Caveolin Involvement and Modulation in Breast Cancer

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Abstract: Caveolae are highly enriched in numerous membrane-bound proteins and caveolin-1 is their major component. Caveolae and caveolin proteins are involved in a variety of cellular processes including lipid homeostasis, endocytosis, signal transduction, and tumorigenesis. Breast cancer is one of the most common cancers in women throughout the world. Clinical studies have shown that the correlation of caveolin-1 expression with tumor progression varies with tumor type. The data presented here extend the findings that caveolin-1 suppresses breast cancer but there are controversial studies. The potential function of caveolin-1 in scaffolding signaling factors also demonstrates the importance of its expression control and modulation, correlating with physiological or pathological conditions. Based on current research, this review presents the current understanding of their function and the involvement of caveolin-1 in breast cancer pathogenesis.

Keywords: Caveolin-1, breast cancer, caveolae.

INTRODUCTION

Caveolae, also called “little caves” due to their flask-like invagination form, are located in the plasma membrane with an average diameter of 50–100 nm, and were identified more than 50 years ago [1, 2]. Their major component, caveolin protein, was detected in 1992 [3]. The proteins are characterized by a size from ~21 to 24kDa, occupy up to 20% of the plasma membrane and are normally expressed at high levels in adipocytes, stromal cells and normal mammary epithelia, and are probably absent in lymphocytes and mature neurons [4-8]. Moreover, their number, density and morphology can vary greatly depending on the specific cell type and physiological status of the cell [8].

Caveolin-1 belongs to a highly conserved gene family and is co-expressed with caveolin-2 in cells and tissues. The caveolin-1 gene consists of three exons that are alternatively translated into the endoplasmatic reticulum as a full-length 178 aminoacid α-isoform and β-isoform, lacking the first 32 aminoacids. The protein also has a hydrophobic putative membrane-spanning sequence and three palmitoylation sites at the C terminal domain, that contribute to stabilizing the characteristic hairpin loop formation of the protein associated with the membrane, providing that both the C and N regions are exposed to the cytoplasm [8]. It binds to cholesterol and sphingolipids within “lipid rafts” which are considered as specialized ‘‘detergent-insoluble cholesterol- and glycolipid-rich” (DIG) membrane microdomains and it appears to have diverse functions, including vesicular transport, cellular cholesterol balance maintenance, and signal transduction [9-11] (Table **1**).

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It is a complex structure due its interaction with many others cell components, such as NF-kappaB, a pleiotropic transcription factor, that controls many gene expressions and plays an essential role in immune response and cellular adhesion. It also inhibits apoptosis and favors cancer cell survival. MCF7 cells show caveolin-1 as a target gene of NF-kappaB after stimulation with TNF-alpha and IL-1beta, demonstrating that it controls genes involved in tumor angiogenesis and cell transformation [19].

Caveolin-1 also serves as a scaffold for several proteins that can thus be concentrated within caveolae membranes [8]. This scaffold function is due to interaction with partner proteins within its ‘scaffolding’ domain (CSD) (aa 82–101) that binds short peptide motifs rich in aromatic residues [20].

CAVEOLIN AND TUMORIGENESIS

There are reports in the literature that suggest involvement of caveolin-1 in tumorigenesis and conflicting results have been reported about its expression in tumors [7; 21]. Furthermore, it is still unclear whether caveolin-1 acts as tumor suppressor or as an oncogen and there is evidence that cav-1 exerts an ambivalent role in tumorigenesis [22]. These contradictory results probably reflect the dynamic changes of caveolin-1 expression during oncogenic transformation [23] and its expression level depends on the type of neoplastic cell investigated [24]. Indeed, it may exercise tumor suppressor activity by inhibiting the signaling products of several proto-oncogenes [25]; on the other hand, its tyrosine-14 phosphorylation results in growth stimulation [26], suggested that cav-1 may also perform as a protumorigenic factor.

