Caveolae and Caveolins: Multiple functions and Architectural Adaptation in various Head and Neck Diseases

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Abstract

The cell consists of numerous small invaginations of the plasma membrane, among which caveolae describes its novel role in transport, in communication between intra- and extracellular environment and transport of macromolecules across endothelial cell layer. Caveolin 1 is a major component of the caveolae, allowing purification of caveolar membranes and establishment of a list of caveolar proteins. Numbers of signaling components are present in caveolae which serve as organizing platform of signaling components. Caveolae are remarkably stable structures but their functional significance in cells and organisms remains poorly understood. Hence, this article emphasize the importance of mysterious, hidden, two fold and secrete functions brought about in the caveolae and by caveolae proteins at the plasma membrane in different diseases affecting head and neck region.

Keywords: Cell; Caveolae; Extracellular; Invaginations; Intracellular; Plasma membrane

Introduction

Cancer is a heterogeneous, multifactorial, multifaceted and multistep process. It is a complex process involving the contribution of many different factors. The suggested categories of cancer causation include spontaneous, hereditary, environmental and interaction of gene and environment. Head and Neck Cancer has become one of the chief causes of death worldwide and mortality has doubled in the last 20 years. It is the sixth most common human malignancy and global incidence is rising at an alarming rate due to environmental changes. The spread of cancer from its primary site and its subsequent growth in secondary sites is the most fatal aspect of the disease [1]. Many genes and proteins, which are known to play essential roles during embryonic development, are altered or abnormally expressed in cancerous cells.

Caveolae (plasmalemmal vesicles) were first identified in 1953-1955 as endocytic structures that transport molecules across endothelial cells [2]. In Latin "Caveolae" means "little caves", which are small plasma membrane lipid rafts of 50 – 100 nanometer in size and was discovered by electron microscopy in 1953. Under transmission electron microscopy, it appeared as flask-shaped plasma membrane invaginations of strikingly

regular shape and size (≈ 70 nm average outer diameter) that lack a membrane dense coat. The caveolae are present in most of the cells but are predominant in terminally differentiated mesenchymal cells including endothelial cells, vascular smooth muscle cells, adipocytes, fibroblasts, type I pneumocytes of the lung and striated muscle cells [3,4,5].

Caveolin (Cav) and Cavin are the key protein components of caveolae which are supplemented in glycosphingolipids and cholesterol. Caveolins are proteins closely associated with caveolae and are also known as Cav – 1 or VIP21 (Vesicular Integral-membrane Protein of 21kDa). VIP21 was cloned as component of the vesicular transport machinery localized at the plasma membrane, Golgi bodies and on vesicular structures [6,7]. Cav – 1 was the first member of caveolin family to be identified as a tyrosine-phosphorylated protein in cells transformed by the Rous sarcoma virus. In mammals, three caveolin genes are expressed (caveolin 1, caveolin 2, caveolin 3) and it codes for 4 protein isoforms (caveolin- 1 α , caveolin- 1 β , caveolin 2 and caveolin 3). Most of the body tissues express at least one of these isoforms. Caveolin – 1 high level expression was observed in adipocytes, fibroblasts, endothelial cells, smooth muscle cells and type 1 pneumocytes. Caveolin – 2 expressions was observed with caveolin – 1 for membrane localization and stability whereas caveolin – 3 was predominantly expressed in heart and skeletal muscle cells. So, among these three caveolins, only Cav 1 and Cav 3 are required for the caveolae biogenesis. Monier, et al. showed high affinity of Cav 1 for cholesterol which helps it to form oligomer and this Cav 1 oligomerization is a key feature for the formation of complete caveolae structure [8].

Cavins are other heteromeric protein complexes that are recruited to the caveolae. Four Cavins are identified so far in mammals these includes Cavin 1/PTRF (Polymerase I and Transcript Release Factor), Cavin 2/SPDR (Serum Deprivation Response Protein), Cavin 3/SRBC (Sdr-Related Gene Product That Binds To C-Kinase) & Cavin 4/MURC (Muscle – Restricted Coiled - Coil Protein) [9].

Structure & Formation of Caveolae

Caveolae are the non-coated pits showing close resemblance to the clathrin – coated pits which are present in all the cells

and have many receptors responsible for receptor – mediated endocytosis whereas caveolae plays quantitatively minor role in endocytosis. These are secured at the plasma membrane via cytoskeleton of the cell. Actin and actin connectors such as filamin A, Eps-15 Homology Domain – containing protein 2 (EHD2) helps attachment of caveolae at the membrane site. Cavin proteins are believed to be the important organizational element of the caveolae. Liu L, et al. & Hill MM, et al. (2008) showed that cavins are recruited to the membrane site by the caveolins and these both are required for caveolae formation [10,11]. Ludwig G, et al. and Gambin Y, et al. in their ultrastructural study by electron microscopy and X-ray crystallography proposed that cavins along with Cav 1 plays an crucial role in caveolae assembly. Also showed that the association of cavin 1 to its counterpart cavin 2 & 3, is in the ratio of 2 to 3 : 1, whereas its association with Cav 1 showed the ratio of 1 : 4 [12,13].

