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Review

Getting rid of caveolins: Phenotypes of caveolin-deficient animals

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Abstract

The elucidation of the role of caveolae has been the topic of many investigations which were greatly enhanced after the discovery of caveolin, the protein marker of these flask-shaped plasma membrane invaginations. The generation of mice deficient in the various caveolin genes (cav-1, cav-2 and cav-3) has provided physiological models to unravel the role of caveolins or caveolae at the whole organism level. Remarkably, despite the essential role of caveolins in caveolae biogenesis, all knockout mice are viable and fertile. However, lack of caveolae or caveolins leads to a wide range of phenotypes including muscle, pulmonary or lipid disorders, suggesting their implication in many cellular processes. The aim of this review is to give a broad overview of the phenotypes described for the caveolin-deficient mice and to link them to the numerous functions so far assigned to caveolins/caveolae.

Keywords: Caveolin; Caveolae; Knockout mice; Metabolic disorders; Signaling

1. Introduction

Since the first morphological description of caveolae more than 50 years ago, the elucidation of the role of these plasma membrane invaginations in the cell has been the topic of many investigations. This interest has been greatly enhanced after the discovery of caveolin, the major protein constituent of caveolae [1,2]. In the following years, numerous cellular functions have been assigned to caveolae; however, the physiological relevance of these suppositions remained controversial. Already the fact that not all the commonly used genetic animal models have caveolin genes (i.e. *S. cerevisiae* or *D. melanogaster*) indicated that the function of caveolae in the cell should be organism specific rather than general like that of mitochondria or lysosomes.

Advances in unraveling the function of caveolae came through investigations of the consequences of caveolin gene product disruption in animals that normally express members of this protein family. First, disruption of caveolin-1 gene function was assessed in *C. elegans* by RNA interference [3]. Remarkably, the phenotype observed was very mild: morphology, behavior or lifespan of worms were not affected to any extent. However, lack of cav^{ce}-1 resulted in a burst of egg-laying by hermaphrodites due to the acceleration of the meiotic cell cycle progression, a process which is regulated by *let-23*, a homologue of the mammalian ras oncogene. Thus, cav^{ce}-1 appeared to be dispensable for the life cycle of a worm. Indeed, it was later shown that a line-bearing deletion in the two caveolin-like genes cav^{ce}-1 and cav^{ce}-2 was viable and displayed no visible defects (*C. elegans* Gene Disruption Consortium). Given the variety of possible physiological tasks attributed to caveolae and caveolins, the generation of mice lacking caveolins was eagerly awaited. First, caveolin-3 knockout mice were produced [4,5] followed by caveolin-1 [6,7] and caveolin-2 knockout animals [8]. Later on, other caveolin-1 deficient mice were independently generated, allowing the comparison of the observed phenotypes and judging their specificity [9,10] (see Fig. 1). Despite the essential role of caveolins in caveolae biogenesis, all knockout

Abbreviations: GPI, Glycosylphosphatidylinositol; DRMs, Detergent Resistant Membranes; IR, Insulin Receptor; VLDL, Very low density lipoprotein; MEFs, Mouse embryonic fibroblasts

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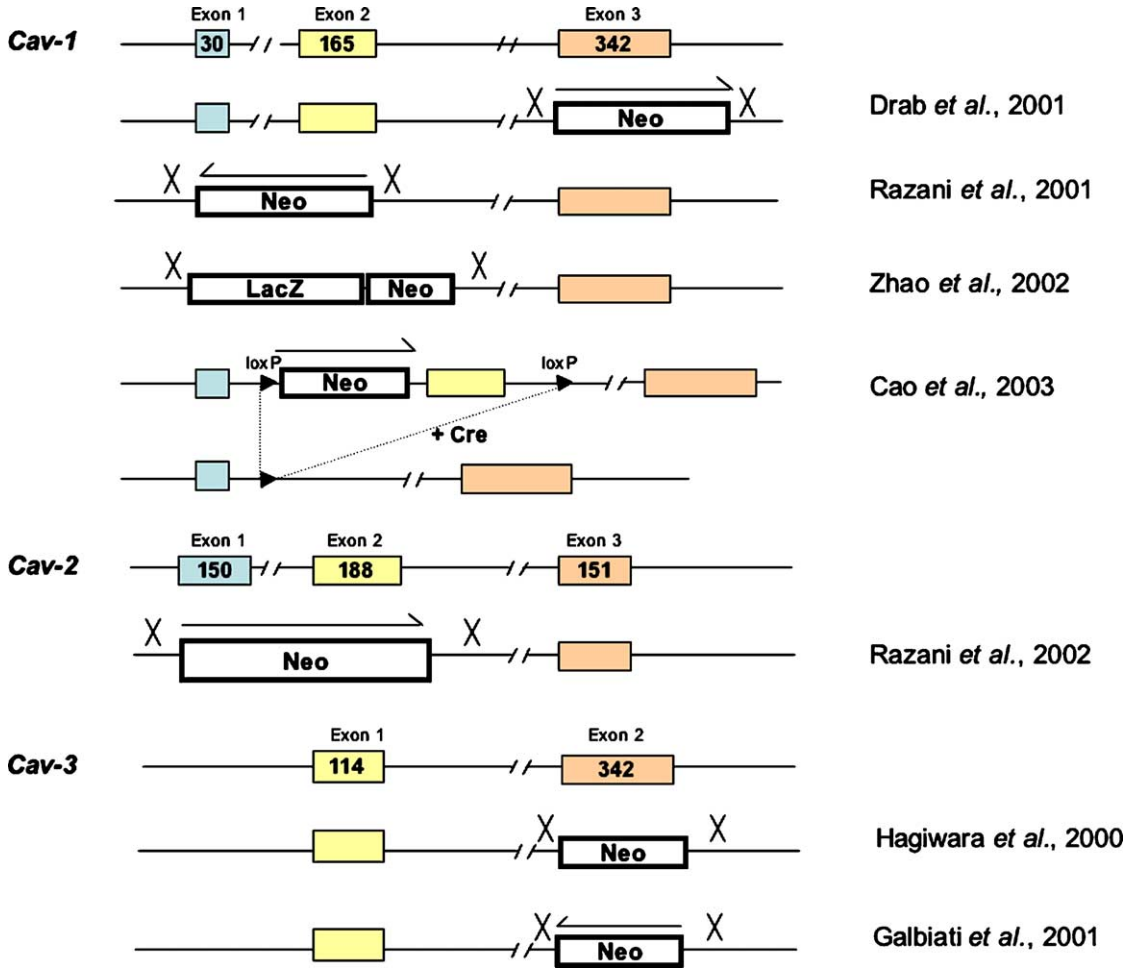