This dual action of caveolin-1 as a tumor suppressor or an oncoprotein may be due to its scaffolding domain or the phosphorilation of the aminoacid tyrosine-14. Historically, caveolin-1 was first described as a major tyrosine

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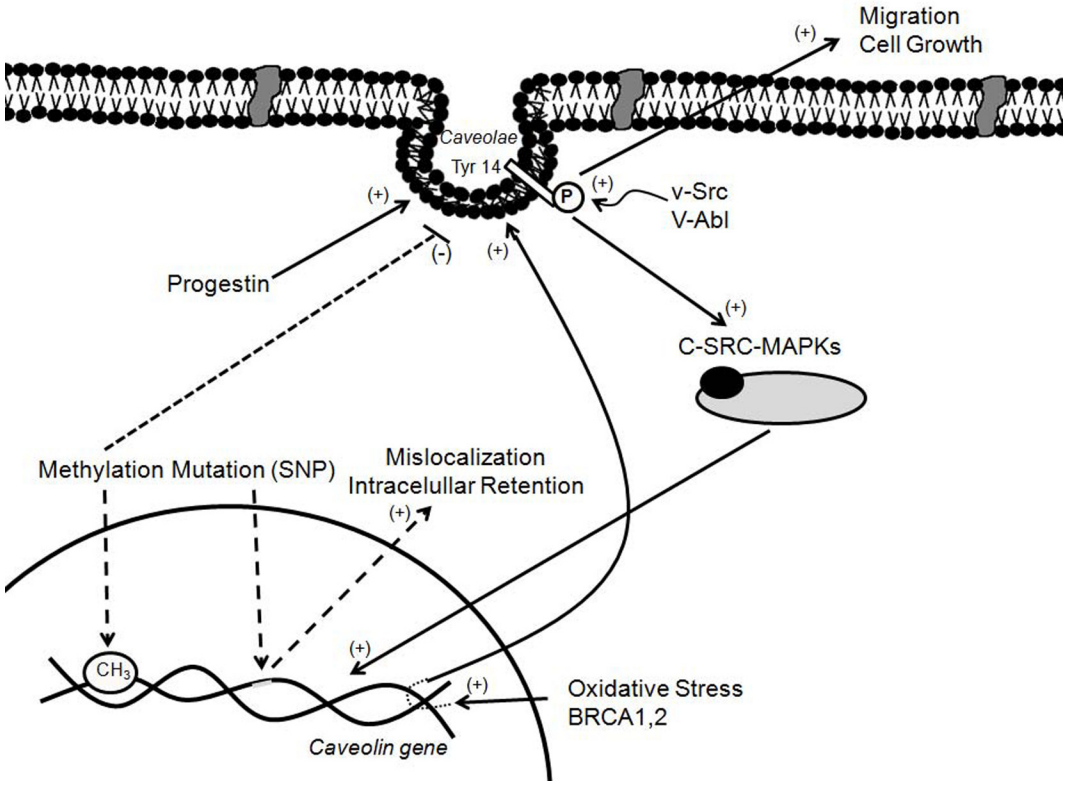

Fig. (1). Schematic overview of biological effect of Caveolin 1. CAV1 protein phosphorilated at tyrosine 14 by v-Src and v-Abl and its subsequent reactions that, acting like a oncogene. A single nucleotide polymorphism (SNP) in the caveolin1 gene, resulting in a change in the aminoacid acts in a dominant-negative manner, causing mislocalization and intracellular retention. BRCA1 hereditary breast cancer can express CAV1. Oxidative stress induces up-regulation of caveolin-1.

phosphorylated protein in v-Src-transformed chicken embryo fibroblasts and may represent a critical target during cellular transformation [27]. At steady state, caveolin-1 is not phosphorylated on tyrosine 14 [28], a situation that contrasts with v-Src transformed cells where it is constitutively phosphorylated on tyrosine 14 [29]. However, caveolin tyrosine 14 phosphorylation also occurs in normal cells but in a tightly regulated fashion [30]. Phosphorylation on tyrosine 14 leads to 2-fold anchorage-independent growth and foci formation in 293T cells. When these cells expressed c-Src, caveolin-1 and growth factor receptor-bound protein 7 (Grb7), foci formation was 7-fold and cell migration 2- to 3-fold compared to cells without caveolin-1 expression. In contrast, coexpression of c-Src, Grb7 and caveolin-1 Y14A, with a change of the tyrosine in position 14 for alanine, had little or no effect on foci formation and cell migration [26].