Hansen CG, et al. identified two new caveolar proteins namely EDH2 protein and Pacsin2/syndapin-11. EDH2, an ATPase, does not form caveolae but helps in its stabilization at the membrane site and is present in the neck of caveolae. Any defect either increase or decrease of EDH2 will directly affect the endocytosis function brought about by caveolae. Pacsin 2 plays its role in morphogenesis by localizing itself partially with caveolae through a membrane curvature binding domain (BAR). Alterations in pascin 2 results in altered morphology of caveolae thereby causing decrease in Cav 1 and cavin proteins [14,15,16].

Cytoskeletons of the cell like actin filaments, intermediate filaments and microtubules, are all associated with caveolae. Caveolae involves them in intracellular signaling by uniting signaling boards at the cell membrane. It has been showed that these caveolae have been involved in several signaling pathways such as MAPK (Mitogen Activated Protein Kinase), AKT/PKB (Protein kinase B), Src Kinases (sarcoma family kinases), Rho and Rac 1 GTPase.

Caveolin and Caveolin hypothesis

Caveolins isoform, the Cav1 and Cav2 genes are in close proximity on human chromosome 7q31 .1, while Cav3 is located on the 3p25 chromosome and are predominantly expressed in plasma membrane, but can also be found in the Golgi, the endoplasmic reticulum, at cytosolic locations, and in vesicles [17,18]. Lisanti b, et al. suggested that all the three isoforms have both an N and C-terminus in the cytoplasm and a long membrane spanning hairpin-like domain. Also, Cav proteins exist as monomers in the Golgi apparatus but undergo homo-oligomerization upon being transported to the plasma membrane [19]. Cav 1 has high affinity for cholesterol and its oligomerization is key step for the formation of caveolae structure.

Studies on caveolins function have opened a door for some of the proposed hypothesis like caveolin scaffolding domain hypothesis and caveolae signaling hypothesis. These hypotheses were in support to understand the apparent diverse function of Cav 1 & Cav 1 interacting molecules. Recently, Lamaze C and Torino S proposed new hypothesis “mechano-dependent

caveolae disassembly/reassembly” [20].

Caveolin Scaffolding Domain Hypothesis

Oligomerization function of caveolin results in scaffolding of the membrane due to its interaction with several signaling molecules. It has been outlined that Cav 1 Binding Motif (CBM) is present in several signaling proteins which allows the interaction in the précised region with Cav Scaffolding Domain (CSD) near the plasma membrane. Although these interactions often suppresses the signaling activity or stimulates the scaffolding domain activity or has no effect at all. So it is suggested that the scaffolding domain might only be a sticky region that binds nonspecifically to many proteins present within the cell. Fernandez et al. [21,22] & Schlegel A et al in their study on the structure of caveolin showed that amino acids 79 – 96 of the scaffolding domain have α – helix secondary structure and the principal interacting surface of the scaffolding domain is a simple helix which mediates lateral interactions between caveolins and a tendency to interact non – specifically with membranes.

Cav 1 modulates signal transduction at the scaffolding region through an interaction with lipids mainly cholesterol. Experimental studies and genetic evidences have shown Cav 1 importance in maintaining caveolae cholesterol levels. It functions to maintain the caveolae lipid scaffold by replacing oxidized cholesterol with the new cholesterol. Hence, in caveolae scaffolding domain hypothesis caveolae lipid itself form a scaffold that systematizes multiple signaling molecules and controls their interactions.

Caveolin Signaling Hypothesis

This hypothesis highlights the role of caveolins mainly caveolin 1 as a negative regulators of diverse signaling molecules. It has been shown that the lipid raft caveolae has highly ordered caveolin proteins micro domains located within the plasma membrane. Their presence in Golgi, endoplasmic reticulum, vesicles and cytosol, extends their potential reach in regulating signals. They can function as scaffold as well as membranous signaling molecules. Specific motifs within the caveolin proteins aid to recruit lipids and proteins to caveolae thus enabling intracellular transferring of cellular apparatus and regulation of signaling pathway [23,24,25]. On the other hand, Machleidt T, et al. [26] in 2000 suggested that there is lack of genetic evidence to assess whether scaffolding domain function as a repressor or activator of cell signaling. Mutating the interacting region of scaffolding domain showed impaired trafficking of Golgi apparatus but the signal transduction through the scaffolding did not show any change. Hence, confirming that Cav 1 may not play strict role in suppressing the signal transduction at cell membrane scaffolding domain site.

