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Caveolae structure and function

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Abstract

Studies on the structure and function of caveolae have revealed how this versatile subcellular organelle can influence numerous signalling pathways. This brief review will discuss a few of the key features of caveolae as it relates to signalling and disease processes.

Keywords: caveolae • lipid rafts • caveolin • eNOS • signal transduction • polymerase I • transcript release factor

Introduction

Caveolae were originally described in the 1950s as *plasmalemmal* vesicles in the endothelium of the heart [1] and it was proposed that these *plasmalemmal* vesicles were capable of shuttling molecules across the cell surface [2, 3]. Two years later membrane-associated *plasmalemmal* vesicles were termed *caveolae intracellulare*, because they resembled little caves when they were observed in the gall bladder epithelium [4]. It was also proposed at that time that these *caveolae intracellulare* were able to communicate with the outside of the cell as well as shuttle molecules across the cell, however a molecular mechanism was lacking. Caveolae are found in the plasma membrane of a variety of cell types and tissues, in fact most, but not all, cell types contain caveolae [5–8]. Also, it is of note that different cell types differ in the number of caveolae on the membrane surface, for example, it has been shown that

caveolae can increase the surface area of the plasma membrane of adipocytes by up to 50% [9], whereas a separate study suggests that only 3–5% of fibroblast plasma membrane is caveolae [10]. Caveolae are most often described as flask shaped, however the morphology of caveolae is dependent on the physiological status of the cells [1, 11–15]. For instance, caveolae depleted of cholesterol appear flat [16], caveolae can form transport vesicles [17, 18] and caveolae can form tubular structures [19]. To understand caveolae functions it is important to consider caveolae as dynamic structures that can have several morphologies and different components. In the past decade a great deal of effort has been expended to determine the biological role of caveolae. Some of the processes associated with caveolae include endocytosis, exocytosis, cholesterol homeostasis and signal transduction [20].

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Lipid rafts and caveolae

To understand the function of caveolae it is necessary to have a clear understanding of its structure within the plasma membrane. On a basic level it is also necessary to recall that the plasma membrane itself is a highly organized structure that provides a barrier for the cell that protects it from the exterior environment. The plasma membrane has hundreds of lipid and protein species [21, 22]. An understanding of how these basic components interact is required to delineate the role of the plasma membrane in the exchange of materials and signals across its bilayer. The fluid mosaic model of the plasma membrane describes the organization of plasma membrane components including the extracellular matrix, lipid bilayer, proteins and cytoskeleton [23]; however it assumes that the membrane is homogeneous and lacks a consideration of ordered domains. It has long been recognized that lipids are involved in the regulation of signal transduction and one way in which this occurs is through microdomains within the plasma membrane called lipid rafts [24, 25]. It has been relatively difficult to define rafts though it is apparent that they are regions of the plasma membrane that are more ordered than the rest of the membrane. It has also proven a challenge to distinguish between different types of lipid rafts [26]. For example, it is generally accepted that caveolae are lipid rafts, but it is important to recognize that not all lipid rafts are caveolae. Also, while the plasma membrane has distinguishable microdomains within them, intracellular organelles also have microdomains that may not be comparable at a functional level to those found in the plasma membrane. Plasma membrane lipid rafts contain regions that are enriched in sphingolipids which associate laterally with each other through their head groups and rafts are also enriched in cholesterol which is situated between the interacting sphingolipids [27, 28]. The lipids are organized and assemble to form microdomains within the plasma membrane on the exoplasmic leaflet. The lipid composition of lipid rafts provides a degree of insolubility in non-ionic detergents, a property that is often exploited to isolate lipid rafts [29–32]. In fact, this property is often used as the sole method to identify a membrane domain as a lipid raft. However, these detergent-based methods can alter the molecular composition of lipid rafts [33, 34], including caveolae, thus non-detergent methods are now often used to isolate caveolae [35].