Caveolin-1 has a scaffolding domain (CSD) located between aminoacids 82-101, a region that has the ability to kidnap and compartmentalize activators and effectors that regulate cellular signaling and may play an important role in its regulation of tumor progression [31]. Growth factor receptors (ex: EGFR), endothelial nitric oxide synthase (eNOS), G proteins, G-protein coupled receptor, c-Neu and H-Ras are examples of molecules that can be influenced by CSD [32-38]. The eNOS act as a target downstream of activated Ras and Akt that is required for tumour growth and maintenance [39], c-Neu is a cell surface protein-tyrosine kinase receptor that is found to be overexpressed in a significant number of adenocarcinomas [40] and EGFR is a cell surface that leads to DNA synthesis and cell proliferation in Wilms tumor [41].

Nevertheless, the role of caveolin-1 in cancer development still remains unclear, but most research shows that it can act a tumor suppressor gene and influence the tumorigenese process. Some facts have been reported that support this idea, such as its genetic location at human chromosome 7, region q31.1, locus D7S522, frequently deleted in a variety of human cancers, including head and neck, prostate and breast [37], and the observation that oncogene-transformed and tumor-derived cells present a down-regulation of caveolin-1 expression [42] and oncogene overexpression, including H-ras, v-abl, and bcr-abl that present an inverse correlation with its expression [43].

Caveolin-1 protein can be regulated in some forms so that its cellular location and function are modified, such as the phosphorylation of the amino acid Tyr-14 [26]. Furthermore, expression of p21 is regulated by caveolin-1 phosphorylation in a p53-dependent manner [44]. It also modulates store-operated Ca(2+) entry (SOCE), shown by Zhu *et al*. [45] using Hs578/T breast cancer cells, expressing three different caveolin-1 protein levels, generated by overexpression and shRNA knockdown. Overexpression could increase SOCE activity, while caveolin-1 knockdown significantly reduced SOCE activity.

Table 1. Caveolae Functions Recently Related in Various Studies

Reference	Caveolae function	Observation
Chang et al., 2009 [12]	Vascular permeability	Caveolae are thought to be the transcellular pathway by which plasma proteins cross normal capillary endothelium
Rivera et al., 2009 [13]	Vessel formation	Coordinated role in determining vessel identity, not only during embryogenesis but also during adult vascular remodeling and angiogenesis.
Balijepalli et al., 2009 [14]	Ion channel control	Several ion channels and exchangers have been localized at caveolae, and others have been associated with noncaveolar lipid rafts in different cells types
Burgermeister et al., 2008 [7]	Cell adhesion	Targeted to zonula, in order to maintain epithelial and endothelial barriers in vitro and in vivo
Medina et al., 2007 [15]	Infection pathway	Viruses, bacteria, toxins, and parasites have evolved to co-opt this function of the caveolae in order to gain access to mammalian cells
Luoma et al., 2008 [16]	Signaling	Essential role in membrane estrogen receptor function in non-neuronal cell types and nervous system
Trigatti et al., 1999 [17]	Lipid regulation	Lipid regulation in adipocytesas, as well as in other cell types. Interacts with cholesterol and binds to fatty acids
Vogel et al., 2006 [18]	Mechanosensors	Flow sensors in endothelial cells. The surface of endothelial cells is sensitive to changes in hydrostatic pressure and shear stresses are related with caveolae

Caveolin-1 transfection into MCF7 cells resulted in less proliferation on the fourth day regarding the cell growth rate. A soft agar assay of these transfectants showed less growth with a lower number of colonies [46].

Caveolin-1 can also influence anoikis, an important step in the metastasis process, and enhances matrix-independent cell survival that is mediated by upregulation of the IGF-I receptor expression and signaling in MCF7/cav-1 cells. Caveolin-1 inhibition is associated with suppression of detachment-induced activation of p53 and of the consequent induction of the cyclin-dependent kinase inhibitor p21 [47]. Cell cycle progression regulation and activation of the apoptotic signaling molecules Bcl2, p53, and p21 are also caveolin-1 roles that sensitize cells to apoptosis [44].