Mechano-Dependent Caveolae Disassembly/ Reassembly

This hypothesis suggests that caveolae play an important role in adaptation of cells to mechanical stress. Cells tolerate many forces during its activity such as compressive, tensile, shear and

hydrostatic, and all these are involved in shaping, developing and maintaining the tissue structure. It was suggested that in the tumor, the physical forces within the complex microenvironment change with a myriad level of effect on the cells and tissues [27,28]. Paszek MJ, et al. examined tissue rigidity, tumor behavior and matrix stiffness and suggested that these are directly related to tumorigenesis. Lopez JI, et al. suggested that the Cav 1 presents dual role in tumorigenesis, first, as loss of expression during transformation and second, as a re-expression in the advances stages of tumor growth. Sinha B, et al. in 2011, presented with a threefold increase in free Cav 1 at the plasma membrane under hypo – osmotic shock condition which lead to mechanical disassemble of the caveolar structure. Once the mechanical force was removed the caveolae reassembled, maybe due to reduction of the amount of free cav 1 at the plasma membrane. Hence, the On/Off cycle of caveolae disassembly/reassembly due to mechanical force may cause an impact on either activation or inactivation of the signaling molecules. So, at early stages of tumorigenesis, the mechanical forces encountered by the tumor cells and the supporting stromal cells would be minimal to which the caveolae responds by flattening out and releasing free cav 1 at the plasma membrane, which in turn will activate anti tumoral effect by inhibiting the pro tumoral signaling activity. But in the later stages of tumorigenesis, the mechanical force and tension would increase to an extend that caveolae would activate pro tumoral signaling and suppress anti tumoral signaling effects [20,29,30,31].

Functions of Caveolin

The most appreciated function of caveolae in wide variety of cellular events includes endocytosis, transcytosis of proteins, cholesterol haemostasis, cell migration, cell polarity, calcium signaling and other signaling events. Recent evidences indicated that caveolins 1 confers long lasting stability and is an unusual protein that can be an integral membrane protein or a soluble membrane protein in multiple cellular compartments. In addition, it can interact with both lipids and lipid anchored on several proteins and these interactions make its prime function in lipid transport, membrane traffic and signal transduction [32].

Smart E, et al. in 1996 [33] strongly suggested that caveolin 1 plays a role in import and export of cellular cholesterol. He observed that in normal human fibroblasts cholesterol moves directly to the plasma membrane after being synthesized in the endoplasmic reticulum and then to the extracellular space. Plasma membrane cholesterol can also move directly to the endoplasmic reticulum. Graf GA in 1999, [34] showed binding of SR-B1 (Scavenger receptor class B membrane 1) to free cholesterol, cholesterol esters and cholesterol ethers in High Density Lipoproteins (HDL) which allows their movement to the intracellular sites from caveolae. Caveolin 1 is also present in the cytosol of many cells, where it gets associated with the cholesterol and behaves like a protein that is embedded in a particle with the size and light density of HDL. Ito J, et al. [35] showed that apoA-1(apolipoprotein A 1) binding to SR-B1 which stimulates the formation of cytosolic lipid particle containing caveolin 1,

cholesterol and phospholipid. Hence, both intracellular and extracellular lipid transport function is carried out by caveolin 1 Figure 1 & 2.

Membrane trafficking takes place at the caveola where caveolin 1 attracts proteins and functions as molecular engine that powers membrane invagination and budding. The exact way by which caveolin 1 performs this function is not known but the little evidence suggests its interaction with several tyrosine kinases and EGFR (Epidermal Growth Factor Receptor). Li PU, et al. [36] challenged the role of caveolin 1 in caveolae internalization and suggested that the Autocrine Motility Factor (AMF) is transported through caveolae pathway directly to the Endoplasmic Reticulum (ER) without the involvement of caveolin 1 and this delivery of AMF to ER is 5 times faster than the

cells expressing caveolin 1. The delivery of AMF to ER appears to occur in cells deficient in caveolin 1. In the experimental study on caveolin 1 deficient cells, numerous flask shaped membranes that were morphologically similar to caveolin 1 expressing cells were observed which contained AMF receptors. This suggests that internalization of protein through the caveolae pathway is the same regardless of whether the cells express caveolin 1. Internalization of caveolae is tightly regulated by PKC (Protein Kinase C) and tyrosine kinase. The unknown pathogens control this regulatory system and enhance their own internalization through caveolae by activating an unknown tyrosine kinase. Hence, caveolin 1 indirectly regulate the interaction of caveolae with the actin rich region and /or with actin binding proteins thereby controlling caveolae at the cell surface or in the interior of cell. The overall interaction at the membrane site is controlled by the caveolin 1 Figure 3.