Fig. 1. Generation of cav-1, cav-2 and cav-3 knockout mice. Schematic representation of genomic locuses of different caveolins (cav-1, cav-2 and cav-3) and of the targeting constructs which have been used for the generation of knockout mice. The arrangement of exons is represented by color-boxes with numbers of nucleotides composing each exon. White boxes indicate the placement of the *Neo* cassette (containing the neomycin resistance gene with flanking segments homologous to the locus) that has been used in all generated knockout mice. References to original publications are given on the right side.

mice are viable suggesting the presence of compensatory pathways that allow a cell to function without caveolar structures. Yet, studying phenotypes of caveolin knockout mice has considerably enhanced our understanding of the function of these proteins in diverse tissues and cellular processes in vivo. Moreover, cells isolated from caveolin-deficient mice are excellent tools for studying caveolae-dependent or caveolae-independent processes in situ.

2. Caveolin-1 knockout mice

The generation of cav-$1^{-/-}$ mice was achieved either by the targeted disruption of the exon 3 [6], exon 2 [10] or the first two exons of caveolin-1 [7,9] (Fig. 1). Cav-$1^{-/-}$ mice show a remarkable lack of caveolae in all non-muscle tissues confirming the necessity of this protein in caveolae biogenesis but the functional requirement of these membrane invaginations remains mysterious (Fig. 2). As mentioned above, all cav-$1^{-/-}$ mice generated independently are viable and fertile. Although one group has reported a reduction of the life span of these animals, this finding could be attributed to secondary complications such as pulmonary fibrosis, hypertension or cardiac hypertrophy [11] (see below).

One of the surprising findings observed in all knockout mice was the drastic reduction of caveolin-2 expression, without any noticeable change at the transcription level. It is known that caveolin-1 and caveolin-2 have the same tissue pattern of expression and they can hetero-oligomerize [12]. Moreover, caveolin-2 localization is determined by the presence of caveolin-1; otherwise, caveolin-2 remains trapped within the Golgi complex [7,13,14]. In addition, caveolin-2 seems to be degraded in the absence of caveolin-1, since proteosomal inhibitors are able to restore caveolin-2 expression to nearly wild-type levels in cav-$1^{-/-}$ MEFs [7]. Thus, regarding protein expression, cav-$1^{-/-}$ mice can be seen as cav-1/cav-2 double-knockout.

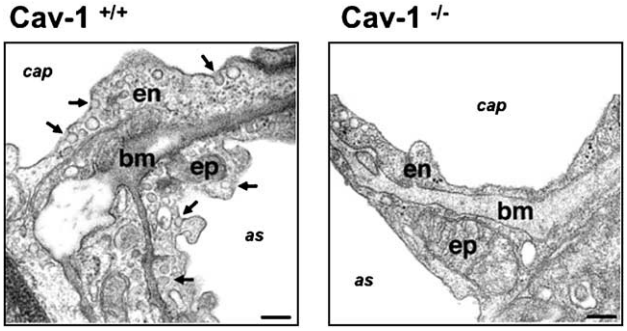

Fig. 2. Disruption of caveolin-1 results in the absence of caveolae. Electron micrographs of lung tissue from cav-$1^{+/+}$ and cav-$1^{-/-}$ mice. The alveolar space (as) is separated from the blood capillary (cap). The septum consists of a layer of epithelium (ep), a basal membrane (bm) and a layer of endothelium (en). Both epithelial and endothelial cells are covered with caveolae (arrows) in cav-$1^{+/+}$ whereas these invaginations are absent in cav-$1^{-/-}$ mice. Scale bar, 200 nm.

