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Clinical and Translational Implications for the Caveolin Gene Family: Lessons from Mouse Models and Human Genetic Disorders

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Abstract

Here, we review the clinical and translational implications of the caveolin gene family for understanding the pathogenesis of human diseases, including breast and prostate cancers, pulmonary hypertension, cardiomyopathy, diabetes, and muscular dystrophy. Detailed phenotypic analysis of caveolin knock-out mice has served to highlight the crucial role of a caveolin-deficiency in the pathogenesis of many human disease processes. Mutations in the human caveolin genes are associated with a number of established genetic disorders (such as breast cancer, lipodystrophy, muscular dystrophy, and cardiomyopathy), making the caveolins important and novel targets for drug development. The implementation of new strategies for caveolin-replacement therapy—including caveolin-mimetic peptides—is ongoing.

Keywords

Caveolae; Caveolins; Human Disease Pathogenesis; Mouse Animal Models

Overview

Caveolins are the signature proteins of specialized invaginations of the plasma membrane, named caveolae, that function to regulate signal transduction within the cell. Three members of the caveolin family protein have been identified, caveolin-1, -2 and -3. Caveolin-1 and -2 are co-expressed in a wide range of tissues, whereas caveolin-3 is muscle specific. The generation of caveolin null mice has demonstrated clear roles for the caveolin proteins in mammalian physiology. Caveolin-1 null mice present several pathological phenotypes, suggesting that caveolin-1 has pleiotropic functions in various organs. Caveolin-1 null mice show increased susceptibility towards the development of epidermal and mammary tumors, but exhibit decreased tumor formation in the prostate, demonstrating that caveolin-1 behaves as a tumor suppressor or a tumor promoter depending on the cellular context. Moreover,

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several other phenotypes of caveolin-1 null mice indicate that caveolin-1 plays an important role in the regulation of insulin signaling, and in pulmonary and cardiac function.

Caveolin-2 null mice reveal unique functions of caveolin-2 in the lung and in the skeletal muscle. Indeed, caveolin-2 null mice display lung defects with increased alveolar septa and fibrosis, and exhibit a peculiar skeletal muscle phenotype, with tubular aggregates formation and mitochondrial proliferation. Caveolin-3 null mice exhibit pathological changes in the skeletal muscle and the heart, and show an unexpected metabolic phenotype, with increased adiposity and insulin resistance.

Importantly, data from human samples confirm that caveolins are involved in human pathology. Mutations in the caveolin-1 gene are found in breast cancer as well as in oral squamous cell carcinomas. Caveolin-1 mutations are associated exclusively with estrogen receptor positive breast tumors, suggesting that caveolin-1 normally regulates estrogen receptor expression and signaling. Mutations in the caveolin-3 gene are detected in a wide spectrum of skeletal muscle disorders, including limb girdle muscular dystrophy, distal myopathy, idiopathic hyperCKemia, rippling muscle disease, as well as in cardiac pathologies such as familial hypertrophic cardiomyopathy, Long-QT Congenital Syndrome, and sudden infant death syndrome.

Thus, modulation of caveolin expression may represent a novel therapeutic approach for the cure of a number of pathological conditions. Preliminary studies have shown that a caveolin-1 mimetic peptide may be successfully used for the treatment of cancer and of pulmonary hypertension. More studies will be required to evaluate the possibility of using caveolin-1 mimetic peptides in other pathological contexts.

Caveolae and the Caveolin Gene Family

Caveolae are 50-100-nm omega-shaped invaginations of the plasma membrane. Due to their high content of cholesterol, glycosphingolipids and sphingomyelin, caveolae are considered a specialized subset of detergent-insoluble plasma membrane microdomains, named lipid rafts (1). The signature proteins of caveolae are a family of proteins called caveolins (2). Structurally, the formation of caveolae requires the capability of caveolin to bind cholesterol and to oligomerize (Figure 1) (3-5). Three members of the caveolin family have been discovered so far. Caveolin-1 (Cav-1) is widely expressed and is most abundant in adipocytes, fibroblasts, endothelial cells as well as epithelial cells such as mammary epithelial cells and type I pneumocytes (6). Cav-1 is essential for caveolae formation, and Cav-1 expression levels correlate with caveolae numbers (7, 8). Caveolin-2 (Cav-2) is expressed in the same cell types as Cav-1 (9, 10). Caveolin-3 (Cav-3) is the muscle specific family member and is mainly found in heart, skeletal muscle, as well as smooth muscle cells (11). Caveolins are more than just caveolae-associated proteins. They help the formation of a microenvironment to compartmentalize several signaling molecules, thereby facilitating the cross-talk between different signaling pathways (12). The caveolin-scaffolding domain (CSD) serves as a docking site for the binding and tonic inhibition of several classes of molecules belonging to pro-proliferative and pro-survival pathways. Thus, deregulation of caveolin expression and function is involved in many pathological conditions. The physiological implications of caveolins became evident with the generation of null (-/-) mice for each caveolin.

Cav-1 in Tumor Biology: Is Cav-1 a Tumor Suppressor, an Oncogene or Both?

