Caveolin-1 (CAV1) is an integral membrane protein, however, it is also found less concentrated in other subcellular localizations. CAV1 is a scaffolding protein and is involved in several vital functions including receptor-independent endocytosis, vesicular transport, and signal transduction. Studies in the last two decades have identified CAV1 as an important factor in tumor growth and development. CAV1 expression and its role in cancer are highly variable and largely dependent on the context of the cell. Thus, there are a number of seemingly contradictory findings; however, when considered case by case we may find promising applications for specific CAV1-mediated signaling in specific cancers at particular stage or treatment condition. In this review, we focus on our current understanding of CAV1 function and its potential role in drug responsiveness in breast cancer.

MOLECULAR CHARACTERISTICS OF CAVEOLINS

CAVs line and permeate the caveolar membrane, both the triply palmitoylated C-terminus [9] and the N-terminus of CAV1 face the cytoplasm while a hairpin loop is formed near the N-terminal end within the membrane [10]. In addition to forming caveolae, CAVs are also structural components of Golgi apparatus and network-derived transport vesicles [1] used to bind and traffic molecules including various cholesterols, proteins, and signaling ligands [11]. This binding property is accomplished via the CAV1 scaffolding domain (CSD) sequence as well as a cholesterol binding domain, both of which are found in the N-terminus of the protein [12,13].

MOLECULAR CHARACTERISTICS OF CAVEOLINS

All three proteins in the CAV family possess an identical amino acid motif near their N-terminus, labeled the caveolin signature motif, which repeats as follows (using the single letter amino acid coding): FEDVIAEP [14], however, researchers have yet to find a significant importance for this homology. While there is a slight variation in the sizes of the CAV proteins, all the functional domains are relatively homologous in size with the N-terminal domain comprising 70-86 amino acid residues, the C-terminal domain comprising 43-44 residues, and the transmembrane domain comprising 33 residues [15].

CAV1 is a 22kDa protein found on the chromosome 7q31.1 [16], and contains 3 exons. Due to alternative splice sites, CAV1
has 2 isoforms, α and β; the β isoform has a truncated N-terminus roughly 31 amino acid residues shorter than α isoform [15,17] (Figure 1). The N-terminal domain of CAV1α is marginally larger than that of CAV1β, CAV2, and CAV3, comprising the first 101 residues of the protein [15]. CAV1β does not possess the phosphorylation site at tyrosine 14 that the other caveolin proteins possess [17]. CAV1 oligimerizes with several other monomers to form a 200-400kDa complex which usually includes CAV2 [18]. CAV1 is expressed at its highest levels in adipocytes, but is also seen in fibroblasts, endothelial cells, epithelial cells, and smooth-muscle cells [19].

CAV2, which encodes a 20kDa protein, is found at the same locus as CAV1, separated by about 19 kilobases [16] and contains 2 unique exons with the last exon of CAV1 and CAV2 being analogous [16]. CAV2 is usually co-expressed along with CAV1 and the pair often co-localize [14]. While CAV2 is a component of caveole formation, it requires CAV1 for proper membrane localization and can only create caveoles if CAV1 is also being expressed [21]. A splice variant of CAV2 has also been identified in mice, it possesses an intron in place of the 3rd exon, effectively removing its C-terminus [22]. The absence of the C-terminus prevents the splice variant from localizing to the caveole (even in the presence of CAV1) and promotes its localization to the endoplasmic reticulum while the authentic CAV2 localizes to the plasma membrane and Golgi apparatus.

CAV3, gene found on chromosome 3p25, is expressed primarily in skeletal and heart muscle cells [23]. CAV3 oligimerizes into a large 350-400kDa complex [24]. CAV3 localizes to the plasma membrane similarly to its sister proteins, but it uniquely associates with T-tubules that are a product of the caveole in the muscle cells [25]. Because of this association, CAV3 mutations play an important role in various degenerative muscle diseases such as muscular dystrophy [26].