2.1. Caveolae and vesicular trafficking

Already in very early studies, caveolae were suggested to serve as transport vesicles involved in transcytosis of solutes through endothelial cells [15,16]. Recent work extended their role to the internalization of membrane components such as glycosphingolipids or glycosylphosphatidylinositol-anchored proteins, viruses (Polyoma virus or Simian virus 40), bacterial toxins (cholera toxin or tetanus toxin) and extracellular ligands (albumin, folic acid or autocrine motility factor) introducing caveolae as an alternative pathway to the established clathrin-dependent endocytic pathway (see review in [17]).

The contribution of caveolae to transcytosis of albumin through endothelia was first examined by Drab et al. [6]. Despite the complete loss of caveolae from the capillary network of cav-$1^{-/-}$ mice, the albumin concentration in the cerebrospinal fluid was not different between knockout and wild-type animals. However, it was recently found that the uptake of radioiodinated albumin in the aortic segments of cav-$1^{-/-}$ mice was dramatically lower than in wild-type mice [18]. Also, the uptake of albumin in MEFs derived from the knockout mice was defective [7]. These discrepancies could be explained by an increase of microvascular hyperpermeability in cav-$1^{-/-}$ mice due to more efficient paracellular transport via intercellular clefts and tights junctions [19]. Considering the essential role of albumin as a serum transport-protein for numerous endogenous molecules, these parallel pathways would then compensate for the lack of caveolae.

Caveolae membranes are enriched in lipids and proteins found in detergent-resistant membrane domains (DRMs) like GPI-anchored proteins [20–22]. Sorting of GPI-anchored proteins at the Golgi complex has been proposed to use caveola-like vesicles as exocytic vesicular carriers to be transported to the cell surface [23,24]. Drab et al. compared the levels of GPI-anchored proteins between DRMs derived from lungs of wild-type or cav-$1^{-/-}$ mice and detected no appreciable differences [6].Thus, caveolin-1 does not seem to be required for the maintenance of the association of GPI-anchored proteins with DRMs. Sotgia et al. confirmed these results but showed an intracellular retention of GPI-linked proteins within the Golgi complex in cav-$1^{-/-}$ cells [25]. The authors suggested that caveolins might play a role in the transport of raft-associated proteins from the trans-Golgi network (TGN) to the plasma membrane. The latter results, however, could not be reproduced in caveolin-1 deficient MEFs derived from mice produced by Drab et al. (A. Manninen et al., submitted manuscript). In fact, one would expect the mislocalization of GPI-anchored proteins to be lethal since this class comprises more than 100 proteins with a wide variety of physiological functions including signal transduction and cell adhesion. A total block of the transport of GPI-anchored proteins is, thus, hardly conceivable. Even if such a deficiency existed in vitro, alternative pathways should be able to compensate for this defect in vivo.

In addition to plasma membrane and TGN, caveolin-1 is also associated with the endosomes, lipid bodies and a unique endocytic compartment called caveosomes [26]. Simian Virus 40 (SV40) has been shown to enter host cells via caveolar endocytosis followed by transport through caveosomes to the endoplasmic reticulum [27]. This process has been shown to involve tyrosine phosphorylation of proteins in caveolae and recruitment of actin and dynamin [28,29]. Remarkably, a recent study shows that SV40 can still be internalized into cav-$1^{-/-}$ cells via a pathway independent of clathrin [30]. Also, cholera toxin B (CTB) has been reported to be endocytosed via caveolae in wild-type MEFs and via a clathrin and caveolin-independent pathway in cav-$1^{-/-}$ MEFs [31]. Thus, both studies report that the two pathways can exist in parallel in wild-type cells and are both involved in the uptake processes [17,31]. Future studies will have to clarify to which extent these endocytic pathways overlap functionally and mechanistically.

2.2. Lipid disorders

2.2.1. Metabolic disorders associated with adipose tissue abnormalities

In addition to being involved in transport and signaling events, caveolae have also been linked to the regulation of cholesterol homeostasis. This assumption is based on observations that firstly, caveolin-1 can bind directly to cholesterol [32] and secondly, is involved in cellular sterol transport [33,34]. Moreover, cellular cholesterol balance is important for the maintenance of caveolae [2,35] and the expression of caveolin-1 can be regulated through two sterol regulating binding elements (SRE) at the transcription level [36,37]. Despite this link, initial analysis of cav-$1^{-/-}$ mice did not reveal any differences either in the lipid contents of DRMs, or in intraperitoneal adipose tissue appearance [6,7]. However, as they grow older, the knockout mice tend to be

leaner. The difference in weight becomes even more striking when mice are challenged by high-fat diet [38] (Le Lay et al., unpublished data (Fig. 3A)). Histological investigations of fat pads from high-fat diet fed cav-$1^{-/-}$ mice showed a reduced diameter of adipocytes, reflecting smaller lipid droplet sizes. Knockout animals also display marked hyperplasia of the brown adipose tissue (BAT) [38]. One could envisage that the observed resistance to obesity could be based on the increased metabolic activity of the BAT. However, a recent study has shown that the BAT is relatively inactive in knockout animals and that caveolin-1 expression is essential for proper nonshivering thermogenesis [39].