Several lines of evidences indicate that Cav-1 may act as a tumor suppressor or a tumor promoter depending on the organ. Cav-1 expression is downregulated in NIH-3T3 cells transformed with various oncogenes such as H-Ras (G12V), Bcr-Abl and v-Abl (13). In soft agar, the anchorage-independent growth of transformed cells can be reversed by Cav-1 re-expression (14, 15). In NIH-3T3 cells, antisense-mediated down-regulation of endogenous Cav-1 induces anchorage-independent growth, promotes tumor formation in nude mice with hyperactivation of the MAP kinase pathway (16). Importantly, loss of Cav-1 in mice was shown to promote cellular growth in several contexts, including the mammary gland and the skin. For example, although the ablation of Cav-1 is not sufficient to induce spontaneous tumor formation, Cav-1 null mammary glands display cellular hyperplasia, and accelerated mammary gland development during pregnancy (17, 18). In the MMTV-PyMT (mouse mammary tumor virus-polyoma middle T antigen) mouse model background, loss of Cav-1 accelerates dysplastic lesion formation, promotes premature development of mammary tumors, and increases metastatic potential (19, 20). Additionally, the combined loss of INK4a, a tumor suppressor, and Cav-1 lead to severe mammary hyperplasia with increased side branching and fibrosis (21). In the skin, Cav-1 ($-/-$) mice demonstrate hypersensitivity to carcinogen-induced epidermal tumors. Following 16 weeks of treatment with the carcinogen DMBA, Cav-1 ($-/-$) null mice show very significant increases in tumor incidence, tumor area and tumor number per mouse when compared to wild type counterparts (22). Mechanistically, in the mammary gland and in the skin, Cav-1 is thought to inhibit proliferation and cellular growth. Indeed, in the context of Cav-1 genetic ablation, carcinogen-induced epidermal tumors and oncogene-induced mammary tumors display hyperactivation of the mitogenic p42/44 MAP kinase pathway, with increased levels of Cyclin D1 (20, 22).

However, Cav-1 does not behave as a tumor suppressor in all cellular contexts. A growing body of evidence derived from cellular, mouse, and human studies clearly indicates that Cav-1 acts as a tumor promoter in other organs, such as the prostate. In the TRAMP (TRAnsgenic Adenocarcinoma of Mouse Prostate) mouse model of prostate cancer, Cav-1 expression is increased when compared to the normal prostate epithelium (23). Genetic ablation of Cav-1 in TRAMP mice decreases incidence of prostate tumors at 28 weeks of age and reduces metastasis to regional lymph node and to distant organs, such as the lungs (23). In patients with prostate cancer, Cav-1 expression is increased in 13% of well-differentiated tumors, 24% of moderately differentiated tumors and 39% of poorly differentiated tumors (24). These results suggest that Cav-1 over-expression in prostate cancer cells correlates with tumor progression (24). Other studies have shown that high levels of Cav-1 are associated with high Gleason's score, reduced survival and poor prognosis in prostate cancer (25, 26). Finally, a higher incidence of Cav-1 positivity is observed in metastatic lesions and metastasis-derived cell lines, as compared to primary tumors and primary tumor-derived cell lines, respectively (27).

In the mammary gland and skin, Cav-1 negatively regulates proliferation. However, the anti-apoptotic properties of Cav-1 seem to contribute to the development of prostate cancer. Prostate tumors derived from TRAMP/Cav-1 null mice exhibit increased apoptosis as assessed by TUNEL staining, with increased levels of prostate apoptosis response factor-4 and PTEN (23). However, the role of Cav-1 in apoptosis in different cell types still remains largely unexplored and highly controversial. In prostate cancer cells, it is believed that Cav-1 can shift from its conventional membrane-bound position to a secreted form outside the cell. Indeed, Cav-1 has been detected in the serum of patients with prostate cancer (28). It was also found that the Cav-1 secreted form is bioactive since it could promote cell

survival (28). The phosphorylation of Cav-1 on serine 80 seems to be involved in the conversion of Cav-1 from an intracellular protein to a secreted form (29).

Table 1 summarizes the main phenotypes of Cav-1 ($-/-$) mice and the implications for different human pathologies.

Cav-1 and Adult Stem Cells

Studies have shown that Cav-1 may normally regulate the proper differentiation of stem/progenitor cell populations in organs, such as the skin, the mammary gland, and the intestine, that are physiologically subjected to constant self-renewal (Figure 2). For example, Cav-1 deficiency leads to an amplification of an adult mammary stem cell population, both *in vivo* and *in vitro*. The expression of stem cell markers, such as Sca-1 and Keratin 6, is greatly increased in the hyperplastic mammary ducts of Cav-1 deficient mice, as well as in 3D cultures of Cav-1 ($-/-$) primary mammary epithelial cells. Such an amplification of progenitor cells is functionally associated with the abnormal presence of myoepithelial cells in the hyperplastic lesions of Cav-1 deficient mammary glands (30).

In addition, genetic ablation of Cav-1 induces an abnormal amplification of small intestine crypt stem cells, resulting in increased susceptibility to gamma-radiation. Cav-1 null small intestine crypt stem cells display higher proliferation rates, as compared to wild-type controls. Because of its fast renewing nature, the small intestine constitutes one of the main targets of radiation. After gamma-radiation exposure, Cav-1 deficient mice display a decreased survival rate, as compared to wild-type mice (31). Mechanistically, Wnt/beta-catenin signaling, which normally controls stem cell self-renewal, is up-regulated in Cav-1 null mammary and crypt stem cells. The longevity and slow-dividing properties of stem cells facilitates the accumulation of genetic alterations, and renders progenitor cells the likely precursors of malignant derivatives. As such, loss of Cav-1 may induce the accumulation of stem cells, and that this event may be an initiating factor during tumorigenesis.