Do Cav 1 behave like a tumor suppressor!!

Cav 1 gene is located at D7S522 locus of human chromosome 7q31.1 and is suggested to be erased in various human cancers such as head and neck cancers, breast, kidney, ovary, prostate and colon, thereby highlighting it to be a tumor suppressor [37, 38]. Lee H, et al. [39] and Rieth MD [40] in their experimental studies supported the hypothesis of Cav 1 as tumor suppressor by describing sporadic negative mutant of Cav 1 P132L. They showed that expression of P132L mutant resulted in the absence of caveolae at the plasma membrane but the mutant itself resided in the golgi apparatus. If the mutation of both the mutant and wild type Cav 1 is expressed in the cells and localized to the golgi apparatus then this results in mispleating of the proteins. The mutant causes up regulation of several signaling molecules like EGF, HGF etc and cellular revolution. Bonuccelli G, et al. [41] demonstrated that Cav 1 P132L acts as a loss of function mutation thereby promoting the recurrences in breast cancer. Hayashi, et al. reported 16 % mutation in a sample of 92 breast cancer patients whereas Li T, et al. [42] observed mutation in only 9 %. Most of the tumors presented Cav 1 activity combined with oncogenes and signaling molecules which either results

in down regulation of Cav 1, over expression of cyclinD1, ERK hyper activation and/or hyper proliferative activity. Glenney JR [43] suggested that Cav 1 may be a target for inactivation during oncogenesis; hence in most of the cancers the Cav 1 is down regulated. However, elevated expression of Cav 1 has been associated with the metastasis and negatively correlated with patient survival [44,45]. Hence, Cav 1 is considered as "conditional" tumor suppressor.

Do Cav 1 is an oncogene!!

Despite number of evidences that support Cav 1 as a tumor suppressor, there are evidences that suggest a drastically different role of Cav 1. Cav 1 expression may vary (reduced, unchanged or over expressed) depending on the tissue involved. Li L, et al. and Tahir, et al. [46,47] stated that in early stages of tumorigenesis, Cav 1 inhibits cell cycle progression and cell proliferation whereas in advance stages it progresses cancer and metastasis.

A variety of mechanisms have been recommended to elucidate how caveolin-1 may stimulate tumorigenesis. In prostate cancer cells, increased caveolin-1 levels were found to favor growth factor release and regulation by a positive feedback loop that enhances tumor cell invasiveness [48] and VEGF-associated angiogenic signaling [49]. In breast cancer cells, caveolin-1 was shown to associate with type 1 matrix metalloproteinase, thereby promoting invadopodia formation and matrix degradation, and this provides a mechanism for increased invasiveness [50]. Successful tumor cell migration requires proper cell polarization, directionality, and the ability to invade new surrounding matrices and cav 1 is one among them, proved in different experimental research studies [51,52].

Cav 1 which is highly expressed in endothelial cells is down regulated during the proliferative phase and up regulated during the differentiation phase of angiogenesis. In transmigrating endothelial cells, caveolin-1 was shown to interact with intermediate filaments [53]. In provision to this, an evidence involves caveolin-1 in regulating the small GTPases Rho and Rac, which are required for actin dynamics, cell polarization, and directional migration [54,55,56]. The role of caveolin-1 in tumorigenesis is not limited only on mechanisms of migration and invasiveness. But it was associated with various expression linked to the attainment of qualities associated with malignant cell behavior, including Multidrug Resistance (MDR) and metastasis, as well as poor prognosis.

The caveolin-1 plays a dual role in cancer. The uncertainty of caveolin-1 function is best resolved by the model for colon cancer. Early on the cellular environment, caveolin-1 is re-expressed, it develops traits consistent with a role as a tumor suppressor. Regulation of the Wnt signaling pathway is depicted as one of the possibility. Caveolin-1 into tumor cells that still express E-cadherin leads to the repression of beta - catenin-Tcf/Lef-dependent transcription of genes, such as cyclin D1, cox-2, survivin and c-myc. However, during tumor progression multiple changes occur at the molecular level (i.e., genome

Figure 3: Role of caveolae at the membrane site.

instability, genetic mutations and/or epigenetic alterations). One such possibility is the loss of E-cadherin. In this situation of cellular environment, caveolin-1 can no longer repress pathways associated with its role as a tumor suppressor. Instead, traits of the molecule prevail that favor the development of Multi-Drug Resistance (MDR) and metastasis. Therefore, it was suggested that caveolin-1 is a “conditional” tumor suppressor and that its ability to promote or suppress tumor development depend both on the cellular context and environmental conditions [57] Figure 4.

Role of Cav 1 in tumor microenvironment!!