Razandi *et al.* [48] showed the co-location of ER-alpha with the caveolae structural coat protein, indicating that caveolin-1 can interact with this receptor and is capable of facilitating its translocation to the membrane. The deregulation of caveolin-1 can interfere in ER-alpha expression and its activation at the start of breast tumorigenesis [49]. They also observed that ER-alpha, but not ER-beta, expression was constitutively activated in caveolin-1 haploinsufficient cells. Treatment of these cells with beta-methyl-cyclodextrin, a chemical that can displace caveolin-1 from the plasma membrane, stimulated ER-alpha expression. Caveolin-1 dominant-negative mutations were found exclusively in ER-alpha positive breast cancer samples and ER-alpha expression was increased in cav1 -/- null mammary epithelia. Estrogen stimulation further enhanced the growth of cav-1-deficient three-dimensional epithelial structures. In addition, when caveolin-1 was inactivated, it induced the accumulation of a cell population with the characteristics of adult mammary stem cells, promoted premalignant alterations in mammary epithelia and induced increased ER-alpha expression levels [50].

The caveolin-1 regulation and expression levels can also represent important and complex factors in homeostasis and diseases and may also play a role in cell resistance to drugs and treatments. Its overexpression in Hs578T doxorubicin resistant cells, which contain low levels of endogenous caveolin-1 and high levels of P-glycoprotein, resulted in a 97% and 64% reduction in resistance to doxorubicin and cisplatin, respectively. It also resulted in a significant decrease in P-glycoprotein activity [51, 52] suggesting a possible physical interaction between caveolin-1, P-glycoprotein [51] and breast cancer resistance protein in the caveolae membrane [53].

It is known that oxidative stress influences caveolin-1 expression, as reported by Dasari *et al.* [54], who showed that subcytotoxic oxidative stress generated by hydrogen peroxide application promoted premature senescence and stimulated the activity of a caveolin-1 promoter reporter gene construct in fibroblasts. It induced p38-mediated up-regulation of caveolin-1 and premature senescence in normal human mammary epithelial cells.

CAVEOLIN-1 IN BREAST CANCER

The influence of caveolin-1 expression in breast cancer appearance, development and progression remains controversial, and mutations on it have been observed in some breast cancer studies. A single nucleotide polymorphism (SNP) in the caveolin-1 gene, resulting in a change in the aminoacid proline for lisine at position 132 (P132L), was detected in 16% of 92 primary human breast cancers [55]. Li *et al.* [56] studied this mutation and found a similar mutation rate, but only in estrogen receptor alpha-positive breast cancers, not in ER-alpha-negative breast cancer patients. They also found that caveolin-1 mutation reaches 35% in breast cancer, including six novel SNP (W128Stop, Y118H, S136R, I141T, Y148H, and Y148S). Lee *et al.* [57] transfected and expressed caveolin-1 P132L

in HEK293 and Cos-7 cells, showing that this cellular genotype leads to the formation of misfolded caveolin-1 oligomers that are retained within the golgi complex, that it are not targeted to caveolae or the plasma membrane. They confirmed that the P132L allele acted in a dominant-negative manner, causing P132L/WT cav-1 mislocalization and intracellular retention.

BRCA1 belongs to a class of genes known as tumor suppressors, which maintains genomic integrity to prevent uncontrolled cell proliferation. The multifactorial BRCA protein product is involved in DNA damage repair, ubiquitination, transcriptional regulation and other functions. Many papers have been published that focus cancer risk and BRCA mutations. It is known that caveolin-1 expression in breast cancer may be influenced by parameters, such as BRCA1 or BRCA2 mutations. Pinilla *et al*. [58] showed for the first time cav-1 expression in BRCA1 and BRCA2 hereditary breast cancer and identified cav-1 as a marker associated with a basal-like phenotype in both hereditary and sporadic breast cancer.

Caveolin-1 can also act as a stimulation factor for BRCA1 protein and mRNA levels, *via* a mechanism that involves transactivation of the BRCA1 promoter and p53-dependent [59]. The other possible way would be the increase of caveolin-1 mRNA by BRCA1 levels *via* transactivation of the caveolin-1 promoter region. Additionally, BRCA1 might inhibit the invasiveness and metastatic abilities of mammalian cells by inducing the redistribution of caveolin-1 from the cytoplasm to the cell membrane [60]. MPA (medroxyprogesterone acetate), a synthetic progestin, increased caveolin-1 expression in tumors treated with it, an effect abolished by pre-treatment with progestin antagonist RU486, demonstrating that caveolin-1 expression was upregulated by progestin [61].