Cav-1: Insulin Signaling, Pulmonary and Cardiac Function, & Ischemia

The analysis of Cav-1 ($-/-$) mice has highlighted how loss of Cav-1 function may lead to a number of important pathological conditions. In addition to its involvement in breast, skin and prostate cancer, Cav-1 also plays important roles in diabetes, lung and heart disease, and ischemia (Table 1).

Cav-1, Insulin Signaling and Diabetes

Cav-1 ($-/-$) mice show resistance to diet-induced obesity, and display adipose tissue atrophy (32). Metabolically, plasma levels of insulin and glucose are normal in Cav-1 null mice (32). However, an insulin tolerance test revealed abnormally low glucose uptake in young Cav-1 ($-/-$) mice, suggestive of impaired insulin signaling (33). This defect in glucose uptake is due to a severe deficiency of insulin receptor protein expression in Cav-1 ($-/-$) adipose tissue (33). In direct support of these findings, Cav-1 null adipose tissue displays decreased insulin signaling, as assessed by phosphorylation of insulin receptor and its downstream targets (33). Although loss of Cav-1 is not sufficient to induce diabetes, it may function as a predisposing factor for the development of insulin resistance in humans.

Cav-1 and Pulmonary Function

Cav-1 is highly expressed in the lung, and is found in several pulmonary cell types, including endothelial cells and pneumocytes. Histological analysis of Cav-1 null mice reveals a deeply modified lung morphology with reduced alveolar spaces, increased wall

thickening, fibrosis, and hypercellularity (34, 35). In addition, Cav-1 null mice develop pulmonary hypertension (36, 37). Reduced Cav-1 levels in the lung have been documented in several animal models of pulmonary hypertension and in patients with severe pulmonary hypertension. These findings may have important implications for understanding human respiratory pathologies, such as pulmonary hypertension, fibrosis, as well as acute respiratory syndrome.

Cav-1 and Cardiovascular Function

Cav-1 is implicated in several cardiovascular pathologies, including cardiac hypertrophy, neointima formation and atherosclerosis. Cardiac hypertrophy is a critical pathology leading to heart failure. Cav-1 ($-/-$) mice show progressive concentric left ventricular hypertrophy, as well as right ventricular dilation (36, 38). Cav-1 expression is restricted to the supporting cells of the heart, such as fibroblasts and endothelial cells. Excessive activation of the Ras-p42/44 MAP kinase cascade in Cav-1 ($-/-$) cardiac fibroblasts is considered one of the upstream key factors promoting hypertrophy and fibrosis in the adjacent myocytes (39). Notably, Cav-1 ($-/-$) cardiac fibroblasts exhibit p42/44 MAP kinase hyperactivation when compared to wild type fibroblasts (38), suggesting that the hypertrophy of Cav-1 null hearts occurs via a paracrine mechanism.

Neointimal hyperplasia is the principal cause for clinical failures in angioplasty and is a critical component of re-stenosis. During the development of neointimal hyperplasia, the arterial wall thickens and the lumen narrows as a consequence of smooth muscle cells (SMC) accumulation and proliferation in the intima (40, 41). Genetic ablation of Cav-1 in mice facilitates SMC proliferation and neointima formation. Four weeks after ligation, Cav-1 ($-/-$) carotid arteries showed significantly more neointimal hyperplasia with subtotal luminal occlusion, as compared to wild-type mice. Mechanistically, the development of neointimal hyperplasia in Cav-1 ($-/-$) mice is mediated by elevated levels of phospho-p42/44 MAP kinase and cyclin D1 (42).

Atherosclerosis is characterized by the accumulation of modified lipoproteins in the subendothelial space followed by the recruitment and proliferation of monocytes/macrophages and SMC. Genetic ablation of Cav-1 in mice confers protection against atherosclerosis. In the apolipoprotein E-deficient (ApoE $-/-$) atherogenic mouse model background, loss of Cav-1 prevents the development of aortic atheromas, with a ~70% reduction in atherosclerotic lesion area. Mechanistically, loss of Cav-1 is associated with the down-regulation of proatherogenic molecules, namely CD36 and vascular cell adhesion molecule-1 (VCAM-1) (43).

Thus, Cav-1 ($-/-$) mice are more sensitive to the development of neointimal hyperplasia (luminal narrowing), but are strongly resistant to the development of atheromas, clearly demonstrating the different patho-physiology of sub-endothelial *versus* mural narrowing of the arterial lumina.

Cav-1 and Ischemia

Recent studies have directly addressed the functional role of Cav-1 in ischemic injury. First, Cav-1 ablation in mice was shown to promote ischemic injury in a model of hindlimb ischemia (44). Similarly, in a model of cerebral artery occlusion, Cav-1 null mice display a significant increase in the volume of cerebral infarcts, as compared with wild-type mice. Mechanistically, Cav-1 null ischemic brains exhibited decreased proliferation of endothelial cells and an elevated apoptotic index, as compared with wild-type counterparts (45). Ischemic pre-conditioning has previously been shown to increase the cardiac phosphorylation of Cav-1 and SRC in mice subjected to myocardial ischemia-reperfusion.

These interesting observations suggest that phosphorylation of Cav-1 and SRC might play a cardio-protective role in ischemic injuries. Hence, genetic ablation of the Cav-1 gene in mice was shown to attenuate the protective effect of myocardial ischemic pre-conditioning (46).

