

Review

## Caveolin-1, Breast Cancer and Ionizing Radiation

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**Abstract.** Breast cancer (BC) recovery has increased in recent years thanks to efforts of Omics-based research in this field. However, despite the important results obtained, BC remains a complex multifactorial pathology that is difficult to treat appropriately. Caveolin-1 (CAV1), the basic constituent protein of specialized plasma membrane invaginations called caveolae, is emerging as a potential therapeutic biomarker in BC. This factor may modulate BC response to chemotherapy and radiation therapy. In addition, recent reports describe the key role of CAV1 during cell response to oxidative stress. The aim of the present review was to describe the biological roles of CAV1 in BC considering its contrasting dual functions as an oncogene and as a tumor suppressor. In addition, we report on how CAV1 may contribute to tumor cell response to ionizing radiation treatment. Finally, new roles of CAV1 in BC both on epithelium and stroma may be useful as prognostic indicators for patient treatment and help clinicians in the selection of the best personalized therapy.

It is well-known that breast cancer (BC) is not a single disease but a neoplastic disorder which is highly heterogeneous at both the molecular and clinical level,

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comprising of different molecular subtypes that generally correspond to different prognoses and responsiveness to therapy (1-4).

Although therapeutic treatment plans for BC have recently made great progress, recurrence and death rates remain unacceptably high (5). Molecular biomarkers of BC could help clinicians identify new diagnostic and therapeutic strategies for predicting clinical outcome and response to therapy schedule (6).

Caveolae are plasma membrane invaginations rich in proteins involved in the pathogenesis of several human diseases that are emerging as potential BC biomarkers (7). The formation and maintenance of caveolae is primarily due to caveolin proteins. The caveolin protein family consists of the following three members: caveolin-1 (CAV1), CAV2, and CAV3. CAV1 is widely expressed in various tissues, whereas CAV3 is muscle-specific. CAV2 is co-expressed with CAV1 and requires CAV1 for stabilization and plasma membrane localization (8).

### Caveolin-1 and Breast Cancer

CAV1 plays an important role in carcinogenesis because it interacts with many factors involved in mitogenic signaling, angiogenesis, and senescence processes. In this direction, many data describe its involvement in BC as a possible molecular target, able to predict the patient's response to treatment (9).

CAV1 protein coats the caveolae, acting as a scaffolding protein to organize and concentrate specific lipids (*e.g.* cholesterol and glycosphingolids) and signaling molecules, such as SRC-like kinases, Harvey rat sarcoma viral oncogene homolog (H-RAS), endothelial nitric oxide synthase, and G-proteins, within caveolae membranes (10-11). The CAV1 scaffolding domain (CSD; amino acid residues 82-101)

interacts with the binding motif contained within the catalytic site of specific signaling molecules. On binding it forms an inactive complex. In fact, following an appropriate stimulus, CAV1 is released from the binding motif, causing the propagation of downstream signals. More precisely, it has been reported that the CSD inhibits SRC family tyrosine kinases (i.e., c-SRC/FYN), epidermal growth factor receptor (EGFR), ERB-B2 receptor tyrosine kinase 2 (alias HER2/neu), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) proteins (10-12). CAV1 is also able to maintain a specific active v-Akt murine thymoma viral oncogene homolog 1 (AKT) status able to guide pro-survival signaling. This action is probably facilitated by inhibitory binding of the AKT-phosphatase protein 1 and -2A (PP1A and PP2A) to the CSD domain of CAV1 (13).

A link between CAV1 and E-cadherin is also reported. CAV1 seems to inhibit the expression of the anti-apoptotic factor Survivin only in the presence of E-cadherin *via* the wingless-type MMTV integration site family (Wnt)/ $\beta$ -catenin-pathway, with the involvement of cyclo-oxygenase-2 and prostaglandin E2, modulating cancer cell radio- and chemoresistance (14).

The CAV1 gene is localized at the D7S522 locus on chromosome 7 (7q31.1). Interestingly, D7S522 spans a known common fragile site (called FRA7G) that is frequently deleted in a variety of human neoplastic diseases, including squamous cell carcinoma, prostate cancer, renal cell carcinoma, ovarian and colon carcinoma, as well as BC. This evidence has suggested that the coded protein may act as a tumor-suppressor at the D7S522 locus (15).