Caveolin-1 in Breast Cancer – Current Understanding

While both CAV1 and CAV2 are implicated in tumorigenesis, in this review we will focus on the role of CAV1 in human breast cancer. CAV1 is located on chromosome at the D7S522 locus; 7q31.1. This region spans a fragile site on the chromosome, labeled FRA7G, and is frequently deleted in various human malignancies including breast and ovarian cancers [20]. Presently, there is much debate regarding the precise role of CAV proteins in human breast cancer. While there are several studies that suggest CAV1 as a tumor suppressor, many other studies propose CAV1 as an oncogene in breast cancer [20,27-29]. Nonetheless, the differential expression pattern of CAV1 may not be random. For example, researchers have noted an inverse association between CAV1 expression and estrogen receptor α (ERα) or HER2 [28,30]. Additionally, we see a correlation between increased CAV1 expression and clinical outcome in ERα-negative tumors confers a poorer prognosis [39].

A change in CAV1 expression patterns was noted in the tumor microenvironments that favor cell elongation and microenvironment stiffening. In metastasized human carcinomas, CAV1 expression is enhanced in carcinoma-associated fibroblasts (CAFs) [31]. Both in vitro and in vivo models, CAV1 expression in CAFs consistently favors tumor invasiveness, thus promoting metastasis and further progression of the disease and a poor survival rate. A clinical study in patients with triple negative (ERα-, PR, and HER2) and basal-like breast cancers, the two most aggressive types, showed an association of loss of CAV1 expression in the stroma with poor prognosis [32].

Several technical factors, for example, a lack of a standardized approach for immunohistochemistry protocols and scoring systems, and controversial studies associated with the CAV1 P132L mutation [33-35] has confounded progress towards underscoring the role of CAV1 in specific breast cancers based on subtypes, stages or post-treatment conditions. However, the diverse signaling properties of CAV proteins warrants further investigations to determine the relevance of CAV1 as a useful clinical marker for breast cancer subtypes.

Regulation of cav1 in breast cancer

CAV1 gene silencing can occur via epigenetic DNA methylation. While epigenetic alterations (including nucleosome and histone modifications in addition to DNA methylation) are widely accepted as being benchmarks of tumor progression, patterns of DNA methylation are still being investigated to find
predictive elements that confer influence on tumor development [36]. DNA methylation, taking place at specific regions in the DNA composed of a high frequency of CpG dinucleotides (cytosine followed directly by a guanine) called CpG Islands (CGIs), is directly responsible for the alteration of the interaction between transcription factors and DNA usually near gene promoters, thus it is capable of altering the expression of a gene based on the pattern of methylation, whether it be hypermethylated or hypomethylated [37]. Methylation patterns of CGIs are important in predicting gene silencing, however methylation of additional regions of DNA now coined CpG Island “shores” are now being considered for their correlational to gene expression. These “shores” occur a short distance from the CGIs (both 5’ and 3’) rather than within them [38]. Shore methylation has been correlated to increased CAV1 expression in breast cancer. In aggressive basal-like breast cancer cell lines, hypomethylation of CGI shores of CAV1 has been reported. Thus, shore methylation for CAV1 may be a potential prognostic biomarker in specific subtypes of breast cancer.

While CAV1 proteins are mostly localized within caveolae in the plasma membrane, they are also found localized elsewhere in the cell. Evidence from other cancer models shows that specific subcellular localization can then play an influential role on the aggressiveness of tumor progression. In clear cell renal carcinoma, high expressions of CAV1 in the cytosol correlate with poor outcome in patients [40]. To date, nearly all of CAV1-focused cancer research has focused on total CAV1, without discerning expression levels of its variants. We have shown differential roles of CAV1 variants (α/β) in apoptosis in ER+ breast cancer cells [41]. CAV1β lacks Tyr-14 residue that is phosphorylated by Src kinase, and structural homology modeling suggest that phosphorylation on Tyr-14 promotes a favorable conformation for other proteins to bind to the CAV1 scaffolding domain [41-43]. Thus, increasing CAV1β over CAV1α may alter signal transduction and dependent cellular phenotypes.

**Caveolin-1 and breast cancer therapy**

Emerging data from various cancer models suggest different ways that knowledge of CAV1 expression and function can help improve efficacy of existing cancer therapies. CAV1 knockout mice with hyper proliferating intestinal crypt stem cells show increased sensitivity to wholebody gamma-irradiation displaying increased apoptosis as compared to CAV1 wild-type mice [44]. Increased radio-sensitivity was also proved using siRNA silencing of CAV1 in pancreatic cancer cells [45] by changing several cancer related proteins, including: AKT, Paxillin, MAPK, JNK, and Src. Inhibition of CAV1 expression induced DNA double strand breaks following irradiation, establishing a connection between CAV1 and the DNA damage repair processes [46]. CAV1 is suggested to help mediate transport of EGFR of into the nucleus to aide in the removal of DNA lesions through the activation of DNA-protein kinase [45,47]. Although these are interesting observations, unfortunately, there is not a great deal of knowledge of how CAV1 protects cells from nuclear damage.