Caveolin-2: Pulmonary and Skeletal Muscle Functions

Cav-2 requires the presence of Cav-1 for stabilization and membrane targeting, such that in the absence of Cav-1, Cav-2 is retained in the endoplasmic reticulum/Golgi complex and undergoes degradation through a proteasomal pathway (47, 48). As a consequence, Cav-2 expression is nearly abrogated in Cav-1 null mice (34, 35). Thus, Cav-2 was considered an “accessory” protein that just functions in conjunction with Cav-1. However, the generation and analysis of Cav-2 null mice revealed unique roles for Cav-2 in the physiology of the lung and of the skeletal muscle. Cav-2 null mice show normal caveolae formation and nearly normal levels of Cav-1 expression (49). Cav-2 null lungs show thickened alveolar septa with increased number of endothelial cells. Consequently, Cav-2 null mice show exercise intolerance, suggestive of an impaired gas exchange that is often associated with human pulmonary diseases (49). Interestingly, the Cav-2 null lung phenotypes are identical to the ones observed in Cav-1 null mice (34, 35). Because Cav-2 expression is severely reduced in Cav-1 null mice, a Cav-2 deficiency is considered to be the root cause of this lung disorder.

Cav-2 null mice also exhibit an unexpected skeletal muscle phenotype. Cav-2 null skeletal muscles show peculiar abnormalities, such as tubular aggregate formation, and mitochondrial proliferation/aggregation. In addition, Cav-2 deficiency induces increased numbers of satellite cells, which are skeletal muscle specific stem/precursor cells (50). For a summary of Cav-2 null mice phenotypes and their implications in human pathologies, please refer to Table 2.

Caveolin-3: Skeletal Muscle Disorders, Heart Disease and Diabetes

Caveolin-3 is the muscle specific family member, and is necessary for caveolae formation in muscle tissues. Indeed, electron microscopy revealed a lack of caveolae in all striated and cardiac muscle cells of Cav-3 null mice (51, 52). Cav-3 null skeletal muscle shows signs of mild myopathic changes, such as mononuclear cell infiltration, variable fibers size, and presence of necrosis. These phenotypes are similar to the ones found in a human disease, termed limb-girdle muscular dystrophy (LGMD-1C), which is due to mutations in the *Cav-3* gene.

A Cav-3 deficiency also affects cardiac function, with the development of cardiac hypertrophy, dilatation, and reduced fractional shortening at 4 months of age (53). Cav-3 null cardiac myocytes show hypertrophy, with cellular infiltrates and progressive interstitial and perivascular fibrosis. Hyperactivation of the Ras-p42/44-MAP kinase pathway is associated with these cardiac phenotypes (53).

Interestingly, transgenic over-expression of either wild-type or dominant-negative mutant Cav-3 was shown to induce cardiomyopathy, with cardiac tissue degeneration, and reductions in cardiac functions (54, 55). These results suggest that maintenance of normal Cav-3 expression levels is essential for proper cardiac function.

Surprisingly, Cav-3 null mice also show an interesting metabolic phenotype. Cav-3 null mice demonstrate increased adiposity and develop insulin resistance, as shown by decreased glucose uptake and reduced glucose metabolic flux in their skeletal muscles. During fasting, Cav-3 null skeletal muscles exhibit normal insulin receptor protein levels. However, insulin stimulation induces a severe reduction in insulin receptor levels in Cav-3 null mice,

suggesting that Cav-3 may function to stabilize insulin receptor. These results show that Cav-3 contributes to the regulation of whole body glucose metabolism *in vivo* and indicates that Cav-3 may play a role in the development of insulin resistance (56, 57).

For a summary of Cav-3 null mouse phenotypes and their implications in human pathologies, refer to Table 2.

Caveolin Mutations in Human Disease

Over the last few years, mutations in the caveolin genes have been detected in several types of human diseases, clearly indicating the importance of these proteins for normal human physiology, and demonstrating that dys-regulation of caveolin function is a critical factor for the development of human disease.

Cav-3 mutations

The first mutations in a caveolin gene were identified a decade ago, when mutations in the *Cav-3* gene were found in patients with limb-girdle muscular dystrophy (LGMD-1C) (58). Since then, several other mutations have been found in patients with skeletal muscle disorders. The spectrum of disease phenotypes associated with Cav-3 mutations is quite variable, ranging from LGMD-1C, to idiopathic hyperckemia, rippling muscle disease, and distal myopathy. For a comprehensive review of Cav-3 and muscle disorders, please consult the GeneReview database available at <http://www.genetests.org> (search CAV3). Most of the Cav-3 mutations are heterozygous and cause a dramatic decrease in Cav-3 expression in skeletal muscle, thus acting in a dominant-negative fashion (59). Finally, mutations in the Cav-3 gene have been found in familial hypertrophic cardiomyopathy (60), in the arrhythmogenic syndrome Long-QT Congenital Syndrome (LQTS) (61), as well as in sudden infant death syndrome (SIDS) (62).

