while caveolin-3 is limited to cardiac, skeletal, and vascular smooth muscle cells [27]. The role of caveolin-2 is unclear since it is not essential for caveolae formation, while in contrast, caveolin-3 is essential for caveolae formation in muscle. This is supported by the finding that caveolae are not present in vascular endothelial cells or adipocytes from caveolin-1 knockout (KO) mice but are still present in skeletal muscle [28]. Caveolin-1 directly binds cholesterol which is essential for caveolae formation. In fact, overexpressing caveolin-1 dramatically increases (70%) cholesterol levels within plasma membrane [29].

Caveolins have been highlighted as a drug target for cardiovascular disease and has been recently reviewed [30]. Caveolin-1 is critical in regulating inflammatory signalling *via* sequestration and inhibition of eNOS via the caveolin-1 scaffold domain (amino acids 82-101) [31]. As such, loss of caveolin-1 leads to enhanced AKT and ERK1/2 signalling resulting in several cardiovascular phenotypes [32–34]. For example, while caveolin-1 KO mice models are viable, they develop pulmonary hypertension and cardiac hypertrophy [35]. These phenotypes can be rescued by either expression of a peptide containing the scaffold domain or genetic deletion of eNOS [36], suggesting a functional link between the two.

In contrast to its protective effects, caveolin-1/caveolae are thought to regulate the transcytosis of LDL in blood vessels resulting in the accumulation of pro-atherogenic lipids in the sub-endothelial space, which is important for lesion formation [37]. As such, loss of caveolin-1 has been suggested to be protective against atherosclerosis [37].

The cavin family

The cavin family has four members with predicted molecular weights ranging from 31 to 47kDa, cavin-1 (PTRF, polymerase I and transcript release factor), cavin-2 (SDPR, serum deprivation response protein), cavin-3 (SRBC, sdr-related gene product that binds to-c-kinase), and cavin-4 (MURC, muscle restricted coiled-coiled protein) where cavin-1 is the most highly expressed and most intensively studied. Cavin-1-3 are expressed ubiquitously at differing levels in a cell/tissue-specific manner but are found most abundantly in endothelial cells, adipocytes, fibroblasts, and epithelial cells [38,39] while cavin-4 is restricted to striated muscle. A 3D structure of members of the cavin family is lacking, common structural features include leucine-zipper motifs, PEST (Pro-Glu-Ser-Thr-

rich) domains, and phospho-regulatory sites [3,38]. Cavins aggregate into large 60S oligomeric complexes (the cavin complex)[38] and can do so in the absence of caveolins [13], although the significance of this is not known. Each cavin family member also binds phosphatidylserine (PS) which is enriched within the inner leaflet of the plasma membrane. By making several weak interactions with PS, the cavin complex can strengthen its association with caveolae. This also opens up the possibility that altering the lipid environment might regulate the association of the cavin complex with the plasma membrane.

Functionally, while cavin-4 seems to substitute for cavin-1 within muscle tissue each member provides non-redundant functionality. Cavin-1 stabilises caveolae by anchoring caveolin-1 to the cytoskeleton via a C-terminal region [40]. Initially, only cavin-1 was thought essential for caveolae formation, however recent data has shown cavin-2 to be essential in lung and adipose tissue[3]. Furthermore, cavin-2 modulates the size of the cavin complex and regulates caveolae depth. Thus the degree of cavin-2 expression might create heterogeneous caveolae complexes and functionality in a tissue-specific manner [3].

While functional studies into the significance of cavin-2-4 are on-going, this review will focus on cavin-1, the most intensively studied of the cavin family.

Cavin-1/PTRF

Cavin-1 (Figure 1B) was first identified in 1998 as PTRF involved in the dissociation of paused ternary transcription complexes [41]. Cavin-1 has since been revealed as an essential component of caveolae [42]. Supporting this dual role, Lui et al showed that only 50% of cellular cavin-1 co-localises with caveolin-1 in detergent-resistant rafts isolated from adipocytes via gradient centrifugation [40]. Within caveolae, cavin-1 regulates membrane curvature by stabilising caveolin-1, the main structural component of caveolae [40]. Expression of cavin-1 and caveolin-1 are tightly linked such that overexpression of cavin-1 results in a concomitant increase in caveolin-1 while its loss results in a global loss of caveolae due to increased lateral motion and lysosomal degradation or mislocalisation of caveolin-1/2 [43]. Furthermore, genetic deletion of cavin-1 in mice leads to impaired caveolae formation and loss of stability of all three caveolins [43]. Interestingly, cavin-1 like caveolin-1 is stably expressed but are also induced by stress conditions including starvation [44], catecholamines [44], and oxidative stress, leading to increased numbers of caveolae [45].