Today, estrogen receptor status (ER) is a well-defined biomarker routinely tested in BC diagnosis and treatment. ER $\alpha$  and ER $\beta$  are nuclear protein targets of estrogen action, very important factors for normal development and maintenance of female characteristics and sexual reproduction. While ER $\alpha$  is expressed at low levels in normal mammary epithelial cells, its up-regulation is found in pre-malignant hyperplasia and during the development of BC. One-third of human ER $\alpha$ -positive BCs harbor CAV1 mutations (16). In particular, the CAV1 (P132L) mutation, which causes a proline to leucine change at amino acid position 132 of the transmembrane domain, is found in more than half of all ER-positive BC cases (13, 16). This mutation leads to ER $\alpha$  overexpression and increased sensitivity toward estrogen therapy, but on the other hand, it could represent an important risk factor for breast tumor development (13, 17).

Until recently, the role of CAV1 in cancer was strictly focused on its epithelium-dependent functions, disregarding completely its effect on the tumor-associated stroma. However, stromal CAV1 expression has also been reported as a biomarker predictive of poor prognosis in BC (9, 18-19). Today, it is well-known that the tumor microenvironment plays an important role in BC onset and progression (20-21),

in particular, the tumor stroma can drive invasion and metastasis, hallmarks of malignancy responsible for cancer treatment failure, recurrence and death.

Loss of expression of CAV1 in BC-associated fibroblasts (BCAFs) has been associated with increased tumor progression, the presence of local metastases and ER negativity, all features related to poor outcome (18-22). However, the role of CAV1 in BC is still very controversial because it has been shown to have a dual opposing role, acting as a tumor suppressor and as an oncogene.

### Tumor Suppressor or Oncogenic Factor?

Many data suggest anti-proliferative and transformation-suppressive roles of CAV1 in mammary epithelial cells. Indeed, reduction of CAV1 protein level is a common event in transformed cell lines, suggesting that it might be inactivated during tumorigenesis and that it may be associated with known tumor-suppressor proteins (23, 24). In the MCF7 BC cell line, CAV1 transfection up-regulates breast cancer 1 (BRCA1) protein expression through a p53-dependent mechanism. In addition, with a positive feedback mechanism, BRCA1 protein also increases CAV1 mRNA expression by promoter gene transactivation (25, 26).

CAV1 is also involved in regulation of phosphatase and tensin homolog (PTEN), a protein phosphatase with a tumor-suppressor function (27). Interestingly, ERK1/2 is compartmentalized within caveolae and the up-regulation of CAV1 expression down-modulates ERK1/2 kinase activity (28-30).

CAV1 can also regulate cell-cycle progression by acting on cyclin D1 protein. More precisely, cyclin D1 is responsible for the G<sub>1</sub> to S phase transition, forming an active complex that promotes cell-cycle progression by phosphorylating and inactivating the retinoblastoma (Rb) factor (31-34). The cyclin D1 gene (CCND1) is amplified or overexpressed in almost 50% of BC cases. *In vitro* evidence has shown that this gene is transcriptionally repressed by CAV1 overexpression, preventing cell transformation (35).

In general, CAV1 expression correlates positively with the recovery of cell adhesion and reduced cell motility (36, 37). CAV1 may have a pro-apoptotic role, indeed, its overexpression induced apoptotic cell death through inhibition of PI3-kinase and activation of caspase-3 (38, 39).

Several studies have reported that CAV1 plays a role as a suppressor of BC metastasis, controlling activity of metalloproteinases (MMPs) which are able to enhance tumor invasiveness and metastasis formation (40-42). Metastatic mammary tumor cells that expressed recombinant CAV1 showed significant reduction in matrigel invasion and dramatically reduced MMP2 and MMP9 activities (35).

On the other hand, certain data demonstrated that CAV1 may also have oncogenic properties in human cancer

including breast, colorectal, lung, and other (43). P132L mutation of *CAV1* is believed to cause the mislocalization and intracellular retention of wild-type *CAV1*, therefore acting as a dominant-negative mutant (44). Some reports described the presence of the P132L mutation in primary breast tumors whose overexpression in mammary tumor cells caused a significant increase in cell migration, invasion and metastasis formation. This genetic alteration is clinically relevant: patients harboring the P132L mutation have an 82% chance of cancer recurrence (44-47). Moreover, six *CAV1* mutations associated with ER $\alpha$ -positive BC (W128Stop, Y118H, S136R, I141T, Y148H, and Y148S) were also described, with an overall frequency of 35%, suggesting that sporadic *CAV1* mutations may act as an initiating event in BC pathogenesis (16, 48-49).