Lipid rafts are believed to consist of lipids in a liquid-ordered (l_o) form and it has been demonstrated that mixtures of phospholipids, sphingolipids and cholesterol can form ordered domains that resemble rafts as well as non-raft domains [24, 27, 33, 36–38]. However, while this is valid when the mixtures were characteristic of lipids found in the outer leaflet of the plasma membrane it was not found to be valid of lipid mixtures characteristic of the cytoplasmic surface of the plasma membrane [39]. These results suggest that additional components on the cytoplasmic surface other than phospholipids and cholesterol are needed for spontaneous formation of liquid-ordered domains. One way this can be accomplished is through the association of proteins to the inner leaflet of the membrane which may be capable of organizing lipid rafts or it is possible that molecules from the outer leaflet may penetrate to the cytoplasm to further organize rafts. Lipid raft microdomains bind to proteins such as glycosylphosphatidylinositol (GPI)-anchored proteins, transmembrane proteins and dually acylated proteins such as src kinases [40, 41]. In an effort to understand the organization of lipid rafts, researchers characterized the content of the inner cytoplasmic leaflet of the plasma membrane. In liquid ordered domains, there are several acylated proteins that are anchored to the cytoplasmic surface. One of these acylated proteins is H-Ras whose binding is dependent on its guanosine-5' triphosphate (GTP)/guanosine diphosphate (GDP) bound state and has been reviewed [26]. Another group showed that acylated proteins can cluster on the inner leaflet of the plasma membrane using fluorescent resonance energy transfer (FRET) between non-dimerizing cyan fluorescent protein (CFP) and yellow (YFP) variants of green fluorescent protein [42].

As the evidence supporting the role of caveolae in critical cellular functions accumulated, the need for a method to isolate caveolae from the rest of the cell membrane was required. The first methods used Triton X-100 to solubilize the cells followed by centrifugation to separate the Triton resistant membranes from the soluble components [43–45]. Some of the proteins that were also isolated by this method were GPI-anchored proteins (later shown to be in lipid rafts but not in caveolae [46, 47]) along with caveolae specific proteins (*i.e.* caveolin). While this method dramatically advanced the molecular characterization of caveolae, there were some drawbacks. The most serious drawback to this method is that

both caveolae and lipid rafts are isolated together. This is important because while caveolae and other lipid rafts have overlapping functions, they also have distinct functions [48]. Caveolae is distinct in that it has a striated coat and contains the protein, caveolin [16]. Caveolin influences numerous cellular processes by forming homo- and hetero-oligomers [49–53], by directly interacting with signalling molecules [54–57], and by regulating the cholesterol content of caveolae [58]. Thus, caveolin containing lipid rafts (*i.e.* caveolae) and non-caveolin containing lipid rafts are not functionally the same. In an effort to isolate the portion of the plasma membrane that was only caveolae another method was devised in which the plasma membrane is isolated on a Percoll gradient, then sonicated to release caveolae. Finally, the caveolae are collected by centrifugation on an OptiPrep gradient [35]. Shortly after this detergent-free method of isolating caveolae was devised, another group developed an additional detergent-free method which replaces Triton X-100 with sodium bicarbonate buffer [59]. In addition, a method for the isolation of caveolae that avoids physical disruption (sonication) of the membrane was also developed [47]. In this method, GTP is added to the plasma membrane which induces budding of caveolae which are then released through the hydrolysis of GTP by dynamin. The free caveolae vesicles are then isolated by density centrifugation. Another procedure was developed in which caveolae were isolated from rat lung endothelial cells by coating these cells with cationic colloidal silica particles and then separating caveolae from other detergent insoluble membranes [48]. Finally, the use of a caveolin-specific monoclonal antibody has allowed for isolation of caveolae by binding oligomeric caveolin which associates with caveolae [60].