CAV1 expression has been implicated in drug resistance in various cancer models. CAV1 knockdown in mice with renal cell carcinoma was able to sensitize the tumor cells to doxorubicin, clinically administered pro-apoptotic anti-cancer drug, and prevent the incidence of lung metastasis [48]. It has been suggested that CAV1 confers a drug resistant phenotype through its role in drug endocytosis and trafficking into the cell [49,50], however, a mechanistic pathway for this suggestion remains unknown. Furthermore, even though a loss of CAV1 expression has been shown to confer sensitivity to drugs and radiation, there are still contexts in which loss of CAV1 can confer resistance, such as in ER+ breast cancer cells that are resistant to the clinically administered antiestrogen drug, Tamoxifen [51]. Interestingly, our research has shown that post-translational modification such as Src-mediated phosphorylation on Tyr-14 on CAV1 sensitizes ER+ breast cancer cells to anti-microtubule drug paclitaxel, which is commonly used in the clinic for treatment of various types of cancers [41,42,51].

Knowledge of CAV1 expression and function in breast cancer has proven helpful in guiding the choice of anti-cancer therapy. The role of CAV1 is well-established in albumin transcytosis, particularly in endothelial cells [52-54]. This knowledge of CAV1 function has been used in testing the efficacy of paclitaxel conjugated to albumin nanoparticle (ABI-007; Abraxane) in high CAV1 expressing tumors [55,56]. In ductal in situ carcinoma (DCIS) of the breast, the anti-autophagy drug chloroquine can restore stromal expression of CAV1, and this knowledge has direct relevance for ongoing clinical trial involving chloroquine in DCIS [57]. Additionally, multi-kinase inhibitors such as dasatinib show increased sensitivity in breast cancers with triple-negative or basal-type breast cancer that shows high CAV1 expression [58]. Unfortunately, while the use of CAV1 expression has been used to correlate specific subtypes of breast cancers and existing anti-cancer drugs, its use as a direct anti-cancer target has been limited. One encouraging example employs caveolin-1 scaffolding domain (CSD) mimetic peptides, which have been used to inhibit hepatocarcinoma and lung tumors by inhibiting endothelial nitric oxide synthase (eNOS) and vascular permeability [59-61]. Further in vivo studies are needed to prove efficacy of CSD mimetic peptides in breast tumor subtypes that over express CAV1. Thus, knowledge of CAV1 function has the potential to impact development or refinement of novel anti-cancer therapies.

**DISCUSSION AND CONCLUSION**

CAV1 has diverse functions in relation to tumorigenesis, growth, progression, and treatment. Unfortunately, there has been much discord between researchers in providing an absolute set of definitions to CAV1 in breast cancer considering is variable effects based on tumor subtype, stage, microenvironment and treatment. CAV1 expression depends of a wide variety of factors and it is becoming evident that any clinical use of CAV1 as a biomarker in conjunction with therapy has to be tumor subtype- and treatment-specific. A large volume of work so far suggests an important role for CAV1 in different subtypes of breast cancer, perhaps more as a potential clinical biomarker rather than a therapeutic target. With consensus and collaboration among researchers regarding the standardization of immunohistochemistry techniques, for example, it is possible to use CAV1 as a tool to select therapeutic options in breast cancer. Moreover, there are still many unanswered questions.
for CAV1 function in regulating cancer progression, for example in regulating cellular metabolism. Thus, with a clearer understanding of CAV1-mediated signaling in specific subtype of breast cancer, we will be better equipped to use CAV1 as a clinical biomarker for choosing therapeutic options for specific tumors.

ACKNOWLEDGEMENTS

We thank Dr. Robert Clarke, PhD, DSc (Georgetown University) for helpful discussions for this review. This work was supported, in whole or in part, by National Institutes of Health Grant U54-CA149147 (to RC) from the USPHS and American Cancer Society Institutional Research Grant IRG 92-152-17 (to ANS-H).

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