The approaches that cancer cells procure to achieve the invasive properties require genetic and molecular alterations that are highly complex. Cancer growth is never a symmetrical growth instead it is an asymmetrical growth involving the growth of not only the tumor cells but also the supporting cells such as inflammatory cells, immune cells and the stromal fibroblasts. Cav 1 is shown to be expressed in the stromal fibroblasts (which tend to be myofibroblasts showing increased expression of a smooth muscle actin) [58,59,60,61] the loss of which could promote tumor growth by up regulating the (Extracellular Matrix) ECM components. In vitro studies showed that Cancer Associated Fibroblasts (CAFs) consistently expressed Cav 1 to guide the migration and invasiveness of the cancer cells. In vivo studies reported that Cav 1 remodels the peritumoral and intratumoral microenvironment so as to facilitate tumor invasion and the metastatic potency [62].

Various treatments can enhance the ability of fibroblasts to stimulate tumor cell invasion which include irradiation [63] and reactive oxygen species [64,65,66] as well as lifestyle-correlated factors, such as cigarette, smoke [67] and areca nut extract [68]. Fibroblasts have also been shown to encourage tumor cell invasion through secretion of a number of factors, including chemokine (C- C motif) ligand 2, stromal cell-derived factor-1, TGFb, IL-33, MMP2, EGFR ligands, and Hepatocyte Growth Factor (HGF).

Figure 4: Dual role of caveolin 1 in tumor growth.

Role of Cav 1 in angiogenesis!!

Angiogenesis is another major factor in the process of tumor growth, motility and invasion. Cav 1 is shown to control several aspects of endothelial cell functions in normal as well as pathological condition. Endothelial cells can secrete (epidermal growth factor) EGF to induce EMT and invasion [69]. Under hypoxic condition, tumor cells will secrete angiogenic factors such as Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF) which induces neovascularization. Tumor-associated endothelial cells has shown experimentally to stimulate invasion in vitro through the secretion of IL-8 (Interleukin - 8) [70] and CXCL1 [Chemokine (C-X-C motif) ligand 1] [71]. Hypoxia in the tumor microenvironment can induce Notch and EMT factors, such as Slug and Snail, to increase invasion [72,73]. In addition, MMP-9 can be induced by hypoxia to enhance invasion in a Na⁺/H⁺ exchanger 1- dependent fashion [74]. Various other studies showed that Cav 1 co- localizes with VEGF in tumor associated endothelial cells and promotes angiogenesis whereas the depletion of Cav 1 in endothelial cells affect cell migration and reduced angiogenesis [75,76].

Tkachenko E, et al. showed that in prostate cancer Cav 1 stimulate specific angiogenic activities through the modulation of P13K-eNOS (Phosphatidylinositol 3-Kinase-endothelium nitric oxide synthase) pathway [77]. NO (Nitric Oxide) was discovered by Furchgott and Zawadzki as “Endothelium-Derived Relaxing Factor” (EDRF). It soon became obvious that EDRF, like nitroglycerine, activates soluble guanylate cyclase in vascular smooth muscle by binding to its active haeme centre. The three NOS isoenzymes (neuronal, endothelial and inducible) and among all eNOS is responsible for maintaining low vascular tone and preventing leukocytes and platelets from adhering to the vascular wall [57] Table 1.

Caveolae role in Inflammation!!

The functional importance of caveolins in inflammatory responses is yet to be elucidated. Yan SR [78] documented the expression of cav 1 in neutrophils but fails to explain its functional role. Hu, et al. [79] described the unique role of caveolin 1 in regulating antimicrobial responses of neutrophils, which under pathological circumstances may contribute to inflammatory tissue injury. The author experimented on cav 1 deficient null mice to study the functional response of neutrophils derived from the bone marrow. He observed that the cav 1 null neutrophils are defective in agonist induced oxidant production, adhesion and transendothelial migration compared with wild type neutrophils. The author also studied the perfused murine lung model in which cav 1 null or wild type neutrophils are perfused through wild type (cav 1 sufficient) and observed that cav 1 null neutrophils are activated with the combination of formyl-Met-Leu-Phe (fMLP) and Platelet Activating Factors (PAF) thereby causing less micro vascular injury than the wild type neutrophils.

Caveolae role in inflammation was partly explained by the “Caveolae Signaling Hypothesis”. Antibacterial response of neutrophils can be induced by activation of the NADPH oxidase,

Table 1: Distribution of Cav 1 and Caveolae in NOS Isoenzymes.