Cav-1 mutations

Somatic mutations in the *Cav-1* gene are involved in human cancer. We and others have shown that a proline to leucine substitution at position 132 (P132L) in the Cav-1 gene is found in 16-20% of breast cancers (63, 64). This heterozygous mutation acts in a dominant-negative fashion and induces the intracellular retention of the normal Cav-1 protein at the level of the ER/Golgi complex (17). Other mutations were also found in human breast cancer samples, such as W128stop, Y118H, S136R, I141T, Y148H and Y148S (64). Most interestingly, Cav-1 mutations only correlate with estrogen receptor alpha (ER-alpha) positive breast tumors, and are found in up to 35% ER-alpha positive tumors (64). These results suggest that Cav-1 may regulate ER-alpha expression. In fact, we have shown that ER-alpha is dramatically upregulated in the luminal mammary epithelial cells of Cav-1 knockout mice, as compared to their wild type counterparts (64). Because ER-alpha expression increases as mammary cell transformation progresses, these results suggest that Cav-1 mutations may be an initiating event in the development of human breast cancers. Thus, screening of patients biopsies for Cav-1 mutations could become a routine analysis as a preventive measure to detect estrogen-dependent breast cancer at an early stage. These findings provide new insights into the regulation of ER-alpha in breast cancer. Finally, mutations and abnormal expression of Cav-1 have been also found in human oral squamous cell carcinomas (65).

Recently, a homozygous mutation in the Cav-1 gene has been detected in Berardinelli-Seip Congenital Lipodystrophy, a rare genetic disorder characterized by near absence of adipose tissue, severe dyslipidemia, and insulin resistance. This mutation induces a complete loss of Cav-1 expression in skin fibroblasts (66). These findings provide human genetic evidence to directly support a critical role for Cav-1 in regulation of adipocyte function and insulin

signaling. Additional heterozygous Cav-1 mutations have now been observed in patients with atypical partial lipodystrophy and hypertriglyceridemia (67). See Garg et al, 2008, for a recent review on this subject (68).

Caveolin Mimetic Peptides: A Novel Therapeutic Strategy for Human Pathologies

Cav-1 functions as a powerful tumor suppressor in certain types of cancer and its re-expression in tumor cells could potentially become a new therapeutic avenue for the treatment of human cancers. Tumor progression in mice can be blocked by the administration of a cell-permeable peptide, containing the homeodomain of the antennapedia (penetratin) coupled to the Cav-1 scaffolding domain (CSD) (69). After subcutaneous implantation of Lewis lung carcinoma cells (LLC), the injection of the CSD peptide reduced tumor size and tumor vascular permeability, and increased tumor necrosis, suggesting that Cav-1 prevents tumorigenesis by affecting tumor blood supply (69). Similar experiments in WT and Cav-1 (−/−) mice further demonstrated the role of Cav-1 in vascular permeability and tumor development. In Cav-1 KO mice, LLC-derived tumors showed significantly higher growth rates, with increased angiogenesis and decreased apoptosis than tumors implanted in WT hosts. Most interestingly, administration of CSD peptide was able to reverse tumor hyperpermeability as well as to attenuate the increased tumor growth (70).

Therapeutic administration of the Cav-1 scaffolding domain peptide has been employed in other experimental models. Indeed, administration of CSD peptide has been successfully used to prevent the development of pulmonary hypertension (PH), right ventricular hypertrophy, and pulmonary artery medial hypertrophy in a monocrotaline-induced PH rat model (71). Administration of CSD peptide to monocrotaline-treated rats significantly prevented PH and normalized pulmonary artery medial hypertrophy and right ventricular hypertrophy (71). Finally, in an experimental mouse model of inflammation, the administration of the CSD peptide has also been shown to decrease inflammation, reduce edema formation and vascular permeability (72). Thus, the administration of Cav-1 peptide mimetics might become a novel alternative treatment for cancer as well as pulmonary hypertension patients.

Future Directions: Role of Caveolins in the Cancer Stroma

Normal epithelial cells are often surrounded by fibroblasts which serve mainly as support to protect the tissue from damage. Fibroblasts can proliferate and secrete extracellular matrix proteins as a response to injury. Once the wound healing process is complete, they undergo apoptosis and the ECM production lessens. Several lines of evidence now suggest that stromal fibroblasts become “activated” and play a very dynamic role in tumor initiation and progression. These cancer-associated fibroblasts (CAFs) express smooth muscle actin, have contractile functions and secrete ECM proteins. Unlike normal fibroblasts involved in wound repair, these CAFs do not undergo spontaneous apoptosis, rather they remain activated and hyper-proliferate. Studies suggest the recruitment of CAFs is not only a secondary event to tumor initiation, but is very dynamic as CAFs can increase the growth of adjacent epithelial cells through paracrine mechanisms. For a complete review on stromal fibroblasts and cancer please see Kalluri *et al* (73). However, very little is known about the mechanism(s) controlling the proliferation of CAFs and their clinical implications in cancer progression and outcome. Recently, it was shown that Cav-1 protein expression is decreased in primary cultures of CAFs isolated from patients with invasive breast cancer and that loss of Cav-1 function might play a role in their hyper-proliferation (74). Accordingly, treatment of these cells with a cell-permeable Cav-1 mimetic peptide decreased the phosphorylation of RB (retinoblastoma protein) as well as PCNA (Proliferating Cell Nuclear

Antigen) and MCM7 (MiniChromosome Maintenance) proteins levels, two pRB target proteins. Therefore, caveolin proteins might play an important role in cancer through their novel “tumor suppressor” role in the neoplastic stroma (Figure 3).