Cav-$1^{-/-}$ mice display several metabolic disorders: elevated levels of free fatty acids and triglycerides, decreased expression of leptin and ACRP30, although there are no changes in insulin, glucose and cholesterol levels [38]. Caveolin-1 knockout mice show markedly decreased glucose uptake as assessed by an insulin tolerance test and develop post-prandial insulinemia when placed on a high fat diet [40]. Since caveolin has been shown to be a positive regulator of insulin signaling [41], Cohen et al. investigated the expression of the insulin-receptor (IR) in caveolin-1 knockout animals [40]. Although no changes were observed in insulin receptor gene expression, IR protein levels were reduced dramatically (>90%) in cav-$1^{-/-}$ adipose tissues. Ectopic expression of caveolin-1 in cav-$1^{-/-}$ MEFs was sufficient to restore IR expression to wild-type levels, whereas a caveolin-1 mutant protein lacking the scaffolding domain failed to rescue the defect. Interestingly, cav-$3^{-/-}$ animals have also been shown to develop whole-body insulin resistance associated with impaired glucose tolerance [42,43]. However, in this case, caveolin-3 seems to attenuate insulin-stimulated activation of insulin receptors and downstream molecules in skeletal muscles. Thus, despite the relative importance of caveolins in vivo for insulin signaling, further investigations will be needed to clarify if the mechanisms, which underlie insulin-signaling defects, are tissue- or cell-specific.

Interestingly, some patients suffering from severe insulin-resistance have mutations within the caveolin-binding motif of the insulin receptor which leads to an accelerated degradation of these mutant receptors [44–47]. This is in agreement with a role of caveolin-1 in stabilizing the insulin-receptor [40].

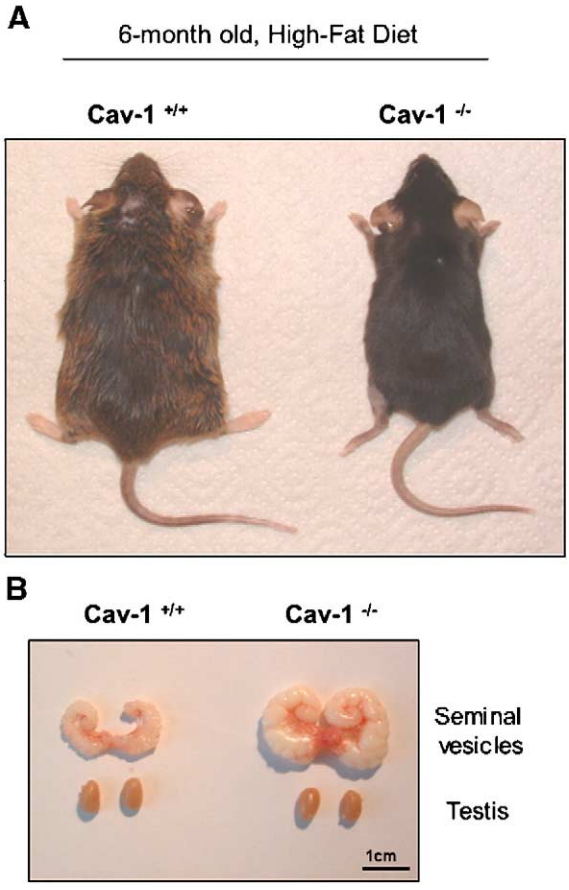

Fig. 3. Some remarkable phenotypes of the Cav-$1^{-/-}$ mice. (A) Cav-$1^{-/-}$ mice are resistant to high-fat diet induced obesity. Photographs of 6-month old cav-$1^{+/+}$ and cav-$1^{-/-}$ mice challenged with a high-fat diet. Due to the atrophy of fat depots, cav-$1^{-/-}$ mice remain leaner than their wild-type littermates. The same phenotype is observed with ageing mice (one-year old mice). (B) Urogenital disorders in aged cav-$1^{-/-}$ males. Disruption of cav-1 leads to an engorgement of seminal vesicles in one-year-old cav-$1^{-/-}$ males. This is mainly due to an accumulation of seminal fluid. No significant changes in sizes of testis are observed.