NOS Isoenzymes	eNOS	nNOS	iNOS
Originally cloned	Endothelial cells	Neuronal cells	Macrophages
Gene encoding and its position	NOS3 7q35-36	NOS1 12q24.2-31	NOS2 17q11.2-12
Major regulatory mechanism	Ca+2 dependent (Ca-calmodulin) Ca+2 independent (phosphorylation, palmitoylation)	Ca+2 dependent (Ca-dystrophin)	Ca+2 independent; transcriptional regulation e.g. by NFB
Subcellular localisation	Golgi apparatus plasmalemmal caveolae	Cytosol endoplasmic reticulum sarcolemma postsynaptic densities caveolae (caveolin 3)	Phagosomes

chemoattractant induced actin reorganization and migration. Also, over expression of recombinant cav 1 in "engineered phagocytes" (Cos-phox cells) resulted in enhanced chemoattractant – induced superoxide production, thereby providing strong evidence for a signal – enhancing role of caveolin 1 [80, 81].

Caveolae in Cancers!!

Huang CF, et al. studied the relation between cav 1 and head and neck squamous cell carcinoma and found a reduced expression of caveolin-1 in cancers including head and neck carcinoma [82] but several other studies recognized cav-1 as a tumor promoter because it was over-expressed in various kinds of cancers, especially in oral cancer [83,84]. Consistent with the previous reports, their findings showed that highly expressed caveolin-1 was in the cytoplasm, and its expression level was stronger in OSCC (Oral Squamous Cell Carcinoma) than in normal oral mucosa. Hung KE. et al, also showed increasing expression of the marker from normal oral epithelium to dysplastic and neoplastic epithelial tissue (squamous cell carcinoma) [85]. Bau, et al. [86] demonstrated that polymorphism of the cav-1 gene in

some genotypes was associated with oral cancer susceptibility.

Keap1-Nrf2 system, as a key signaling pathway that regulates transcription of a series of cytoprotective proteins, plays an important role in oxidative stress, inflammation and carcinogenesis [87]. Keap1 is essential for the regulation of Nrf2 activity; both of them enhanced proliferation, drug resistance and could be potent survival factors in cancers [88]. The strong correlations were observed between caveolin-1 expression and Keap1-Nrf2 system suggesting that caveolin-1 play important role together with Keap1-Nrf2 system in the progression of OSCC.

Gould ML, et al. and Low JY, et al. observed loss of cavin1 expression during the progression of prostate cancer. This down regulation resulted in cell migration, tumor progression and metastasis. All these events occur through the reduce expression of matrix metalloproteases and altered secretion of enzymes, cytokines and growth regulatory proteins. Cavin 1 expression is also decreased in number of cancers like lung cancer and breast cancer, cavin 2 is down regulated in breast, kidney and prostate cancers as well whereas cavin 3 is down regulated in ovarian cancers and breast cancers. Cavin 1 and Cav 1 are co-transcriptionally regulated to modulate oncogenic function and to enhance aggressiveness. [20]

In colon cancer, the established Wnt signaling pathway became one of the attractive potential caveolin-1 targets. This was substantiated by reports by Hult, Bash, et al. 2000, on one hand caveolin-1 expression prevented transcription of cyclinD1 by sequestering β catenin and on the other hand survivin is a β catenin/Tcf/lef target gene [89]. Hence, assuming these roles the authors recognized that caveolin-1 controlled survivin expression by sequestering β catenin at the plasma membrane [90]. Subsequently this ability required the expression of E-cadherin in colon cancer and melanoma cell lines [91]. Lobos-González L, et al. suggested that the combined loss of caveolin-1 and E-cadherin in epithelial cells is likely to promote increased expression of genes relevant to epithelial-mesenchymal transition, loss of cell-cell contacts and cell transformation [92] Table 2.

Role of Cav 1 in Odontogenic Cysts and Tumors!!

Among caveolin proteins, cav-1 is highly expressed in

Table 2: Cav 1 expression in different types of human cancer.

	Caveolin - 1 expression				
Tissue affected	Normal	Cancer – early phase	Cancer – late phase	In vivo study	In Vitro study
Colon cancer	High expression	Low expression	Metastasis	Increased susceptibility to tumors	Not available
Breast cancer	High expression	Low expression	Metastasis	Increased susceptibility to tumors	High susceptibility to chemotherapy; Invadopodia
Lung	High expression	Low expression	Metastasis	Hyperplasia	Cell polarization and migration
Melanoma	Not known	Not known	Metastasis	Not known	Not known
Oral Squamous Cell Carcinoma	High expression	Low expression	Metastasis	Not known	Not known

epithelial cells including keratinocytes, salivary glands and respiratory tract epithelium, as well as, osteoblasts, endothelial cells and skeletal muscles [93,94]. Schwab, et al. reported cav-1 expression in epithelial and mesenchymal cells of tooth germ in various stages of development, suggesting that cav-1 could participate in odontogenesis [95].