In accordance with this idea, we have recently shown that loss of stromal Cav-1 is a novel breast cancer biomarker that predicts early disease recurrence, metastasis, survival, and tamoxifen-resistance (75). For example, lymph-node-positive breast cancer patients showed an ~11.5-fold reduction in 5-year progression-free survival in the absence of stromal Cav-1 (80% *versus* 7% survival) (75). Mechanistically, this appears to be related to the idea that the loss of Cav-1 expression in mammary fibroblasts leads to pRB-inactivation, increased growth factor secretion, and stromal angiogenesis (76, 77) (Figure 3). As such, breast cancer patients lacking stromal Cav-1 might greatly benefit from anti-angiogenic therapy, in addition to standard treatment regimens.

Thus, future studies are warranted to explore this novel role for caveolins in the stromal pathogenesis of human cancers.

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Abbreviations

Cav	Caveolin
CSD	caveolin scaffolding domain
MMTV-PyMT	mouse mammary tumor virus-polyoma middle T antigen
TRAMP	TRansgenic Adenocarcinoma of Mouse Prostate
SMC	smooth muscle cells
LGMD	limb-girdle muscular dystrophy
ER	estrogen receptor
LLC	Lewis lung carcinoma cells
PH	pulmonary hypertension

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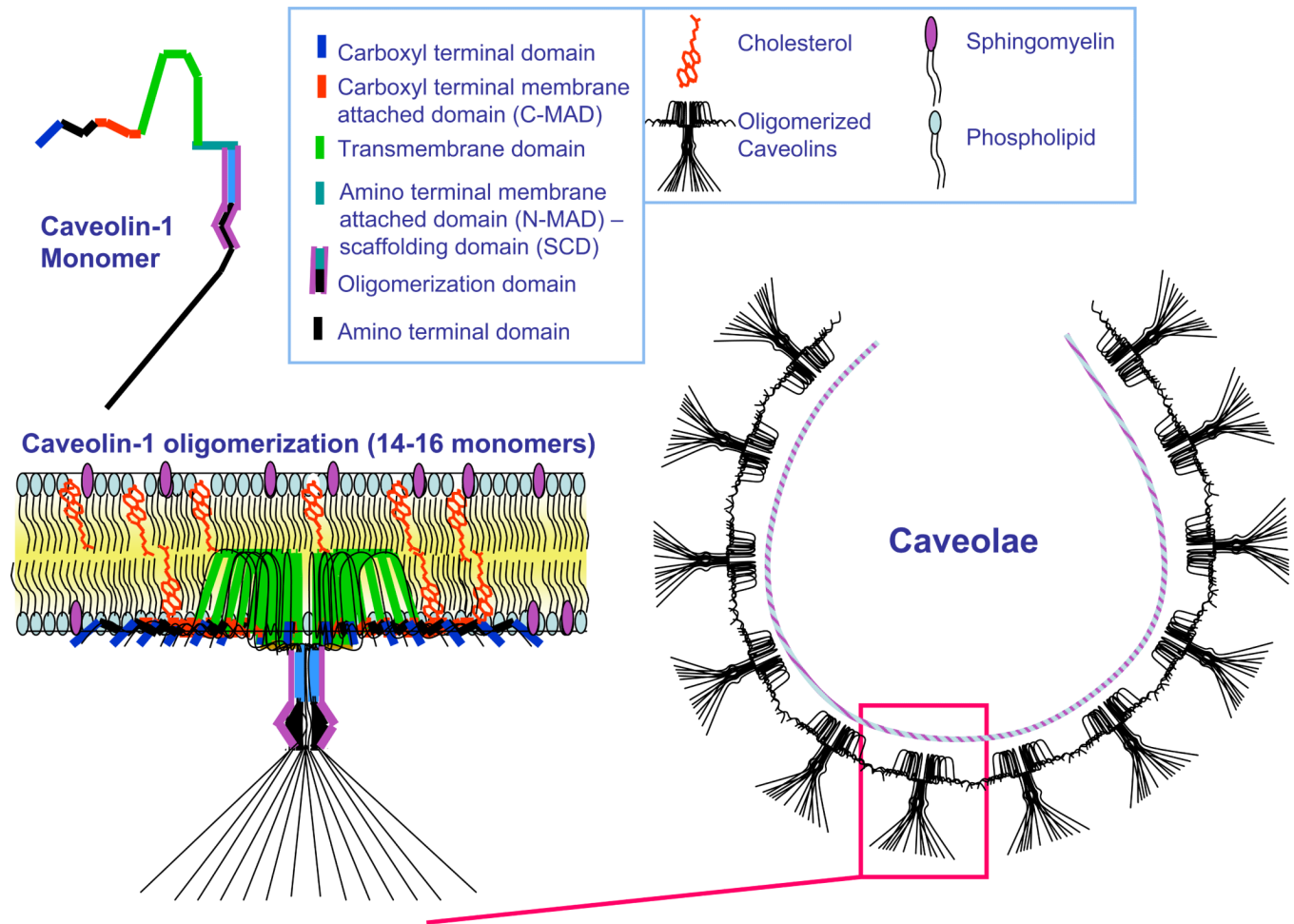


Figure 1. Caveolin Domain Organization, Membrane Topology, Oligomerization, and Caveolar Assembly

This diagram summarizes the domain organization of a caveolin-1 monomer, its self-oligomerization into caveolar assembly units, and the intergration of these assembly units into a caveolar membrane domain. The cytoplasmic surface of the caveolar coat provides an “integrated scaffold” onto which various classes of signaling molecules can assemble at the plasma membrane, such as G-proteins, Src-family tyrosine kinases, endothelial nitric oxide synthase (eNOS), and components of the Ras-p42/44 MAP kinase cascade. Caveolae organelles also function as docking sites for many different classes of cell surface receptors, including G-protein coupled receptors, cytokine receptors, and receptor-tyrosine kinases.