### **CAV1: A Putative Marker for Therapeutic Choices in Breast Cancer**

BC represents a highly heterogeneous group of tumors at both the clinical and molecular level. The choice of a unique therapy common to all patients is not possible because of the presence of several subtypes of BC. Several currently used therapeutic strategies are based on different receptor status and tumor stage (2, 6). *CAV1* is involved in the response to chemo- and radiation therapy used in the treatment of BC. Recent data also show the great involvement of stromal *CAV1* level for predicting BC outcome, indicating how its modulation in response to oxidative stress may lead to the choice of the best therapy (50, 51). In particular, it interacts with the ER and HER2 receptors by modulating their function and conditioning the response to drugs such as tamoxifen and trastuzumab. Trastuzumab is a targeted-drug commonly used for mammary tumors that overexpress the HER2 receptor. The oncogenic tyrosine kinase receptor HER2 can transduce strong mitogenic signals during cell transformation and proliferation by its ability to heterodimerize with EGF and with ERBB3 (52-53). HER2 inactivation is essential for stopping the proliferation signal. Elevated levels of HER2 in tumor cells are associated with its defective endocytosis and deregulation (54). Little is known on the interaction between *CAV1* and HER2 signaling pathways. The kinase domain of HER2 includes a *CAV1*-binding motif, EGFR analogous, and a 20-aminoacid peptide derived from the CSD that can prevent HER2 autophosphorylation and kinase function (54). Caveolae may well function in the endocytotic pathway for HER2 internalization: the endocytosed molecules may be destined to migrate to the intracellular compartments for degradation. Data even suggest HER2 endocytosis to be through a *CAV1*-dependent pathway: HER2 may be retained on the cell surface even after ligand stimulation in caveolae-deficient BC cells (55). Thus, *CAV1* could contribute to inhibition of

growth and proliferation signals from HER2 through its down-regulation, thereby acting as a tumor suppressor. It was shown that upon trastuzumab treatment, HER2 was internalized in the *CAV1*-expressing SKBR3 BC cell line (56). This internalization decreased following siRNA-mediated *CAV1* inhibition (56-57). On the other hand, antibody-dependent cell-mediated cytotoxicity, mediated by human peripheral blood mononuclear cells towards trastuzumab-treated and *CAV1*-transfected SKBR3 cells was also observed. This effect suggests that enhanced endocytosis of HER2 through a potential *CAV1*-dependent pathway is significant for desensitizing cells to the ADCC-dependent cytotoxicity of trastuzumab (57-58). For this reason, *CAV1* has also been described as one of the potential causes of resistance to trastuzumab in BC cells. Thus, *CAV1* and caveolae deficiency might be a prognostic or predictive factor of response to trastuzumab therapy.

Tamoxifen is one of the most effective standard anti-estrogen therapies for treating ER $\alpha$ -positive BC and preventing estrogen-dependent growth. Unfortunately, neoplasia in almost half of the patients under this therapy become resistant and can quickly recur. As described above, a relationship between *CAV1* and ER $\alpha$  status was reported in literature. In ER $\alpha$ -positive BC cells, *CAV1* has a tumor-suppressive role (59, 60). In mammary cells, it is a negative regulator of estrogen-stimulated proliferation through a decreased expression of its coactivators such as coactivator protein for AP1 and ER receptor (*CAPER*), a transcriptional activator of ER $\alpha$  and JUN/AP1 (59). *CAV1* null mammary epithelial cells showed an increased expression of ER $\alpha$  and of its co-activator genes (*CAPER* and forkhead box A1 (*FOXAI*)), and estrogen hypersensitivity (60). Conversely, some reports suggested a potential role of *CAV1* in the development of tamoxifen resistance. *CAV1*-deficient mice had elevated levels of B23 (nucleophosmin), a nucleolar marker predictive of tamoxifen resistance (60, 61). Moreover, only ER-positive BCs generally harbor the *CAV1* P132L mutation. Taking into account that *CAV1* is associated with BC recurrence and almost half of ER-positive patients developed tamoxifen resistance, *CAV1* P132L mutation has been suggested as a predictor of poor response to this drug (46, 62).