2.2.2. Caveolins and lipid bodies

A breakthrough in linking caveolins to cellular lipid homeostasis came from reports that described the presence of caveolins on the lipid droplet surface [48–50]. Later, this finding was confirmed by proteomic screen of lipid droplets derived from Chinese hamster Ovary cells (CHO-K2) [51] or from 3T3-L1 adipocytes [52]. Moreover, a recent report shows that caveolins can relocate from the plasma membrane to lipid bodies in response to fatty acids and return back to the plasma membrane when the lipid source is removed [53]. Considering the fact that caveolins are cholesterol and fatty acid binding proteins [32,54], this caveolin-dynamic pathway argues for a role of caveolin in cellular lipid homeostasis (reviewed in [55]). Indeed, caveolins play a role in regulating the cholesterol content of lipid droplets in adipocytes (Le Lay et al., submitted manuscript). Moreover, studies of lipid droplet metabolism have revealed an impaired lipolytic activity in caveolin-1 knockout animals and perturbations in the architecture of the lipid droplet cortex [56]. Finally, cav-$1^{-/-}$ MEFs stably transfected with perilipin accumulate fewer lipids than perilipin-transfected wild-type MEFs when submitted to loading with fatty acids [56]. Taken together, these results are consistent with a role of caveolin in regulating the lean phenotype of caveolin-1 deficient mice. Further investigations will have to characterize molecular mechanisms that regulate this new pathway between plasma membrane and

lipid bodies and to establish its contribution to lipid balance and signal transduction. The role of this pathway might be important especially in adipocytes, where lipid droplets are playing a key physiological role in lipid storage.

2.2.3. Atherosclerosis

Analysis of lipoproteins in the plasma from cav-1$^{-/-}$ mice revealed a shift in their distribution towards an atherogenic profile characteristic for a hypertriglyceridemia (elevated VLDL/chylomicrons) [38]. Indeed, knockout mice display a marked intolerance in the clearance of an oral fat load independent of lipoprotein and hepatic lipase activities [38]. In order to gain new insights into the role of caveolin-1 in the development of atherosclerosis, Frank et al. interbred cav-1$^{-/-}$ mice with atherosclerosis-prone apo-E$^{-/-}$ mice [57]. Interestingly, these apoE$^{-/-}$/cav-1$^{-/-}$ mice displayed a significant increase in plasma cholesterol and triglycerides compared to apoE$^{-/-}$/mice, with elevated atherosclerotic lesions. Concomitantly, a loss of caveolin-1 in ApoE positive background resulted in a 70% reduction in atherosclerotic lesions in aortas. In parallel, the protein levels of the scavenger receptor for oxidized and modified LDL, CD36 and the vascular cell adhesion molecule-1 (VCAM-1) are markedly reduced in aortic extracts from the double-knockout mice. Thus, caveolin-1 might be required for the proper trafficking of CD36 to the plasma membrane and its stability [58].

2.3. Urogenital alterations

Following up their observations that caveolin-1 expression was increased in prostate cancers (reviewed in [59]), Cao et al. have generated a cav-1$^{-/-}$ homozygous for a null mutation in exon 2 of caveolin 1 using the LoxP/Cre technology [10] (Fig. 1). Disruption of the caveolin-1 gene impaired renal calcium reabsorption and led to hypercalciuria and urolithiasis. Defective calcium reabsorption appeared to be secondary to mislocalization and likely misfunction of PMCA, an important calcium pump protein in the mouse distal nephron.

Another striking phenotype which appears in aged cav-1$^{-/-}$ males is a dramatic enlargement of seminal vesicles due to an engorgement of seminal fluid [60], (S. Le Lay and T.V. Kurzchalia, unpublished results (Fig. 3B)). In addition, these mice exhibit a significant hypertrophy of the bladder associated with a thickening of the smooth muscle layer [60]. Loss of caveolin-1 is also associated with disruption of muscarinic cholinergic activity in the urinary bladder [60], resulting in impaired bladder smooth muscle contraction [60,61].

All these urogenital organ disorders are similar to the lower urinary tract dysfunction (LUTD), observed in elderly male humans. In this regard, cav-1$^{-/-}$ male mice could provide a new animal model for the understanding of these urogenital dysfunctions.

2.4. Vascular system dysfunction

2.4.1. Deregulation of the NO pathway

The lack of caveolae in caveolin-1-deficient mice offers the opportunity to address directly whether this organelle is involved in various signaling events. One signaling pathway which has been widely documented is the interaction between the endothelial form of nitric oxide synthase (eNOS) and caveolin-1, reviewed in [62]. Indeed, the localization of eNOS in caveolae is required for maximal eNOS activity [63,64] which can be negatively regulated by caveolin-1 [65,66]. Isolated aortic rings from cav-1$^{-/-}$ mice failed to establish a steady contractile tone. Moreover, marked relaxation in the response to acetylcholine was observed [6,7] which is rescued by a treatment with the eNOS inhibitor, L-NAME [7]. In fact, the measurement of the basal release of nitric oxide (NO) was higher in cav-1$^{-/-}$ aortic vascular smooth muscle cells (VSMCs) than in plasma samples [9]. Moreover, the content of cyclic guanosine monophosphate (cGMP, the major mediator of NO signaling) was about three-fold higher in knockout animals [6]. Thus, caveolin-1 and caveolae are essential for three major vascular features depending on NO signaling: endothelium-dependent relaxation of arteries, myogenic tone and stimulated contractility.