Caveolin gene family

Caveolae do not contain a clathrin coat; however, the surface of caveolae appear to have a striated coat which can be visualized by rapid-freeze, deep-etch electron microscopy [16]. Upon further investigation of the striated coat, a family of proteins named caveolins were discovered that associate with the coat. Although caveolins are often referred to as the caveolae coat protein, this has not been demonstrated.

However, these proteins serve as markers for the identification of caveolae and are critically involved in caveolae function. There are three identified caveolin proteins, caveolin-1, caveolin-2 and caveolin-3, which have apparent molecular masses between 18 and 22 kD [16, 61–65]. Caveolin-1 is expressed in most cell types including adipocytes, smooth muscle cells, endothelial cells, epithelial cells and fibroblasts [16, 66–70]. Caveolins-1 and -3 can form invaginated caveolae; however, caveolin-2 is not required to form invaginated caveolae [50, 71]. Caveolin-1 is found in two isoforms, caveolin-1 α and caveolin-1 β and these isoforms are located in many of the same cell types [72]. Caveolin proteins are thought to form a hairpin-like structure with a hydrophobic portion that is about 33 amino acids in length and two hydrophilic portions, the N-terminus and C-terminus which are found on the cytosolic side of the plasma membrane [73]. The N-terminal domain contains a caveolin scaffolding domain (residues 82–101) that interacts with other proteins including signalling molecules and other caveolin proteins to form oligomers [74–76]. The C-terminal domain is more conserved among the caveolin family of proteins and aids in anchoring the protein to the plasma membrane [68, 73, 77–79]. Caveolin-1 is also capable of forming hetero-oligomeric complexes with caveolin-2 through the hydrophobic membrane spanning domain [49].

Caveolin-2 colocalizes with caveolin-1 and is found in the same cells as caveolin-1 [62, 71]. Caveolin-2 is unable to form caveolae without the presence of caveolin-1. Vesicular transport and the formation of oligomers also require the involvement of caveolin-1, but not caveolin-3. Without caveolin-1, caveolin-2 is retained in the Golgi complex where it is degraded [49, 51, 71, 80]. Caveolin-2 has three isoforms, caveolin-2 α , caveolin-2 β and caveolin-2 γ where caveolin-2 α is considered to be the full-length isoform. While the removal of caveolin-2 has no effect on the ability to form caveolae, it results in severe pulmonary dysfunction [81].

Caveolin-3 is a muscle-specific caveolin and is found in skeletal, cardiac and smooth muscle cells [82]. Caveolin-3 localizes to the sarcolemma where it associates with the dystrophin-glycoprotein complex [82]. Caveolin-3 is very similar to caveolin-1 both structurally and functionally, but is 27 amino acids shorter [64]. Similar to caveolin-1, caveolin-3 is capable of forming caveolae and is not dependent on caveolin-1 for transport or to form oligomers [64].

Polymerase I and transcript release factor (PTRF)

In 2001 a 60-kD caveolae specific protein was identified on the cytosolic face of the plasma membrane. It was identified using immunogold labelling of epitopes on the cytosolic surface of plasma membranes in adipocyte cells using a monoclonal antibody (2F11) [83]. This protein turned out to be identical to a previously identified protein called polymerase 1 and transcript release factor (PTRF) [84] or alternatively called binding factor of type-1 collagen promoter [85]. Later, it was demonstrated that this protein colocalizes with caveolin-1 in human adipocytes [86] and was later referred to as cavin [87]. The exact role of this protein is not known; however, it is thought to play a structural and possibly a functional role in caveolae. It is expressed highly in adipocytes, lungs, heart and colon though it is also expressed in the thymus, spleen, kidney and testis and has been shown to be weakly expressed in liver and brain [86, 87]. In adipocytes, PTRF colocalizes with hormone sensitive lipase (HSL) which suggests that it plays a role in lipid metabolism [88]. It has been shown that PTRF along with HSL translocated from the plasma membrane to the cytosol when cells were incubated with insulin and further that PTRF translocates to the nucleus in the presence of insulin. These results suggest PTRF's involvement in mediating insulin regulated gene expression. It is possible that PTRF may in fact be the 'coat' of caveolae though further studies are needed.

