Caveolin-1 in oncogenic transformation, cancer, and metastasis

Terence M. Williams and Michael P. Lisanti

Departments of Molecular Pharmacology and Medicine, and The Albert Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, New York

Caveolae are 50- to 100-nm omega-shaped invaginations of the plasma membrane, and are distinct from clathrin-coated pits (77). Since the initial discovery of caveolae ("little caves") in 1953, there has been great interest in elucidating 1) how these vesicular structures are constructed and 2) what cellular functions they perform. Unfortunately, the biochemical components of caveolae remained elusive for the next 40 years.

In 1992, the first caveolar marker protein was identified and cloned, and was termed caveolin/VIP21 (now known as caveolin-1 or Cav-1) (92), thus paving the way for the molecular analysis of caveolar function. Caveolin was first isolated with the use of a screen to identify proteins that are tyrosine phosphorylated upon v-Src-mediated cell transformation (30–32). In contrast, VIP21 was cloned simultaneously as an integral membrane protein of trans-Golgi-derived transport vesicles (50).

In 2001, the development of Cav-1(−/−) null mice demonstrated an absolute requirement for Cav-1 in the formation of caveolae because virtually all tissues in Cav-1 null mice completely lack these organelles (16, 89). Currently, the functional roles attributed to caveolae and Cav-1 are quite diverse, ranging from vesicular transport (transcytosis, endocytosis, and endocytosis) and cholesterol homeostasis, to the suppression of cell transformation and the regulation of signal transduction (for a review of caveolae and vesicular trafficking/cholesterol, see Ref. 90).

The establishment of novel biochemical techniques to purify caveolae (by virtue of their unique biophysical properties) enabled the large-scale preparation of these organelles and the determination of their associated protein components (62, 97). With the use of Cav-1 as a marker protein, caveolae have now been demonstrated to concentrate a wide variety of signaling molecules, including glycosyl phosphatidylinositol-linked proteins, Src-family tyrosine kinases, H-Ras, heterotrimeric G protein subunits, PKC isoforms, and endothelial nitric oxide synthase (eNOS) (for a more complete list, see Ref. 90). These findings led to the proposal of the “Caveolae Signaling Hypothesis”: that caveolae function to compartmentalize signaling molecules and to regulate signal transduction (61).

Interestingly, many signaling molecules have now been shown to directly interact with Cav-1. These interactions occur through a defined modular protein domain, known as the caveolin-scaffolding domain (CSD; residues 82–101).

Caveolin-1 phosphorylation (at Tyr14 and Ser80) and mutations (P132L) may override or inactivate the growth inhibitory activity of the caveolin-scaffolding domain (residues 82–101).

Caveolin-1 has been implicated in the pathogenesis of oncogenic cell transformation, tumorigenesis, and metastasis. Here, we review the available experimental evidence (gleaned from cultured cells, animal models, and human tumor samples) that caveolin-1 (Cav-1) functions as a "tumor and/or metastasis modifier gene." Genetic evidence from the study of Cav-1(−/−) null mice and human breast cancer mutations [CAV-1 (P132L)] supports the idea that caveolin-1 normally functions as a negative regulator of cell transformation and mammary tumorigenesis. In contrast, caveolin-1 may function as a tumor promoter in prostate cancers. We discuss possible molecular mechanisms to explain these intriguing, seemingly opposing, findings. More specifically, caveolin-1 phosphorylation (at Tyr14 and Ser80) and mutations (P132L) may override or inactivate the growth inhibitory activity of the caveolin-scaffolding domain (residues 82–101).

### Caveolin-1, Cell-Cycle Progression, and ERK-1/2 Activation

Caveolin-1 is highly expressed in terminally differentiated or quiescent cells, including adipocytes, endothelia, smooth muscle cells, and Type I pneumocytes, suggesting a possible role for Cav-1 as a negative regulator of cell proliferation. In support of this prediction, several groups have now demon-
strated a role for Cav-1 in modulating cell cycle progression, both in mammalian cells as well as in Caenorhabditis elegans (27, 56, 99).

For example, Galbiati and colleagues (27) have shown that Cav-1 expression levels are negatively regulated by growth factor stimuli and that recombinant overexpression of Cav-1 can inhibit cellular proliferation, by mediating cell cycle arrest in G0/G1. In addition, Cav-1(-/-) null mouse embryonic fibroblasts (MEFs) show increased proliferation rates, concomitant with increased S-phase fractions and decreased G0/G1 fractions, as well as altered expression of several cell cycle-related proteins (89, 121). More specifically, the loss of Cav-1 results in decreased expression of p21Cip1, cyclin D1, and PCNA overexpression, as well as hyperactivation of the Ras-p42/44 MAP kinase cascade.

Elucidation of the mechanisms by which Cav-1 exerts its negative regulatory control of cell proliferation has uncovered some novel molecular pathways. For example, Cav-1 normally functions as a transcriptional repressor of Cyclin D1, an important regulatory component of the cyclin-cdk complex that phosphorylates Rb, thereby controlling entry into S-phase (40). Furthermore, the induction of G0/G1 arrest by Cav-1 overexpression appears to operate through a p53/p21Cip1-dependent pathway (27).