2.4.2. Impaired angiogenic response

Angiogenesis is the process of forming new blood vessels from pre-existing ones and implies a tightly regulated remodeling of the endothelial cell layer (reviewed in [67]). Interestingly, the fusion of caveolae-like vesicles following VEGF stimulation has been shown to be part of the angiogenic response [68]. Further in vitro studies identified caveolin-1 as a positive regulator of endothelial capillary tubule formation [69,70]. Finally, in vivo evidences were brought by the observation that cav-1$^{-/-}$ mice displayed an impaired angiogenic response to exogenous stimuli [71,72]. Especially, Sonveaux et al. emphasized the critical role of caveolae in ensuring the coupling between VEGFR-2 stimulation and downstream mediators of angiogenesis like VEGF-induced ERK and eNOS activation [72]. Additionally, endothelial-specific overexpression of caveolin-1 also impairs VEGF microvascular permeability and angiogenesis [73]. These defects were associated with negative regulation of the PI-3K/Akt/eNOS signaling module, consistent with the established inhibitory action of cav-1 on eNOS [65] and PI-3K activity [74]. Altogether, these data support a role for caveolin in endothelial angiogenesis by regulating protein–protein interactions and activation.

2.4.3. Cardiac diseases

Two studies, performed on independently generated cav-1$^{-/-}$ mice, have reported cardiac diseases in knockout animals: dilated cardiomyopathy and pulmonary hypertension or cardiac hypertrophy [9,75]. Both studies found

right ventricular dilation, decreased systolic function and increased heart-to-body weight ratio. Moreover, they observed an up-regulation of ventricular atrial natriuretic factor (ANF) in caveolin-1-deficient mice which indicates a switch to fetal programming as observed in most forms of cardiac hypertrophia. However, whereas Cohen et al. described a significant left ventricular (LV) wall-thickening, Zhao et al. observed a thinning and dilation of the LV [9,75]. These discrepancies could be explained either by the genetic background of the mice or by the choice of anesthetic.

Because caveolin-1 is thought to act as a negative regulator of the p42/44 MAP kinase, Cohen et al. investigated the status of this cascade in hearts from cav-1$^{-/-}$ mice [76]. They found a hyperactivation of ERK1/2, which are major components of the p42/44 MAP kinase cascade. This finding supports conclusions from earlier in vitro studies that implicated caveolins/caveolae in MAPK activation [77,78]. It also correlates with results obtained from RNAi knock-down of caveolin-1 in *C. elegans* showing that interaction between caveolin-1 and the Ras/MAPK pathway might regulate the meiotic progression [3].

Although caveolin-1 expression in heart is restricted to endothelial cells and fibroblasts and absent from myocytes, caveolin-1$^{-/-}$ mice develop a cardiac disease similar to hypertrophic cardiomyopathy of humans. These phenotypes, as well as the ones described in caveolin-2 and caveolin-3 knockout mice (see below), point out the fact that caveolins/caveolae have a key role in maintaining signaling events in the cardiovascular system.

2.5. Pulmonary diseases

The high density of caveolae in the septa of lung tissue would suggest an important role of these invaginations in the physiology of the lungs. Indeed, lungs from cav-1$^{-/-}$ mice have constricted alveolar spaces, associated with a thickening of the alveolar wall [6]. These abnormal septa showed up-regulation of VEGF-R2 (Flk-1), a marker of non-differentiated endothelial and hematopoeitic cells, whereas von Willebrand factor (vWF), which marks differentiated endothelial cells, is not expressed [6,7]. Interestingly, the same phenotype is observed in caveolin-2 knockout mice [8], implicating the selective loss of caveolin-2 as the primary cause of these abnormalities (see below). The thickening of the septa in knockout animals may be increased by the deposition of extracellular matrix and by uncontrolled hyperproliferation of angioblastic cells [6,8]. This hypercellularity can be correlated with the excessive proliferation of MEFs derived from cav-1$^{-/-}$ mice that is observed in vitro and is consistent with studies presenting caveolin as a tumor suppressor [7]. As a consequence of these pulmonary abnormalities, caveolin-1 knockout mice are exercise-intolerant as demonstrated by the early onset of exhaustion during swimming tests [6,8].

2.6. Susceptibility to tumorigenesis

Caveolin-1 was first linked to cancer due to the fact that it is phosphorylated in *v-src* transformed fibroblasts [79] and that oncogene-mediated transformation of NIH-3T3 cells results in down-regulation of caveolin-1 and loss of caveolae [80]. Later, a number of studies supported a role for caveolin in the oncogenic cell transformation, tumorigenesis and metastasis (reviewed in [81]). Indeed, a dominant-negative mutation in the caveolin-1 gene (P132L) has been detected in 16% of human breast cancers [82].

Caveolin-1$^{-/-}$ mice do not develop tumors spontaneously, but present higher tumorigenicity when exposed to carcinogens compared to their wild-type littermates [7,83]. The tumor formation is preceded by a hyperproliferation of epidermal cell layers and accompanied by an increase in both cyclin D1 and ERK1/2 levels [83]. By interbreeding caveolin-1 knockout mice with tumor-prone transgenic mice (i.e., MMTV-PyMT expressing the polyoma middle T antigen under the control of the mouse mammary tumor virus promoter [84]), Williams et al. showed an accelerated onset of mammary tumors, with significant pulmonary metastases supporting the idea that caveolin-1 normally functions as a negative regulator of mammary tumorigenesis [85,86].