Cholesterol

Cholesterol is the major component of lipid rafts and caveolae [2] and re-localization of caveolae cholesterol or cell deprivation of free cholesterol greatly reduces the number of caveolae [16, 89]. The current data suggest that there is a critical level of cholesterol required for the formation and maintenance of caveolae yet a clear understanding of this relationship has not been uncovered. Increased, levels of low-density lipoprotein (LDL) cholesterol lead to increased formation of caveolae in fibroblasts and smooth muscle cells [90, 91] whereas an increase in pre- β HDL leads to decreased formation of caveolae

[92, 93]. Furthermore, cholesterol levels in the cell do not remain static and cholesterol constantly fluxes into and out of caveolae [94]. This constant turnover of caveolae cholesterol gave rise to the concept that caveolae may function as cholesterol transport vesicles. Cholesterol moves between the cell surface and the endoplasmic reticulum (ER) by multiple mechanisms and one of these mechanisms may involve caveolae. One of the first experiments to demonstrate the involvement of caveolae in cholesterol trafficking used fibroblasts treated with progesterone [95]. Progesterone induced caveolin to leave caveolae and accumulate in internal membranes. Progesterone also induced a decrease in caveolae cholesterol levels without altering the cholesterol levels of the rest of the plasma membrane. Removal of the progesterone from the cells allowed caveolin and cholesterol to return to caveolae. Additional experiments demonstrated that progesterone was preventing the movement of newly synthesized cholesterol to caveolae, however the cholesterol in caveolae was constantly flowing down its concentration gradient into the surrounding plasma membrane. This resulted in a decrease in caveolae cholesterol without a decrease in the cholesterol in the rest of the plasma membrane. In addition to progesterone, cholesterol oxidase has been used to examine the role of caveolae in cholesterol trafficking [94]. Cholesterol oxidase catalyses the oxidation of cholesterol in the outer leaflet of the plasma membrane when added to intact cells. Studies using fibroblasts demonstrated that cholesterol oxidase can oxidize essentially all of the cholesterol in caveolae suggesting that caveolae cholesterol is located in the exocytosolic leaflet of the plasma membrane or that cholesterol can rapidly flip across the membrane in caveolae. Similar to the progesterone studies, the removal of caveolae cholesterol caused caveolin to move to the ER and Golgi. The removal of cholesterol oxidase allowed caveolin to return to caveolae along with cholesterol. Interestingly, inhibitors of Golgi vesicular trafficking did not prevent the return of caveolin or cholesterol to caveolae suggesting a vesicle independent mechanism.

Although it was commonly thought that cholesterol must move in a vesicle, cholesterol circulates in the blood in a lipid-protein complex [92, 96]. Because caveolin re-localized to the ER-Golgi membranes upon caveolae cholesterol depletion, studies were undertaken to determine if caveolin was directly involved in cholesterol trafficking. A series of studies

demonstrated that caveolin directly binds and traffics cholesterol through the cytoplasm in a lipoprotein chaperone complex [97, 98]. The chaperone complex consists of caveolin, cyclophilin A, cyclophilin 40 and HSP56 [98]. It appears that cholesterol interacts with caveolin at sites of caveolin acylation and this complex, by an unknown mechanism, delivers cholesterol to caveolae. Importantly, disruption of this complex does not prevent transport of cholesterol to the plasma membrane because other transport mechanisms such as Golgi-derived vesicles exist. However, disruption of this complex prevents the enrichment of cholesterol in caveolae which subsequently inhibits many caveolae-mediated functions.