While stabilising caveolin-1, cavin-1 appears to not directly interact with caveolin-1 but is instead linked by one of many yet to be identified adaptor protein(s). Thus cavin-1 co-immunoprecipitates with caveolin-1 in Triton-X100 (1%) solubilised cell lysates (intact lipid-raft) but not in octylglucoside treated cells (solubilised lipid-raft) which is indicative of an indirect interaction. Current data suggest that cavin-1 links caveolae to the microtubule network via a C-terminal region since a $\Delta C74$ cavin-1 mutant localises to microtubule bundles [40]. Furthermore, disruption of actin cytoskeleton with latrunculin B or the microtubule network with nocodazole disrupts cavin-1 protein levels but not caveolin-1 suggesting rapid turnover of non-caveolae cavin-1 [40].

Cavin-1 has a predicted mass of 43kDa but is frequently detected at 50-60kDa following SDS-PAGE. Such a shift is characteristic of multiple post-translational modifications, indeed, cavin-1 has been detected in phosphorylated [42], SUMOylated, and ubiquitinated (Williams, J.J.L & Palmer T.M, unpublished) forms. In adipose tissue, cavin-1 acts as an adaptor protein for hormone sensitive lipase (HSL) which regulates the release of triglycerides during periods of fasting. Following starvation or treatment and subsequent elevation in intracellular cAMP, cavin-1 is phosphorylated in a PKA-dependent fashion [44] at multiple sites, with S42, T304, and S368 being essential for HSL activation. Upon re-feeding or treatment with insulin, cavin-1 is tyrosine phosphorylated at Y14, Y158, Y310, and Y318 resulting in translocation of both proteins to the cytoplasm while excess cAMP is removed by PDE3B thus preventing further lipolysis by HSL [44]. Thus, Cavin-1 might function as a general phospho-regulated adaptor protein [44,46] however the full range of cavin-1 binding partners is unknown.

Cavin-1 and disease

Cavin-1 KO mice have a lipodystrophic phenotype i.e. high circulating triglyceride levels, reduced adipose tissue mass, glucose intolerance, and hyperinsulinaemia [40]. This phenotype might stem from the impaired triglyceride uptake and storage by adipocytes due to the lack of caveolae [43]. This phenotype closely matches that of humans with cavin-1 mutations with the addition of cardiovascular and pulmonary disorders found in caveolin-1 KO mice mentioned previously [7,8,47]. Expression of cavin-1 is either lost or C-terminally truncated resulting in loss of caveolae in fibroblasts and muscle tissue [7,8,47]. This effect was also demonstrated in adipocytes using a synthetically generated $\Delta C74$ cavin-1 mutant [40].

Expression of cavin-1 and caveolin-1 are closely linked however this fine balance is lost following diet-induced atherosclerosis. Uyy et al have demonstrated that DRMs isolated from lung endothelial cells of $APOE^{-/-}$ mice fed on a high-fat diet were modified so that caveolin-1 and pAKT1 was up-regulated while cavin-1 levels were reduced [48]. As a result, loss of caveolae and dysfunctional caveolae-dependent signalling e.g. eNOS would be expected. The authors point out that this could account for pulmonary disorders which are associated with cardiovascular disease [48].

Perspectives

It is evident that caveolae have a role in cardiovascular disease but while loss of caveolae is detrimental, the individual roles of cavins and caveolins are unclear. As such, pathologies resulting from loss of caveolae due to loss of either protein family members might only partially overlap since the caveolae-independent roles for each protein is not yet fully appreciated. Thus delineating the roles of the individual caveolae structural proteins will be essential for therapeutic intervention. Furthermore, while cavin-1 is a substrate for multiple post-translational modifications such as phosphorylation, ubiquitination, and SUMOylation, their importance for caveolae stability and signal transduction is underexplored. Also limiting is the lack of structural information that will aid the understanding of protein interaction and regulation.