The odontogenic cystic lesions extensively expressed Cav 1 in the basal cell layer showing both membrane and cytoplasmic staining whereas few odontogenic tumors showed immunoreactivity to a smaller degree and more focally. This suggested that cav-1 could be a tumor suppressor in odontogenic tumors. Jaafari-Ashkavandi Z, et al. [96] demonstrated immunoreaction of cav 1 in the basal cells of cysts and tumors, and reported that this marker is not related to proliferation activity. Various researches have been studied on the expression of proliferation markers (such as Ki-67) in odontogenic cysts and tumors which reported higher proliferative activity in tumors compared to cysts [97].

Expression of cav-1 has been reported in enamel organ, particularly inner enamel epithelium and ameloblast cells of developing tooth germ [95]. It was also demonstrated that cav-1 participated in cell transformation and differentiation [98]. But the immunoreaction of cav 1 appeared clearly in less differentiated cells of cysts and tumors (basal cells, ameloblast-like and some stellate reticulum-like cells) and not in differentiated superficial cells of odontogenic cysts and tumors. So, it is hypothesized that cav-1 may be a useful marker for differential diagnosis of odontogenic lesions [96].

Cav 1 in Salivary Gland Tumors!!

Cav 1 is strongly expressed in the ductal epithelial cells of normal salivary glands but the physiologic function of caveolae and cav 1 in normal salivary gland is yet to be elucidated. It was suggested that, TRPC 1 (Transient Receptor Potential Canonical 1) is a transmembrane protein that is expressed by salivary glands. The TRPC 1 functions in salivary gland secretion and as a store operator for Ca^{2+} influx mechanism. It localizes with cav 1 and its function depends upon the presence of cholesterol and/or on the integrity of the cholesterol rich caveolae [3].

Cohen AW, et al. observed down regulated expression of cav 1 which could be due to transcriptional silencing through hypermethylation of the caveolin 1 gene promoter, which further, may nullify cav 1 expression in human cancers. They observed the down regulation of cav 1 in advanced stage, high grade malignancy or those tumors that had longer duration compared with tumors that had shorter duration. This feature indicates the tumor suppressor activity of cav 1 [99].

Hoffman R in 2004, hypothesized that, in the quiescent vasculature, many factors that regulate angiogenesis normally are held together as part of an inactive modular unit (termed as Angosome); and when, angiogenesis is stimulated, the angosome dissociates, thus enabling angiogenic regulators to become active. It is proposed that angosome is present in the caveolae of

capillary endothelial cells. The presence of caveolin 1 can inhibit proangiogenic factors. It may act as a master-switch, coordinating events during angiogenesis. This was further supported by Liu J, et al. [100] that angiogenesis activators such as VEGF, basic fibroblastic growth factor and hepatocyte growth factor, down regulate cav 1 in endothelial cells and the down regulation of cav 1 may be an important step along the pathway toward endothelial cell proliferation.

Cav 1 in Sarcomas!!

Quest AF, et al. in 2008, proposed that in vivo, cav 1 plays a tissue and stage specific modulatory role in cancer [101]. Wiechen, et al. confirmed cav 1 as a tumor suppressor in sarcomas by immunohistochemistry, where they found high cav 1 expression in fibroblasts, smooth muscle cells, adipocytes and endothelial cells with a fine granular membranous and a diffuse cytoplasmic staining pattern [102]. Various other studies focusing on the expression of cav 1 in specific sarcomas have shown that this protein is expressed in the cytoplasm of cells of the mesenchymal benign tumors, including the adipocyte of all types of lipoma and well-differentiated liposarcoma, the myocyte of angiomyolipoma, leiomyoma, and well differentiated leiomyosarcoma [103]. The immunostaining properties appeared uniform among these lesions, with no implication on the gender and age. Conversely, the malignant mesenchymal tumors which are poorly differentiated and dedifferentiated, including leiomyosarcoma and liposarcoma, showed weak immunoreactivity or failed to stain with cav 1, suggesting that loss of cav 1 might be a necessary step towards a differentiation.

Cav 1 was identified as a metastasis associated gene that is a transcriptional target of EWS/FLI1 (Ewing's sarcoma / friend leukemia integration 1 transcription factor) as well as an important determinant of ESFT (Ewing's sarcoma family of tumors) malignant phenotype and tumorigenicity [104]. Sáinz-Jaspeado M, et al. [105] showed that Cav 1 knockdown led to up regulation of Snail and the concomitant loss of e-cadherin expression. Consistently, loss of Cav1 expression inhibited the anchorage-independent growth of EWS cells and markedly reduced the growth of Ewing's sarcoma cell-derived tumors in nude mice xenografts, indicating that Cav 1 promotes the malignant phenotype in Ewing's sarcoma carcinogenesis. They demonstrated that CAV1 controls migration and invasion in ESFT cells in culture by mechanisms involving the production and activation of metalloproteinases. In another study Cav1 expression determines the sensitivity of ESFT cells to clinically relevant chemotherapeutic agents [106].