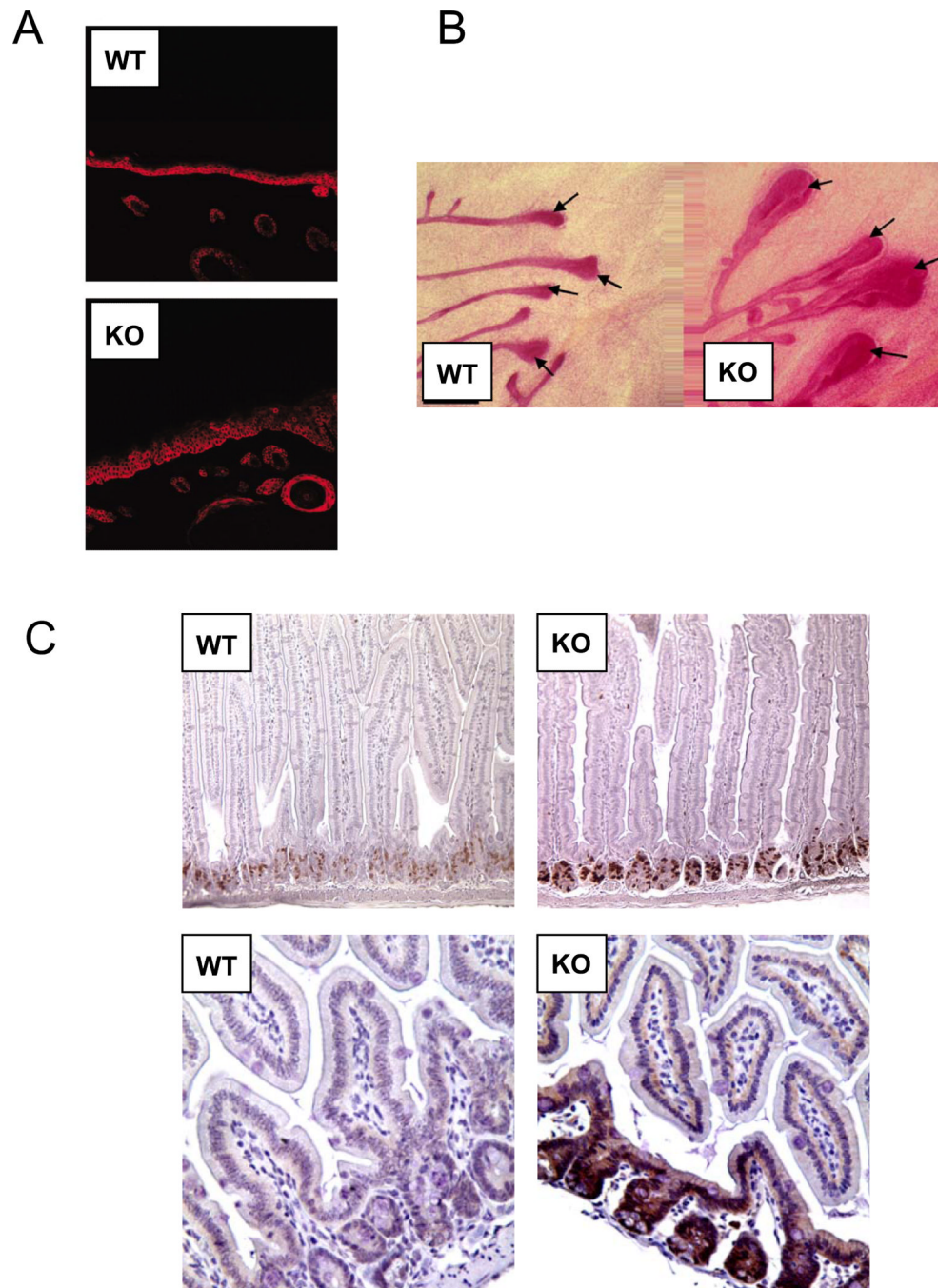


Figure 2. Loss of Cav-1 Increases the Adult Epithelial Stem Cell Compartment

A) *Skin*. After DMBA-treatment, Cav-1 ($-/-$) mice show an expansion of the basal keratinocyte cell layer in the skin, as marked by immuno-staining with anti-keratin-14 IgG.

B) *Mammary Gland*. Cav-1 ($-/-$) mice show an increase in the size of terminal end buds (TEBs), the site of mammary stem/progenitor cells during adult mammary gland development. Ducts and TEBs are stained red with Carmine dye.

C) *GI tract*. The intestinal crypt stem cells from Cav-1 ($-/-$) mice show increases in both proliferation (BrdU incorporation) and expression of β -catenin, a stem cell marker.