While caveolae and caveolins-1 have been demonstrated to be essential for IL6 signalling, the role of cavin-1 has not been assessed. It is intriguing that, all caveolin family members share sequence similarities with SOCS1/3 within the KIR while all JAKs contain caveolin-binding domains [23]. However, the significance of these findings with regard to cytokine signalling has yet to be fully addressed. Doing so might unveil further avenues of therapeutic intervention.

An exciting possibility is that by varying expression of caveolae structural proteins, a heterogeneous group of caveolae organelles can be produced [3]. Each member might then a different function via selection of resident signalling components. Mechanistically, disruption of the cavin complex by mechanical stress and subsequent loss of caveolin-1-dependent inhibition of eNOS is thought to be central to caveolae mechanosensing [49]. It might be possible that there other mechanisms by which this might occur either pathological or other.

Given the multiple roles of cavin-1, if effectively targeted, it might be possible to treat a variety of disorders such as cardiomyopathy and pulmonary disorders, atherosclerosis, and lipodystrophy.

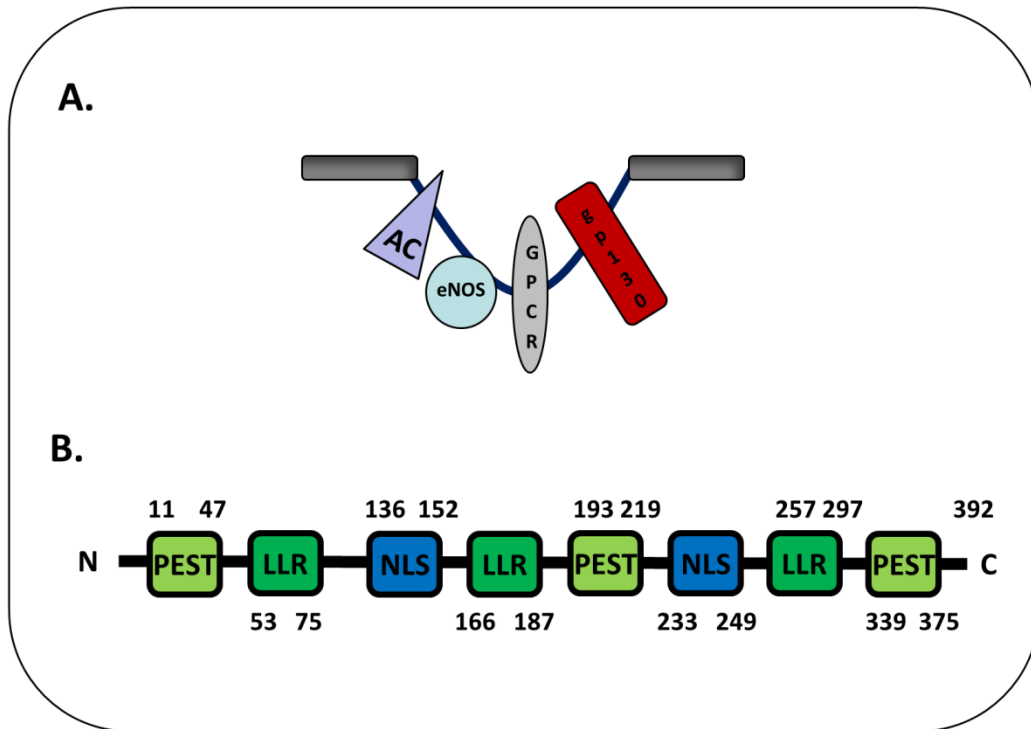


Figure 1: Caveolae and cavin-1

A. Caveolae are curved lipid-raft regions rich in cholesterol and sphingolipids which act as a platform for efficient signal transduction. AC, adenylyl cyclase; eNOS, endothelial nitric oxide synthase; GPCR, G protein-coupled receptor; glycoprotein 130. B. A 3D structure of cavin-1 is lacking, however, predicted structural features include three leucine-rich regions (LLR), three PEST (Pro-Glu-Ser-Thr-rich) domains, and two nuclear localisation signals (NLS).

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