In addition to movement of cholesterol to the plasma membrane it has been demonstrated that a caveolin lipoprotein chaperone complex can also facilitate the uptake of caveolae cholesterol [97, 99, 100]. Using Chinese hamster ovary cells it has been shown that an HDL-binding protein, scavenger receptor class B, type 1 (SR-BI) can deliver cholesterol from HDL to caveolae [99]. This cholesterol is then moved to the ER where it can be esterified and stored. Caveolin facilitates this uptake by forming a lipoprotein complex consisting of caveolin, cyclophilin A, cyclophilin 40 and annexin II. Apparently annexin II provides the specificity for the cholesterol to traffic from caveolae to the ER; whereas, HSP56 provides the specificity to traffic cholesterol from the ER to caveolae. A study also demonstrated that caveolin-1 induces SR-BI to form dimers in HepG2 cells, a liver cell line which subsequently increased selective cholesterol ester uptake [101]. The presence of specific mechanisms to control caveolae cholesterol levels implies that cholesterol is critically involved in the structure and function of caveolae.

Signal transduction

It is now well-established that caveolae compartmentalize and regulate a wide variety of signalling pathways [102–104] although a small minority has questioned the role of caveolae in signalling [105]. However, it is important to understand that many of the signalling pathways in caveolae also function outside of caveolae which most likely explains why caveolin null mice are still viable. In the next section,

a few of the most studied caveolae-associated signalling pathways are reviewed.

G-protein coupled receptors

G-protein coupled receptors (GPCRs) mediate a variety of physiological processes including signalling through neurotransmitters and hormones [106]. G proteins exist as a trimeric unit composed of three subunits α , β , γ which in the inactive state is bound by GDP on the cytosolic surface of the plasma membrane. When an adjacent receptor protein binds a signalling ligand, it alters the conformation of the receptor allowing it to bind to the inactive G protein's α -subunit. The binding of the receptor to the G protein causes GDP to dissociate from the protein which allows GTP to bind making it active by causing the α -subunit to dissociate from the inhibitory β and γ subunits. This multi-step activation is one model of G protein activation and one-step models also exist [107]. In addition, it has been proposed that compartmentalization of these receptors and other proteins in microdomains located in the plasma membrane may lead to more efficient signalling and regulation [104, 108]. There have been several models which suggest that receptors move into or out of caveolae as well as models that suggest that receptors remain in caveolae once activated by agonists [109]. Several studies have suggested that inactive $G\alpha$ subunits may concentrate in caveolae while activation of the subunit causes $G\alpha$ to leave and remain separate from caveolae [110, 111]. In an effort to design a model to predict the role of caveolae in G-protein activation, one group designed a mathematical model to study the effects of compartmentalization of different signalling components under different conditions within caveolae. This model is modifiable and offers a unique approach to understanding signal activation or deactivation under a variety of physiological circumstances [106]. The use of a variety of caveolae/lipid raft isolation techniques have been used to determine which G proteins specifically interact with caveolin and are contained within caveolae. However, despite the high quality of most of these studies, conflicting results have been reported [35, 43–45, 110, 112, 113]. Through the use of immunofluorescence microscopy and subcellular fractionation, one group showed that G_q , a subfamily of $G\alpha$

proteins, concentrates in caveolae and G_{β} , G_i and G_s target non-caveolae lipid rafts. These results may explain some of the discrepancies in the previous studies because the earlier methods to isolate caveolae also isolate lipid rafts [111].

In cardiac myocytes, GPCRs colocalize with caveolin-3. This relationship appears to be important in the regulation of calcium uptake through L-type calcium channels [114]. Calcium increases contraction through a mechanism by which L-type calcium channels are activated through the β -adrenergic receptor signalling pathway. It has been shown that L-type calcium channels can colocalize with caveolin-3 in cardiac myocytes. It was further demonstrated that β_2 -adrenergic receptors also associate with caveolin-3 and the location of the receptor and the L-type calcium channel to caveolae is required for β_2 -signal regulation in cardiac myocytes. Interestingly, in a separate study it was shown that G_{α_s} , a sodium channel and caveolin-3 formed a complex in caveolae [115]. Importantly, caveolin-3 antibodies prevented isoproterenol-induced stimulation of the sodium channels suggesting that the association had functional significance.