In addition, there is growing recognition that the tumor microenvironment can influence tumor cell behavior, where fibroblasts are involved as key modulators of cancer progression (20, 21). Breast tumors grown in a *CAV1*-deficient microenvironment were more than five-fold larger than tumors grown in a wild-type *CAV1*-containing microenvironment. Thus, a *CAV1*-deficient tumor microenvironment provides fertile niche for BC growth (63). The role of *CAV1* in cancer was strictly focused on its epithelium-dependent functions, while completely overlooking its effect on the tumor-associated stroma.

CAV1 has recently emerged as a novel stromal regulator of cancer growth and has been named “stromal gate keeper” (64, 65). Human BCAFs are a subpopulation of fibroblasts that function as supporting cells for cancer progression and provide recycled nutrients to tumor cells. CAV1-deficient CAFs are implicated in the Reverse Warburg Effect (66, 67). This model is based on the original Warburg effect, according to which tumor metabolism switches from oxidative phosphorylation to aerobic glycolysis in order to fuel its own growth. The revolutionary difference is that the Warburg effect occurs in fibroblasts, not in cancer epithelial cells (56). MCF7 cells are able to induce oxidative stress in tumor-adjacent stromal cells that drive the loss of stromal CAV1 in adjacent BCAFs (68). Studies demonstrated that the loss of CAV1 in stromal cells induced the ligand-independent activation of the transforming growth factor beta (TGF $\beta$ ) pathway and that CAV1<sup>-/-</sup> stromal cells exhibited up-regulation of 35 transcripts associated with activated TGF $\beta$  signaling, including the TGF $\beta$  target gene *CTGF* (69). It was also shown that a loss of stromal CAV1 induces the metabolic reprogramming of CAFs with the induction of glycolysis and autophagy (70-72).

The role of CTGF in BC remains controversial. It has both a tumor-promoting and tumor-suppressor role and exerts compartment-specific actions. Overexpression of CTGF in BC epithelial cells inhibited tumor growth, but on the contrary, a tumor-promoting effect was observed when *CTGF* was overexpressed in the tumor fibroblast compartment (73-76). CTGF drives the induction of autophagy in both fibroblasts and BC cells through increased oxidative stress and hypoxia inducible factor 1 alpha subunit (HIF1 $\alpha$ ) stabilization (72). More specifically, CTGF-induced autophagy in CAFs is mediated by mitochondria and thus these cells are forced to undergo aerobic glycolysis. Through this type of metabolic pathway, stromal cells are able to produce elevated levels of L-lactate and pyruvate, becoming a source of fuel for epithelial cancer cells, which become the recipients of stromal catabolites. Conversely, the induction of CTGF-mediated autophagy in epithelial tumor cells may cause self-digestion and inhibition of tumor growth. In conclusion, in the absence of CAV1, CAFs (with the overexpression of CTGF) derived from patients with BC are able to feed epithelial cancer cells with recycled nutrients through metabolic reprogramming of the tumor stroma, driving tumor development without increased neovascularization (77). These data may suggest the use of new therapies, excluding angiogenesis inhibitors when they do not work, and indicate the need for use of antioxidants and autophagy inhibitors to prevent oxidative stress in tumor fibroblasts, cutting off the fuel supply to cancer cells (78-79).

### The Role of CAV1 in Radiobiological Responses of Tumor Cells

Radiation therapy (RT) is one of the most important modalities for treating many types of cancers, including those of the breast, whose goal is local control of the cancer at the site of the tumor (80). Tumor radioresistance and radiosensitivity of normal tissues are the major objects of research in radiobiology. Ionizing radiation (IR) (such as X-rays or high-energy electrons) induces high stress levels in both tumor and normal cells, many of which are dependent upon cell type, genetic background, dose, dose rate and time after irradiation (80-81).

The knowledge in the field of genetics, cell and molecular radiobiology may enable for new approaches to research for developing innovative and predictive tests of radiosensitivity of tumors and normal tissues. Consequently, research efforts are targeted at improving the effects of radiotherapy and realizing greater individualization of treatments.