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Cancer-Associated Fibroblast (CAF) Phenotype

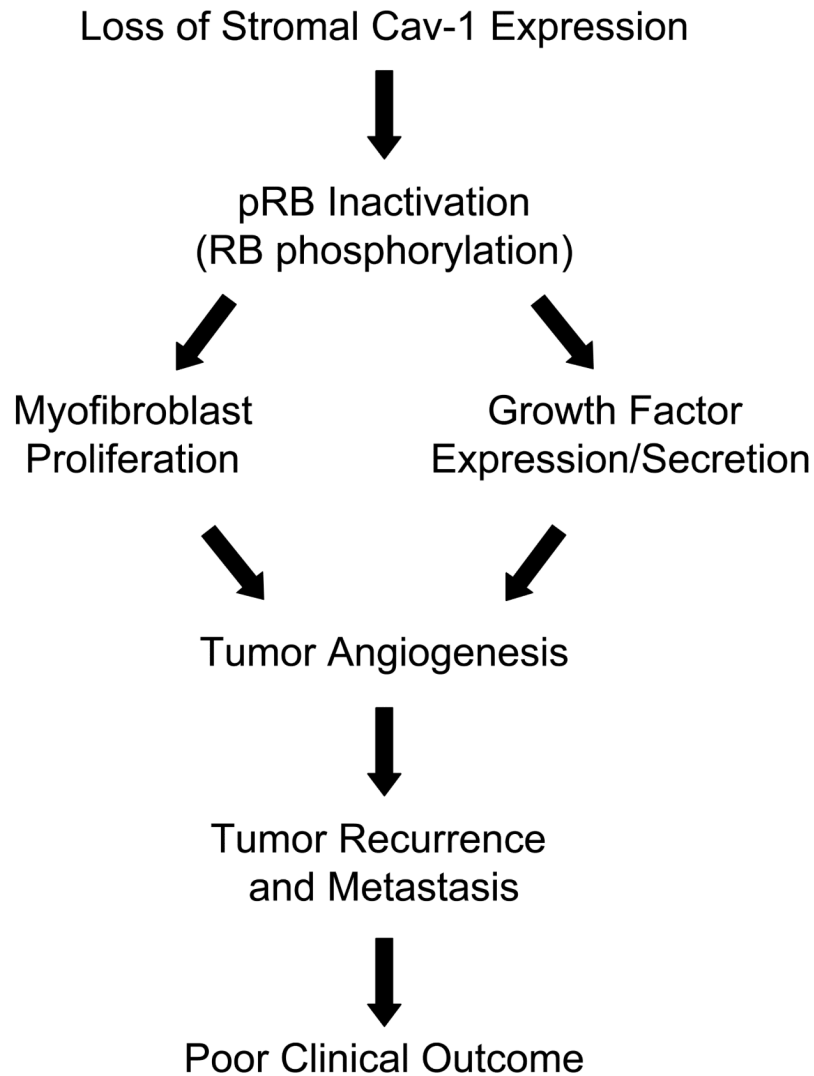


Figure 3. Loss of Stromal Cav-1 Expression Predicts Poor Clinical Outcome in Human Breast Cancer Patients

Mechanistically, loss of stromal Cav-1 expression in the tumor micro-environment leads to RB-inactivation, increased myofibroblast proliferation, and the secretion of angiogenic growth factors. This, in turn, greatly facilitates tumor recurrence and metastasis, leading to poor clinical outcome.

Table 1

Phenotypes of Cav-1 null mice and their implications in human pathologies

Gene	Expression	Knockout mouse phenotypes	Relevance to human pathologies
<i>Cav-1</i>	Epithelial cells Fibroblasts Endothelial cells Adipocytes Type I pneumocytes	Mammary epithelial cell hyperplasia and premature lactation during pregnancy	Breast Cancer
		Enhanced tumor formation and increased metastasis in a mammary tumor model background (MMTV-PyMT)	
		Increased mammary epithelial hyperplasia with fibrosis in INK4a (-/-) background	
		Increased mammary stem cell population	
		Increased DMBA-induced epidermal tumors	Skin Cancer
		Decreased incidence of prostate tumors and metastasis in a prostate cancer model background (TRAMP)	Prostate Cancer
		Reduced pulmonary alveolar space, increased wall thickening, hypercellularity, and fibrosis	Pulmonary Disease
		Concentric left ventricular hypertrophy Right ventricular dilation	Cardiovascular Diseases
		Increased neointima formation	
		Decreased atheromatous lesions in Western type diet fed ApoE (-/-)/Cav-1(-/-)	
		Resistance to diet-induced obesity	Insulin Resistance & Diabetes
		Adipose tissue atrophy	
		Impaired insulin signaling	
		Increased ischemic injury	Cerebral Ischemia

Table 2

Phenotypes of Cav-2 and Cav-3 knockout mice and their implications in human pathologies

Genes	Expression	Knockout mouse phenotypes	Relevance to human pathologies
<u>Cav-2</u>	Same as Cav-1	Reduced pulmonary alveolar space and increased wall thickening with hypercellularity and fibrosis	Pulmonary Disease
	Epithelial cells Fibroblasts Endothelial cells Adipocytes <u>Type I pneumocytes</u>	<u>Tubular aggregate formation, mitochondrial proliferation, increased numbers of satellite cells</u>	<u>Tubular Aggregates</u>
		Skeletal muscle myopathic changes, mononuclear cell infiltration, variable fibers size and presence of necrosis	Limb-Girdle Muscular Dystrophy
<u>Cav-3</u>	Cardiac and skeletal muscle	Cardiac hypertrophy, dilatation, reduced fractional shortening	Cardiomyopathy
		Increased adiposity, Insulin resistance	Diabetes