ENOS

One way in which caveolae can influence oxidative stress which may lead to cardiovascular disease is through the regulation of endothelial nitric oxide synthase (eNOS) [116, 117]. Nitric oxide that is produced from eNOS is important in regulating blood pressure, angiogenesis, inhibition of platelet aggregation and vascular smooth muscle cell proliferation [118]. Several studies have demonstrated that both insufficient levels of nitric oxide as well as excess amounts of nitric oxide can be deleterious [119, 120]. Like caveolae, eNOS is highly expressed in endothelial cells as well as in cardiac myocytes [121]. The mechanism underlying the production of nitric oxide from eNOS or other NOS isoforms is through a reaction where arginine is oxidized to citrulline releasing nitric oxide. Homodimers of NOS bind to cofactors, such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), as well as calmodulin which plays a structural and functional role. The targeting of eNOS to caveolae is through modification by *N*-myristoylation and palmitoylation [56]. Once

associated with caveolae, eNOS interacts with the scaffolding domain of caveolin-1 in endothelial cell and caveolin-3 in cardiac myocytes [122]. The interaction of caveolin with eNOS plays an inhibitory role and it is only upon the release of eNOS from caveolin that the enzyme can be fully activated. Calcium/calmodulin and binding of heat shock protein 90 [123, 124] and dynamin-2 [125, 126] have been shown to increase eNOS activity in caveolae through a variety of mechanisms many of which involve preventing caveolin from binding eNOS. In addition to direct protein interaction, eNOS activity can be regulated by changing the lipid composition of caveolae [127, 128]. For instance, oxidized LDL can promote a decrease in caveolae cholesterol which results in eNOS moving to an intracellular compartment where it can no longer be stimulated by acetylcholine [127].

Caveolae and disease

Caveolae are important in the regulation of signalling molecules, so it is not surprising that human diseases are affected by changes in caveolae function. The fact that caveolae are located in the majority of cell types allows for the potential of caveolae affecting a wide range of diseases. Below, the role of caveolae in prion disease and tumorigenesis is discussed.

Prion diseases

Prion diseases are neurodegenerative disorders that arise as the result of the conversion of the cellular prion protein (PrP^C) to its pathological conformer PrP^{Sc} [129]. Prion proteins are expressed in neuronal tissue as a GPI-anchored protein [130]. When normal prion protein PrP^C is improperly folded it is thought that it becomes pathological and interferes with neuronal function [131–135]. While it is accepted that the onset of this disease is due to the conversion of the prion protein, the mechanism by which this occurs is still not clear. The use of cryoimmunogold electron microscopy made it possible to visualize prion proteins in caveolae in CHO cells and this raised the question of whether the internalization of PrP^C in a caveolae dependent manner was linked to the conversion to PrP^{Sc} [136]. Recently, it was

demonstrated that gangliosides, which are enriched in caveolae, may be contributing to the conversion of PrP^C to PrP^{SC} [137]. This transition is thought to occur through an interaction whereby the positively charged prion protein interacts with the negatively charged ganglioside. These results were obtained using a peptide that represents residues 106-126 of human prion protein by circular dichroism and Raman spectroscopy. In addition to a possible role in the disease process, caveolae may be involved in non-pathological signalling of PrP^C. Caveolin-1 and PrP^C colocalize in a hypothalamic neuronal cell line, GN11, and the interaction of caveolin-1 and PrP^C results in the activation of fyn and erk 1/2 signalling events [138].