In recent years, the molecular mechanisms through which CAV1 is involved in radio- and chemoresistance of cancer cells have been increasingly explored. Hehlhans *et al.* reported radiosensitization and anti-proliferative effects by siRNA-mediated knockdown of *CAV1* in some cancer cell lines, such as Mia PaCa2, Panc1 and PATU8902 (82). Expression of CAV1 was reported to be elevated in cells exposed to IR and its overexpression conferred radioresistance to these cell lines (83). The up-regulation of CAV1 protein in response to DNA-damaging agents such as IR plays an important role in activating the DNA-repair signaling cascade and in promoting the repair of double-strand breaks, through both homologous recombination and non-homologous end joining, thus contributing to the maintenance of genomic integrity. The role of CAV1 in homologous recombination has been shown in the MDA-MB-468 BC cell line transfected with a *CAV1* siRNA and irradiated with 5 Gy of IR. Its effect might be related to the accumulation of BRCA1 foci in the nucleus after DNA damage. BRCA1 is a DNA-repair protein whose expression is controlled by CAV1. In addition, a role of CAV1 in non-homologous end joining has been recorded. Indeed, suppression of CAV1 by siRNA in MDA-MB-468 cells dramatically inhibited the IR-activated phosphorylation (Ser2056) of protein kinase DNA-activated catalytic polypeptide (alias DNA-PK), one of the key factors of the non-homologous end joining system (83).

Unexpectedly, CAV1 was up-regulated within 30 min following IR treatment, a time much earlier than the 24 h as shown in pancreatic carcinoma cell lines. These data suggest that CAV1 may act as a sensor and early mediator in response to DNA damage and that it could be activated before Ataxia telangiectasia mutated (ATM), the key protein kinase activated by DNA damage (83). In MDA-MB-468 cells with *CAV1* silenced, after 5 Gy IR exposure, the activity

of ATM was lower than that in control cells, as demonstrated by decreased levels of phospho-ATM (Ser 1981) and phospho-checkpoint kinase 2 (CHK2) (Thr 68), the downstream target of ATM. Cell treatment with inhibitors of PP2 (SRC tyrosine kinase inhibitor), a phosphatase that reduces ATM phosphorylation, was able to augment the IR-induced phosphorylation of ATM, indicating the involvement of PP2A in the regulation of ATM activity following DNA damage. Co-immunoprecipitation experiments demonstrated an increased physical association between CAV1 and PP2A after treatment with IR. These results suggest that in response to DNA damage, CAV1 plays an essential role in activating the ATM-initiated repair pathway by sequestering and inhibiting the function of PP2A (82, 83).

Nowadays, new therapeutic opportunities and technologies using IR are available in supporting anti-BC treatment. An example is intraoperative electron radiation therapy (IOERT), consisting of the irradiation of the tumor site after surgical removal to destroy residual cancer cells and to avoid tumor relapse, with a unique delivery of a high dose (exclusive treatment of 21-23 Gy) or of a lower one (boost treatment of 9-12 Gy) according to specific clinical features of BC (84). In order to study molecular mechanisms activated by IR during IOERT treatment and to select potential new biomarkers of radiosensitivity or radioresistance, our research group performed gene-expression profiling by cDNA microarrays in BC cell lines irradiated with 9 and 23 Gy doses. We observed consistent differences among the types of treatment and cell lines used, and the magnitude of transcriptional variation was cell type-specific and dose-dependent. In the MCF7 BC cell line, we reported that high IR dose treatment up-regulated *CAV1* transcriptional levels. Based on microarray results and on an as yet not understood role of CAV1 in BC cell response to IR, we analyzed CAV1 protein localization in cholesterol and glycosphingolipid-enriched membrane microdomains, the lipid rafts. In MCF7 cells treated with 9 Gy, we observed a significant recruitment of CAV1 to the low-density fractions containing the lipid raft compartments with respect to untreated MCF7 cells (85). Our data showed an increase of the CAV1 lipid raft localization following IOERT irradiation, suggesting its role as a biomarker of radiosensitivity, since it could contribute to cell death after high-dose IR treatment (85).