Caveolin expression was found in 13.4% of invasive breast cancer cases and was strongly associated with high histological grade, lack of steroid hormone receptor positivity, and expression of basal markers [62]. When caveolin-1 mRNA and protein were inactivated by approximately 50% in human breast epithelial cells, using the retrovirus-mediated poly-A gene trapping approach, significant tumor formation was not induced when tested in nude mice, but it might lead to partial transformation [63].

Using tissue microarray, Liedtke *et al.* [64] found caveolin-1 expression in almost 30% of invasive breast carcinoma, contrary to that observed in normal breast tissue epithelial cells, benign breast disease and ductal carcinoma in situ, where caveolin-1 expression was not detected.

When caveolin cDNA linked to the CMV promoter was transfected into human mammary cancer cells that had no detectable endogenous caveolin, substantial growth inhibition was observed, as seen by the 50% decrease in growth rate and approximately 15-fold reduction in colony formation in soft agar [4].

Wu *et al*. [65] also observed that MCF7/cav-1 transfected cells inhibited invasion and migration and a dramatic delay in tumor progression was verified. They also showed with *in vivo* experiments, employing xenograft tumor models, that caveolin-1 expression resulted in significant breast tumor growth inhibition. The same procedure was used by Glait *et al.* [66] who reported a higher level of IGF-IR protein and mRNA. This transcriptional activation required an intact p53 signaling pathway, since cav-1 was unable to raise IGF-IR levels in p53-null cells.

The expression regulation of the first and second exons of the caveolin-1 gene may be controlled, in part, by methylation. The CGs in the 5' promoter region were functionally methylated in two human breast cancer cell lines (MCF7 and T-47D). In contrast, the same CGs in cultured normal human mammary epithelial cells were non-methylated and these cells expressed high levels of caveolin-1 protein [67].

Caveolin-1 was identified in a screening for genes involved in breast cancer progression and expression of high levels of caveolin-1 also inhibited subsequent metastasis to distant organs [68].

In a clinical study, methylation CpG-island in the caveolin-1 promoter of breast cancer samples was 25.5%, different from 7.3% observed in non-cancer cells. Immunohistochemistry demonstrated that expression of the caveolin-1 gene was correlated with aberrant promoter methylation status [69].

Many studies have been carried out using animal approaches. Cav1-deficient mice showed that inactivation of its gene expression led to mammary epithelial cell hyperplasia, even in 6-week-old virgin female mice [57]. The same procedure was used by Williams *et al.* [24] who showed that mammary epithelia were hyperproliferative, with dramatic increases in terminal end bud area and mammary ductal thickness. Cav-1 -/- mammary stromal cells promoted the growth of both normal mammary ductal epithelia and mammary tumor cells, showing that its expression in both epithelial and stromal cells provided a protective effect against mammary hyperplasia and mammary tumorigenesis.

Primary cultures of mammary epithelial cells derived from caveolin-1 -/- mice were used to identify the role of caveolin-1 in the maintenance of the normal architecture of the mammary acinar unit. This culture presented defect in three-dimensional acinar architecture, including disrupted lumen formation, epidermal growth factor-independent growth and highlighted the ability of growth factors to induce mammary acini branching, indicative of a more invasive fibroblastic phenotype [50].

In caveolin-1 knockout mice interbred with tumor-prone transgenic mice (MMTV-PyMT), that normally develop multifocal dysplastic lesions, a dramatic acceleration was observed of the development of these multifocal dysplastic mammary lesions, even in 3 and 4 week-old animals. It was also observed that cyclin D1 expression levels were extremely high in these null mammary lesions [24].

It has been hypothesized that caveolin-1 in tumor-associated stroma modulates paracrine signaling with tumor cells, leading to a permissive environment for tumor cell proliferation, migration, and local invasion [70]. Primary

cultures of mouse Cav-1 -/- mammary epithelial cells exhibited premalignant changes, such as abnormal lumen formation, epidermal growth factor-independent growth, defects in cell substrate attachment and increased cell invasiveness [71].