In contrast, caveolin-1 is thought to function as a tumor promoter in prostate cancers [87]. Indeed, genetic ablation of caveolin-1 delays advanced prostate tumor development in transgenic adenocarcinoma mouse prostate (TRAMP) mice [88].

Thus, biological functions of caveolin in cancer are complex and the mechanisms underlying these tissue-specific functions are still largely unknown.

2.7. Mammary gland development defects and premature lactation

The potential role for caveolin-1 in mammary gland function arose from screening approaches which identified caveolin as being down-regulated in human mammary adenocarcinoma-derived cells [89] and in a number of human breast cancer cell lines [90]. Further in vitro and in vivo evidences have supported the idea that caveolin-1 can act as a tumor suppressor in the mammary gland (see above) [90–92]. Additionally, caveolin-1 expression is significantly down-regulated in the mammary gland during late pregnancy and lactation [93]. This down-regulation of cav-1 is mediated by the prolactin receptor (Prl-R) signaling cascade via a Ras p42/44 MAPK-dependent mechanism that inhibits cav-1 transcription. Park et al. further demonstrated that caveolin-1 expression blocked prolactin signaling, therefore, functioning as a suppressor of cytokine signaling in the mammary gland [94], akin to the SOCS family of proteins. Finally, physiological relevance for such an inhibitory activity was provided by cav-1$^{-/-}$ mice which

showed an accelerated development of mammary gland and premature lactation during pregnancy because of the hyperactivation of the prolactin signaling cascade [94]. Because a similar premature lactation phenotype is observed in SOCS-1$^{-/-}$ mice, it would be interesting to further question the respective role of each protein in the context of mammary gland development and lactation.

3. Caveolin-2 knockout mice

Cav-2$^{-/-}$ mice have been generated by the disruption of two first exons of the gene which resulted in the loss of the three caveolin-2 isoforms (α, β and γ) [8] (see Fig. 1). The generation of these mice allowed to distinguish between the defects that can be attributed either to the lack of caveolin-1 or of caveolin-2 in caveolin-1-deficient mice, since in the latter mice, caveolin-2 protein levels were dramatically reduced.

Although caveolin-2 is co-expressed and can hetero-oligomerize with caveolin-1 in many cell types, in the absence of caveolin-2, caveolin-1 is properly localized at the plasma membrane and can still form caveolae. Cav-2$^{-/-}$ mice do not show vascular system dysfunctions or lipid disorders like the cav-1$^{-/-}$ mice [6,7,40]. However, caveolin-2-deficient mice display severe pulmonary dysfunction with alveolar septal thickening, endothelial cell hyperproliferation and exercise intolerance [8]. These defects, also observed in cav-1$^{-/-}$ mice, can thus be specifically attributed to the loss of caveolin-2 and, most importantly, are not connected to a loss of caveolae.

4. Caveolin-3 knockout mice

4.1. Loss of sarcolemmal caveolae

Caveolin-3, a muscle-specific form of caveolins [95], shares about 65% identity and 85% similarity with caveolin-1. Caveolin-3 is expressed in most of muscle tissue types (diaphragm, heart, skeletal and smooth muscle) and its expression increases dramatically during the differentiation of skeletal myoblasts in culture [95]. Two independent groups reported the generation of caveolin-3-deficient mice by using targeted disruption of exon 2 (Fig. 1) which encodes the caveolin-scaffolding and membrane-spanning domains and the C-terminal region of the protein [4,5].

The lack of caveolin-3 protein expression results in the loss of caveolae in skeletal muscle fibers without any disturbances in caveolin-1 and -2 expressions or formation of caveolae in non-muscle tissues. The finding that the density of sarcolemmal caveolae is dependent on the amount of caveolin-3 is further supported by the report that transgenic overexpression of caveolin-3 causes an increase in the number of caveolae [96]. This suggests that caveolin-3 is required for caveolae formation in skeletal muscle cells in vivo.

4.2. Mild myopathic changes in skeletal muscle

Hagiwara et al. and Galbiati et al. reported that, on the whole, there were no differences in growth and movement between wild-type and mutant mice [4,5]. However, some pathological lesions which mainly took place in the soleus muscle and the diaphragm, and which were characterized by muscle degeneration, could be observed. These mild myopathic changes are similar to those described in patients suffering from limb-girgle muscular dystrophy-1C (LGMD-1C) caused by a mutation in exon 2 of the human cav-3 gene (3p25) [97]. This mutant caveolin-3 protein acts as a dominant-negative inhibitor, oligomerizing with wild-type caveolin-3 and directing these complexes to proteosomal degradation [98,99]. Consequently, LGMD-1C mutations leads to a reduction of caveolin-3 expression by about 90–95% [97]. Moreover, other human caveolin-3 mutations have been reported; they result in four muscle disease phenotypes: limb girdle muscular dystrophy, rippling muscle disease, distal myopathy and hyperCKemia (reviewed in [100]). Most of these mutations are in residues that cause a dominant-negative version that sequesters the wild-type caveolin-3 [101,102]. On the contrary, caveolin-3 expression is elevated in Duchenne muscular dystrophy [103] and in its genetic homologue mdx mouse that has a deficiency in dystrophin [104]. Taken together, all these observations suggest that proper caveolin-3 expression is required for normal muscle function.