Tumorigenesis

Caveolin-1 has been shown to play a variety of roles in both the prevention and acceleration of cancers [139–144]. While on the one hand, caveolin-1 appears to play a protective role against the onset of certain cancers such as breast cancer [145], it may promote tumour progression in other forms of cancer such as prostate cancer [146]. Caveolin-1 is down-regulated in certain oncogenic cells suggesting that it may serve as a tumour suppressor which lead to further investigation of its direct link in specific cancer cell types such as breast cancer [147]. In 16% of breast cancer patients a mutation occurs in caveolin-1 at codon 132 which has been speculated to be playing an important role in the development of mammary tumours [148]. To investigate this hypothesis further, one group bred a caveolin-1 null mouse with a PyMT transgenic mouse [145]. PyMT transgenic mice express a transforming oncogene specifically in mammary epithelium [149]. An increase in tumorigenesis was observed in these mice compared to those which had the caveolin-1 gene intact. This group also demonstrated that the recombinant expression of caveolin-1 reduced matrix metalloproteinase (MMP)-2 and -9 activities in metastatic mammary tumour cells. When studying breast cancer it is important to consider estrogen exposure because estrogens are essential mitogens of mammary epithelial cells [150]. Estrogen receptors mediate estrogen action and are expressed at low levels under normal conditions but at elevated levels under tumorigenic conditions [151]. Estrogen receptor α (ER α) overexpression is found in the majority of

human breast cancers [152]. This overexpression of ER α is increased and cyclin D1 is up-regulated when the caveolin-1 gene is inactivated in mammary epithelial cells *in vitro* and these findings have been previously reviewed [150].

Cyclooxygenase-2 (COX-2) expression is up-regulated in response to a variety of cancers including colon cancer [153, 154] and COX-2 inhibitors have been used in an attempt to reduce the progression of tumours [155–157] though the mechanism by which this works is unclear. In human colon carcinoma cells, apoptosis events and signalling is clustered in caveolae [158]. In one study, COX-2 was inhibited and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis was increased through the up-regulation of its receptor DR5 which is enriched in caveolae. This occurred through the activation of sphingomyelinase which caused the accumulation of arachidonic acid possibly resulting in the buildup of ceramide in the outer leaflet of the plasma membrane [158]. Caveolae are highly enriched in sphingomyelin which makes them an ideal target for sphingomyelinase-based mechanisms to decrease cancers. In contrast, caveolin-1 expression has been shown to be increased in human prostate cancer [146]. When caveolin-1 null mice were bred with a prostate cancer model, the mice developed less invasive tumours with a reduced metastatic state suggesting that caveolin-1 promoted the development of prostate cancers.

Animal models

The use of animal models has provided an invaluable amount of information concerning the contribution of caveolae and its associated proteins in the development of disease and regulation of signalling molecules. One would predict that animal models of caveolin would not be viable because of the importance of caveolae. Surprisingly, mice deficient in caveolin-1, -2 or -3 as well as double knock outs or caveolin-1 and -3 are both viable and fertile [159]. The generation of caveolin-1 null mice resulted in the inability to form caveolae in tissues specifically expressing caveolin-1, while the formation of caveolae in tissues expressing caveolin-3 was still present [160, 161]. Caveolin-1 null mice also had a reduction in the amount of caveolin-2 protein [159]. Although the caveolin-1 null mice are viable and fertile, they do have an

altered phenotype [159]. For instance, they have abnormal vasoconstriction and vasorelaxation responses which appear to be due to alteration in eNOS regulation [161]. The animals have a thickening of the alveolar septa in the lungs which appear to be due to the lack of caveolin-2 [81, 160, 161]. Caveolin-1 null mice also display an abnormal heart phenotype which includes an enlarged ventricular diameter and decreased contractility [162, 163].

Future direction

A great deal of basic cell biology and signalling research has been done concerning the structure and function of caveolae and lipid rafts. Although many questions remain unanswered the future of caveolae research lies in linking the structure and function of caveolae to specific disease processes. The advent of animal models and more rigorous caveolae isolation methods should greatly facilitate this important and rapidly expanding area of study.

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