CAVEOLIN-1 EXPRESSION IN BREAST CANCER

The role of caveolin-1 in tumorigenesis has been the focus of many studies, but they also have divergent results. Table **2** shows some research made using human breast cancer samples, correlating the caveolin-1 expression level in normal tissues with the clinical stage of the disease.

Caveolin-1 expression in human breast cancer was diminished in some studies. Expression was significantly reduced in human breast cancer cells compared with their normal mammary epithelial counterparts [4]. Using Western Blot, cav-1 expression in breast cancer samples was suppressed compared to normal tissues. Immunohistochemistry revealed that cav-1 proteins were abundantly expressed in mammary gland myoepithelial cells, but only weakly in ductal epithelial cells [77].

Using cancer-associated fibroblasts and normal mammary fibroblasts from human breast cancer lesions, isolated from the same patient, Mercier *et al*. [73] demonstrated that cancer-associated fibroblasts showed dramatic downregulation of caveolin-1 protein expression. The replacement of cav-1 expression in cancer-associated fibroblasts was sufficient to revert their hyper-proliferative phenotype.

Increased expression of caveolin-1, both at the mRNA and protein levels, was found in inflammatory breast cancer cell lines and in human samples of inflammatory breast

Table 2. Caveolin1 Expression Levels in Studies Showing Conclusions Obtained, Method Used and Observations

Study	Expression	Conclusion	Method	Observation
Witkiewicz et al., 2009 [72]	↓	Highly predictive of recurrence and progression to invasive breast cancer	IH	Stromal cav1 level in ER+ Ductal Carcinoma in situ patients
Lee et al., 1998 [4]	↓	Expression significantly reduced in human breast cancer cells	NB/WB IFM	
Sagara et al., 2004 [79]	↓	Cav1 inversely correlated with tumour size, and with hormonal receptor status	qPCR WB/IH	
Mercier et al., 2008 [73]	↓	Highlights the critical role of Cav-1 downregulation in maintaining the abnormal phenotype of human breast cancer-associated fibroblasts	WB MA	Cancer-associated fibroblasts from human breast cancer, compared with normal mammary fibroblasts
Sloan et al., 2009 [70]	↑	Stromal caveolin-1 expression may be a potential therapeutic target and a valuable prognostic indicator of breast cancer progression	IH	Expression in breast luminal epithelium
Perrone et al., 2009 [74]	Not against control	Significant difference between lobular intraepithelial neoplasia and invasive lobular carcinoma; it may play a role in the progression of human breast lobular cancer	IH	Whole human lobular neoplasia spectrum disease
Elsheikh et al., 2008 [62]	Not against control	Expression associated with high histological grade, lack of steroid hormone receptor positivity, and expression of basal markers. Significant association with basal-like phenotype.	TMA	Unselected invasive breast cancer cases
Liedtke et al., 2007 [64]	↑	A significant increase in expression was observed comparing invasive breast cancer to both benign breast tissue and non-invasive breast cancer	TMA	
Savage et al. 2007 [75]	↑	Overexpression associated with high histologic grade, proliferation rates, shorter DFS.	IH IFM IEM	
Van den Eynden et al., 2006 [76]	↑	Overexpression of caveolin-1 and -2 in inflammatory breast cancer (IBC) cell lines and in human samples of IBC	MA qPCR IH	Human IBC and non-IBC samples

IH: Immunohistochemistry; NB: Northern blot; WB: Western blot; IFM: Immunofluorescence microscopy; qPCR: Real time PCR; MA: Microarray; TMA: Tissue microarray; IEM: Immunoelectron microscopy.

cancer, the most aggressive form of locally advanced breast cancer [76].

Caveolin-1 Modulation by Molecules

The caveolin-1 genotype and expression may be a useful marker in cancer emergence, prognosis and treatment. Assuming that caveolin-1 aminoacid 14 may be polymorphic and mostly represented by tyrosine, this variation may play a role in these factors. The already known phosphorilation of this aminoacid results in cell growth stimulus that can be observed in transformed cell lines and tumor samples. An interesting approach in cancer patients could be the genotypic determination and modulation of caveolin-1 expression and the activity of tyrosine 14 protein [77, 78].