### **CAV1, EGFR and RT**

Many tumor cells are characterized by overexpression of EGFR, a protein that promotes growth, aggressiveness and resistance of cancer cells to chemotherapy and RT (86-88). EGFR can be phosphorylated in response to binding of its specific ligands (EGF, TGF $\alpha$  and amphiregulin) (89, 90) and after exposure to non-specific stimuli such as IR, UV-radiation, hypoxia, hyperthermia, oxidative stress and

transactivation by G-protein-coupled receptors (91-97). In these cases EGFR phosphorylation resulted in receptor internalization and intracellular signaling (98), causing its silencing and inactivation (88, 99-101). The two features involved in EGFR internalization are the clathrin-coated pit and CAV1-driven internalization (59, 102-103). The first is associated with EGFR internalization following treatment with EGF (95). Exposure to oxidative stress can lead to internalization of EGFR by caveolae and this process is associated with perinuclear accumulation of EGFR (104). IR leads to a fast activation of SRC, by autophosphorylation of Y416 residue of EGFR (9, 95). IR exposure also mediates the SRC-driven phosphorylation of EGFR at the Y485 residue and of CAV1 at Y14, an event necessary for EGFR internalization into caveolae. Caveolar compartmentation prevents EGFR degradation and simultaneously enables intracellular EGFR signaling (96). Additional EGFR function is further supported by the observation that perinuclear EGFR can be translocated to the nucleus in response to IR. EGFR internalization occurs predominantly following treatment of cells with genotoxic agents, suggesting that internalization and nuclear transport of EGFR are linked with DNA repair processes (105, 106). This assumption is supported by the finding that CAV1-mediated EGFR internalization was not observed in the A549 cell line after EGF treatment (105). In HH4dd and FaDu cell lines irradiated with a single dose of 4 Gy and MDA-MB-468 BC cells irradiated with a single dose of 5Gy, CAV1 controlled non-homologous end joining, modulating the activity of DNA-PK, *via* CAV1-mediated nuclear translocation of EGFR (84, 88). Indeed, nuclear EGFR is linked with the activation of DNA-PK (detected by phosphorylation at T2609) and with the regulation of non-homologous end joining, resulting in increased radioresistance (88). Detection of nuclear EGFR in tumor biopsies correlated strongly with resistance to treatment and a poor prognosis (87). These findings suggest a new function of EGFR depending on its intracellular localization. In response to radiation, not only SRC-dependent EGFR phosphorylation at Y485, but also autophosphorylation at Y992 and Y1173 were observed (105). This finding implies that IR may activate not only SRC kinase, but also EGFR kinase, and both kinases contribute to alter the EGFR phosphorylation pattern following radiation exposure. In A549 and FaDu cell lines, inhibition of SRC activity was shown, with the block of CAV1-dependent EGFR internalization and nuclear transport after irradiation. In addition, activation of DNA-PK was abolished, DNA-repair inhibited and radiosensitivity increased (105). CAV1-targeted siRNA also inhibited the co-translocation of CAV1 and EGFR in MDA-MB-468 cells following IR treatment (84). This EGFR-coupled radiation response mechanism offers new potential molecular targets for cancer treatment and patient care, especially for radiation therapy.

## Conclusion

The choice of a single therapy common to all patients is not possible because of the presence of several types and sub-types of BC. Today, the therapeutic choices used for the treatment of BC are different. CAV1 is emerging as a potential biomarker in BC. Figure 1 summarizes the roles of CAV1 in BC. From its newly-found roles in BC both in cancer epithelium and tumor stroma, CAV1 may be useful as a prognostic indicator for patient treatment, and to help clinicians in the choice of the best personalized therapy and in optimizing results.

## Competing Interests

The Authors declare that they have no competing interests in regard to this this article. All Authors read and approved the final manuscript.

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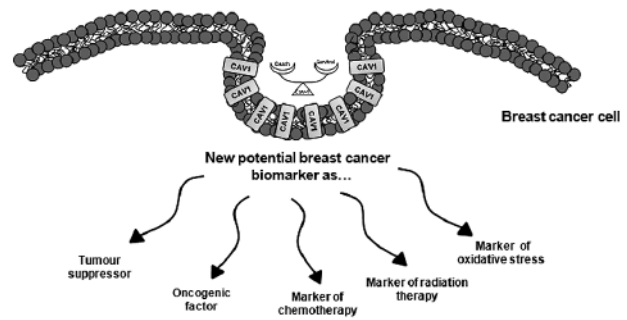


Figure 1. Roles of Caveolin-1 as a new emerging potential biomarker in breast cancer.

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