Cantiani L, et al. demonstrated that cav1 act as an oncosuppressor in human osteosarcoma. The down-regulation of cav 1, is the part of osteoblast transformation and osteosarcoma progression [107]. The 6-year follow-up resulted in a better overall survival for osteosarcoma expressing a level of Cav1. Moreover, the majority of primary osteosarcoma showed significantly lower levels of Cav1 than normal osteoblasts suggesting its role as an oncosuppressor. Mayordomo E, et al.

carried out both in vitro and in vivo study but could not able to conclude that cav 1 is a marker either for good or for bad prognosis. In his, in vitro study, osteosarcoma cell lines forced to over express cav 1, which showed reduced malignancy with inhibited anchorage-independent growth, migration and invasion whereas in vivo study, cav 1 over expression nullified the metastatic ability of osteosarcoma cells. They also showed that c-Src and c-Met tyrosine kinases, which are activated in osteosarcoma, co-localized with cav 1 and were inhibited upon cav 1 over expression [108].

Cav 1 and Caveolin-3, both are expressed specifically in muscle tissue. Cav1 restrict its expression to satellite cells (represents a pool of quiescent reserve elements) whereas Cav 3 expresses in myoblast cells undergoing differentiation and in mature fibers. [109, 110] This suggests that timely coordination of Cav1 and Cav3, contributes to skeletal muscle homeostasis. In skeletal muscle sarcoma, Cav3 is considered a sensitive and specific marker [111] and Cav1 is considered a marker of poor differentiation [112] associating its expression to a better prognostic entity. Hence, depending on the varied role of Cav1 in sarcomas, it may be possible to use different targeting options. For example, the sarcomas in which Cav1 act as a tumor suppressor; either a targeted ectopic re-expression or introduction of a CSD (Caveolin Scaffolding Domain) would be a viable option. Also, cases where Cav1 acts as an oncogene, a direct targeting of Cav1 using antisense or indirectly by chemical inhibition, or lowering cholesterol (that disrupts caveolae) may be of great help [113, 114].

Cav role in miscellaneous!!

Campbell L & Gumbleton M [115] hypothesized that in psoriasis caveolin – 1 negatively regulates key signal transduction pathways in epidermal keratinocytes. The reduced expression in psoriasis, in response to yet unidentified mechanism, a relaxation of caveolin 1 negative regulatory role results in keratinocyte hyperproliferation and development of Psoriasiform hyperplasia. This made them propose that a reciprocal relationship between cav 1 expression and hyperproliferative non-malignant disorder. Pathobiology of psoriasis supports that aberrant signaling through the EGFR is an important basis to the keratinocytes hyperproliferation. Neovascularization mediated by VEGF is an early and critical event in the development of psoriatic plaque. VEGF secreted near the vicinity of epidermis causes down regulation of cav 1 in keratinocytes thus, relaxing the controlled function of EGFR system. Also, abnormal calcium hemostasis within the keratinocytes imparts some role of cav 1. The psoriatic keratinocytes fails to sustain high intracellular levels of calcium, required for normal keratinocyte differentiation, causing dysfunctional or loss of cav 1 [115].

Caveolin expression has also been defined in a variety of vascular neoplasms, including capillary hemangiomas, targetoid hemosiderotic hemangiomas, tufted angiomas, angiosarcomas, Kaposi's sarcoma, and epithelioid hemangioendotheliomas [116]. Bayer-Garner I, et al. studied immunohistochemical analysis

of the scaffolding protein caveolin but failed to discriminate among benign and well-differentiated smooth muscle and adipocyte neoplasms. He observed Caveolin immunostaining in both the nucleus and cytoplasm of cells of mesenchymal tumors, including the adipocyte of all types of lipoma and well-differentiated liposarcoma, the myocyte of angiomyolipoma, leiomyoma, and well-differentiated leiomyosarcoma whereas all the poorly differentiated and dedifferentiated sarcoma, including leiomyosarcoma and liposarcoma, showed weak immunoreactivity or failed to stain with caveolin. So, these observations presented some important pathogenic implications [117].

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Conclusion

The dualistic nature and role of caveolae and caveolin 1, intends to investigate the involvement of caveolae assembly and disassembly in the tumor progression. The ever-changing view of their function in mammalian physiology is in part due to the difficulty of working with such membrane domains and a lack of different but complementary tools available for rigorous analyses. It is now mandatory to put caveolae in the multimolecular signaling platforms like lipid rafts and focal adhesions, as this maybe the potential target for novel therapeutics in the current future.

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