Similar to caveolin-1, caveolin-3 is thought to function as a scaffolding protein concentrating many signaling molecules, in particular components of the dystrophin-glycoprotein complex (DGC) [105]. Despite the normal expression or localization of dystrophin, α-sarcoglycan and β-dystroglycan in skeletal muscle fibers from cav-3$^{-/-}$ mice, the dystrophin–glycoprotein complex is no longer associated with DRMs in the absence of caveolin-3 [5]. Future investigations should clarify if the caveolar localization of components of the dystrophin–glycoprotein complex is critical for the maintenance of the structural integrity of skeletal muscle.

4.3. Cardiomyopathy

Hearts from caveolin-3 knockout mice displayed a mild-to-moderate cardiomyopathy characterized by cardiac hypertrophy [106], a phenotype that was also reported in mice overexpressing caveolin-3 [107,108]. Indeed, the cardiac hypertrophy phenotype in caveolin-3 knockout mice is associated with an hyperactivation of the p42/44 MAPK cascade as observed in caveolin-1 knockout mice [76], supporting the role of caveolin-1 and -3 as negative regulators in this pathway [77,78].

4.4. T-tubule system disorganization

Early morphological studies suggested that T-tubules system is formed from the characteristic muscle-chains of caveolae [109]. Later, caveolin-3 was shown to be associated transiently with T-tubules during the differentiation of primary cultured cells and the development of mouse skeletal muscle fibers [110]. Studies of the T-tubule system in skeletal muscle fibers from cav-$3^{-/-}$ mice revealed that two T-tubule marker proteins (dihydropyridine receptor-1α and ryanodine receptor) are diffusely localized or mislocalized in caveolin-3-deficient mice, reflecting a disorganized immature T-tubule system with dilated and longitudinally oriented T-tubules [5]. Recent work using immortalized skeletal muscle precursor cells from caveolin-3 transgenic and deficient mice showed the importance of caveolin-3 in myoblasts fusion to myotubes [111]. Indeed, caveolin-3-dependent defects of myoblasts fusion may represent important molecular mechanisms underlying the muscle damage observed in DMD and LGMD-1C in humans. This is also supported by the fact that due to caveolin-3 deficiency, a striking disorganization of the T-system in patients suffering from LGMD-1C was observed [112].

5. Caveolin-1/caveolin-3 double-knockout

In order to create truly caveolae-deficient mice, lacking plasmalemmal as well as sarcolemmal caveolae, Park et al. interbred caveolin-1-deficient mice and caveolin-3-deficient mice to generate cav-1/cav-3 double-knockout (cav-$1^{-/-}$/-$3^{-/-}$) mice [113]. As expected, these double-knockout mice failed to form caveolae in all cells. Similar to single knockout animals, double-knockout animals are viable and fertile. Cav-$1^{-/-}$/-$3^{-/-}$ mice exhibited lung, fat and skeletal defects of comparable severity to their single-knockout counterparts. However, these cav-$1^{-/-}$/-$3^{-/-}$ mice develop more severe cardiomyopathy [9,75,106]. This finding stresses the importance of caveolae in the heart, where the loss of the normally high caveolin expression in all cell types leads to the development of a severe cardiac pathology.

6. Conclusion

The generation of animal models that are deficient in different caveolin genes have given some important insights into the function of caveolae. One of the striking observations is that caveolae, as morphological entities, are dispensable for the functioning of the cell. The absence of caveolae or caveolins, however, can impair diverse signaling processes as well as homeostasis of lipids. As a consequence, disorders in muscle, pulmonary or lipidogenic tissues are observed. These effects are especially prominent in ageing animals, indicating the cumulative effect of small deficiencies.

Caveolin knockout mice have provided excellent models to study the relevance of physiological functions assigned to caveolae. However, many open questions remain. Although it is now clearly established that caveolin is essential for caveolae biogenesis, the molecular basis underlying the formation of these structures is still unknown. Moreover, caveolin disruption leads to pleiotropic effects suggesting its implication in diverse cellular processes depending on the tissue. How do caveolae and caveolins organize as signaling platforms and how is their tissue-specific activity regulated? Unraveling the basis of all these mechanisms will constitute an important challenge for the future.

Another conclusion derived from the analysis of these knockout animals is that the organism has powerful compensatory mechanisms for overcoming deficiencies in a particular gene and even an organelle. Only this allows the robust functioning of an organism as a whole. Understanding these compensatory mechanisms will be a wide field for future investigations.

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