Nowadays there are many ongoing studies to determine substances including endogenous, dietary or drugs that may modulate and control caveolin-1 expression, such as in physiological or pathological conditions. There are few studies involving its expression control and modulation in cancer.

Conjugated linoleic acid is a group of biologically active fatty acids that exhibits anticarcinogenic properties, but this mechanism is still poorly understood. Huot *et al*. [80] studied a breast cancer lineage and proposed the hypothesis that it may be due to alteration in the caveolae lipid composition and function. They found that conjugated linoleic acid (CLA) was readily incorporated into caveolae. Buitrago & Boland [81] observed that c-SRC, p38/MAPK and ERK 1/2, participants of the growth stimulus pathway, were stimulated by vitamin D_3 (1α,25-dihydroxyvitamin D_3). This effect was not observed when cells were treated with a caveolae disrupting agent, suggesting that caveolae were involved upstream in c-Src-MAPKs activation by vitamin D_3.

Another study involving cancer was performed by Liu *et al*. [82] who showed that bradykinin increases the permeability of the blood-tumor barrier selectively through the transcellular pathway and caveolin-1 and caveolin-2 levels increased 5 min after bradykinin infusion, peaking at 15 min, showing its involvement in the molecular mechanism of opening the blood-tumor barrier by bradykinin.

Heterologous expression of caveolin has been shown to abrogate anchorage-independent growth and induce apoptosis in transformed fibroblasts and also to suppress anchorage-independent growth in human mammary carcinoma cells. A number of studies suggest that caveolin could function as a tumour suppressor. Caveolin-1 was, however, highly expressed in breast myoepithelial cells and its expression was retained in tumours derived from breast myoepithelium [83].

Yi *et al*. [84] investigated the effect of aspirin on high glucose-induced endothelial cell senescence and observed that it increased NOS activity and NO levels. Consistent with these findings, caveolin-1 expression and caveolin-1/eNOS interaction decreased. The anti-senescent effects of aspirin were by increased NO production *via* the up-regulation of NOS activity that prevented caveolin-1 expression.

Using other models, substances and molecules were administered to different systems to modulate caveolin-1 expression. Green tea polyphenols reduced the levels of caveolin-1 mRNA and protein expression and phosphorylated ERK1/2 expression in microvessel fragments in rats with cerebral ischemia [85]. Conversely, inhalation of the anesthetic isoflurane increased caveolae/caveolin formation in the buoyant membrane of the human renal proximal tubule [86]. An approach modifying caveolin-1 status was proposed by Suh *et al*. [87], where HMG-CoA reductase inhibitor activated the eNOS by phosphorylation related to decreased caveolin-1 abundance, suggesting therapeutic strategies for high blood pressure-associated endothelial dysfunction.

General anaesthetics might disturb the caveolae lipid composition or ordered structure, altering protein–protein interactions or the proximity between signaling proteins, with potential consequences on downstream signaling. Caveolae could well be an important link reconciling discrepant results on the circulatory effects of general anaesthetics. Accumulating evidence obtained either in caveolae research or in anaesthesia research has suggested that caveolae might be disturbed by volatile anaesthetics. It has been suggested that the endothelium-dependent effects of anaesthetics on the cardiovascular system may be caveolae-mediated [88].

In the light of recent developments in caveolae research, a better comprehension of the role of caveolae in the vasculature and how they mediate their activity is needed [89].

The functional subunit of the cystine/glutamate transporter xc- system (xCT) plays a critical role in the maintenance of intracellular glutathione and redox balance. It was verified that caveolin-1 was upregulated and beta-catenin was recruited to the plasma membrane when xCT was deficient and the inhibition of beta-catenin transcriptional activity followed. Further study revealed that caveolin-1 upregulation and tumor cell invasion inhibition were mediated by reactive oxygen species-induced p38 MAPK activation. [90].

Using preclinical cell models for the transition of oestrogen-sensitive (WT-MCF-7 cells) to a tamoxifen-resistant (TAM-R cells) phenotype, Thomas *et al*. [91] examined the role of caveolin-1 in the development of hormone-resistant breast cancer. The WT-MCF-7 cells showed abundant expression of caveolin-1 which potentiated oestrogen-receptor (ERalpha) signalling and promoted cell growth despite caveolin-1 mediating inhibition of ERK signalling. Caveolin-1 expression was negligible in the TAM-R cells, repressed by EGF-R/ERK signalling. Pharmacological inhibition of EGFR/ERK in TAM-R cells restored caveolin-1 and also resulted in the emergence of pools of phosphorylated caveolin-1. Hyperactivation of EGFR/ERK is a feature of tamoxifen-resistant breast cancer cells, a principal driver of cell growth. In this context, these studies defined a novel role for caveolin-1 with implications for the clinical course of breast cancer and identified caveolin-1 as a potential drug target for the treatment of early oestrogen-dependent breast cancers. Further, caveolin-

1 loss may have benefit as a molecular signature for tamoxifen resistance.

It has been reported that filamin A and caveolin-1 co-exist in a complex and the presence of active phospho-Akt has been shown in this complex. Ser-2152 phosphorylation of filamin A has been implicated in cancer cell migration. Accordingly, caveolin-1 expression dramatically enhanced IGF-I-dependent MCF7 cell migration. These data indicated that caveolin-1 specified filamin A as a novel target for Akt-mediated filamin A Ser-2152 phosphorylation thus mediating the effects of caveolin-1 on IGF-I-induced cancer cell migration [89].

Classically, basal-like breast cancers have been characterized by low expression of ER, PR and HER2 neu and high expression of CK5, CK14, caveolin-1, CAIX, p63, and EGFR (HER1), which reflects the mammary gland basal/myoepithelial cell component. In the future, a gene array platform with greater sensitivity for distinguishing the various breast cancer subtypes with the power to predict the molecular biology of the disease, will be an indispensible tool for treatment selection [92].

Stromal caveolin-1 expression can be used to stratify human breast cancer patients into low-risk and high-risk groups and to predict their risk of early disease recurrence at diagnosis. When tamoxifen-treated patients were selected, an absence of stromal cav-1 was a strong predictor of poor clinical outcome, suggestive of tamoxifen resistance. It was concluded that cav-1 functions as a tumor suppressor in the stromal microenvironment [73]. The development of the breast is extremely sensitive to interactions between the epithelium and stroma. Upcoming prevention, diagnostic and therapy strategies and studies should be carried out in an unbiased way, allowing analyses of the stromal compartment in addition to the classical investigations of the epithelial cancer component [91].

Results reported by Martinez-Outschoorn *et al*. [92] suggested that cytokine production and inflammation were key autophagy drivers in the tumor microenvironment. These results may explain why a loss of stromal cav-1 is a powerful predictor of poor clinical outcome in breast cancer patients, as it is a marker of both autophagy and inflammation in the tumor microenvironment. Lastly, hypoxia in fibroblasts was not sufficient to induce the full-blown inflammatory response that we observed during the co-culture of fibroblasts with cancer cells, indicating that key reciprocal interactions between cancer cells and fibroblasts may be required.

Progress in recent years has indicated that caveolin-1 functions as a tumor/transformation suppressor in the mammary gland, presenting a good case for specifically replacing or targeting caveolin-1 and/or its signaling partners as novel therapeutic strategies for breast cancer. As human breast carcinomas demonstrate sporadic dominant negative mutations (P132L) or loss of caveolin-1 expression, these alternations may be a form of acquired resistance to the intracellular effect of this protein on inhibiting cellular proliferation, tumorigenesis, invasion and metastasis [93]. Targeting drugs and gene vectors to tissue-specific proteins in caveolae allowed selective delivery into vascular endothelial cells *in vivo* and might even improve direct access to solid-tumour cells. Therefore, caveolae seem to be rich in potential targets for cancer imaging and therapeutics [94].

Together, these studies show that caveolae and caveolin-1 play an essential role in many molecular, cellular and physiological processes. Cancer is a complex disease, where many alterations should be addressed in transformed cells. Caveolin-1 influences cancer formation, progression and prognosis, but this influence is not so great, in spite of recent results that have clarified many roles. Action such as oncoprotein or tumor suppression may depend on interaction with molecular signaling molecules by specific regions, and this may be modified by genetics changes, mRNA and protein expression level. With a deeper understanding of the role of caveolin-1 in tumorigenesis process, it could be used as a molecular marker in breast cancer diagnoses and prognoses and even in treatment.

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