Cav-1 also controls signaling along the Ras-p42/44 MAP kinase cascade. Many components of Ras-p42/44 MAP kinase signaling appear to be compartmentalized within caveolae, including growth factor receptors (such as Neu/Erb-B2 and EGFR), Ras, Raf-1, MEK-1/2, and ERK-1/2 (64, 67, 68, 72, 93, 105, 106). In addition, Cav-1 directly inhibits ERK-1/2 activation, both in vitro and in vivo (17, 131). In NIH-3T3 cells, antisense mediated downregulation of Cav-1 is sufficient to cause the constitutive hyperactivation of ERK-1/2 signaling (26). Furthermore, hyperactivation of ERK-1/2 also occurs in Cav-1(-/-) null mouse cells and tissues under various conditions (9, 12, 121). Interestingly, there appears to be a reciprocal relationship between Cav-1 and ERK-1/2 because activation of the Ras-p42/44 MAP kinase cascade causes the downregulation of Cav-1 expression in NIH-3T3 cells and in the mammary gland (21). Thus it appears that Cav-1 functions as a natural endogenous inhibitor of the p42/44 MAP kinase cascade.

ROLE OF CAV-1 IN PROGRAMMED CELL DEATH: PRO- OR ANTI-APOPTOTIC?

Cells, including tumor cells, constantly face the decision of whether to survive and proliferate or to undergo programmed cell death (apoptosis). Therefore, identifying the pathways that are pro-apoptotic or anti-apoptotic has important implications for controlling tumor cell growth.

Currently, the role of Cav-1 in apoptosis remains controversial. On one hand, caveolae are highly enriched in both sphingomyelin (a precursor of ceramide) and sphingomyelinase (the enzyme responsible for generating ceramide) (65). Ceramide induces cell death by inhibiting the well-characterized phosphatidylinositol 3-kinase (PI3-kinase)/Akt survival pathway (133). Interestingly, Cav-1 has been shown to interact with PI3-kinase and Cav-1 overexpression sensitizes fibroblasts to ceramide-induced death, through a PI3-kinase-dependent mechanism (134). Furthermore, we have demonstrated that Cav-1 expression sensitizes both NIH-3T3 fibroblasts and T24 bladder carcinoma cells to cell death initiated by staurosporine, a chemical inducer of apoptosis (63).

In contrast, disruption of caveolae using cholesterol-sequestrating agents has been shown to block IL-6 and IGF-1-induced activation of the PI3-kinase/Akt signaling pathway (83). Therefore, caveolae and Cav-1 are required to mediate proper survival signals through the PI3-K/Akt pathway. Moreover, caveolin-1 overexpression in Rat1A cells and human prostate cancer cells (LNCaP), or Cav-1 upregulation in androgen-insensitive LNCaP clones renders these cells more resistant to apoptosis (113, 114). In addition, antisense mediated down-regulation of Cav-1 results in prostate cancer cells that are more sensitive to apoptosis (57, 58, 74). Finally, Li and colleagues (57) have demonstrated that caveolin-1 overexpression mediates cell survival by sustaining Akt activation, through the binding and inhibition of the serine/threonine protein phosphatases, namely PP1 and PP2A. Taken together, these results argue that Cav-1 also has anti-apoptotic activities.

The apparent incongruity of the pro-apoptotic or anti-apoptotic functions of Cav-1 may be explained by cell-type specific effects: anti-apoptotic activity in prostate cancer cells and proapoptotic activity in other cell types. Alternatively, the disparate effects of Cav-1 may be due to the use of different apoptotic inducers. Additional studies will be necessary to distinguish between these two possibilities.

LOSS OF CAV-1 DURING CELL TRANSFORMATION

An inverse relationship between Cav-1 expression and transformation has been clearly established. During the initial characterization of Cav-1, it was shown that Cav-1 levels are reduced in transformed NIH-3T3 cells and that the level of residual Cav-1 inversely correlated with soft agar growth (48). Subsequently, multiple groups (19, 23, 56, 79, 88) demonstrated that forced reexpression of Cav-1 could abrogate anchorage-independent growth in transformed cells with the use of soft agar assays. Interestingly, reduction of Cav-1 expression using an antisense approach was sufficient to induce a transformed phenotype in NIH-3T3 cells, allowing these cells to grow in soft agar and to form tumors in athymic (nude) mice (Fig. 1) (26).

The utilization of Cav-1(-/-) null mice has provided definitive genetic evidence to support the idea that Cav-1 normally functions as a “transformation suppressor” gene. Capozza et al. (9) showed that the skin of Cav-1(-/-) mice is more susceptible to chemical carcinogenic treatment, resulting in the formation of epidermally derived tumors (Fig. 2). Interestingly, epidermal hyperplasia in these 7,12-dimethylbenzanthracene (DMBA)-treated Cav-1(-/-) mice was associated with cyclin D1 upregulation and ERK-1/2 hyperactivation. In addition, genetic ablation of Cav-1 in MEFs renders these cells more susceptible to transformation and in vivo tumorigenesis mediated by transforming oncogenes (Fig. 3) (121). Cyclin D1 overexpression and ERK-1/2 hyperactivation were likewise detected in these Cav-1(-/-) MEFs.

Through the use of mice lacking one or both Cav-1 alleles, these studies mimic the effects of naturally occurring inactivating Cav-1 mutations (such as P132L), adding to the physiological relevance of these mouse models. Indeed, analysis of
Cav-1(−/−) mice has revealed that Cav-1 is critically important for the normal development and physiology of the mammary gland. First, young virgin female Cav-1(−/−) mice demonstrate hyperplasia of the mammary ductal epithelium (Fig. 4A) (52). In addition, complete loss of Cav-1 results in precocious lobuloalveolar development and lactation during pregnancy, with concomitant hyperactivation of the Jak-2/Stat5a and ERK-1/2 signaling pathways (Fig. 4B) (78). In terms of mammary epithelial transformation, we have reported that complete loss of Cav-1 accelerates the appearance of mammary dysplastic lesions in polyoma middle T tumor-prone transgenic mice (MMTV-PyMT) (Fig. 4C) (120). Furthermore, another group has recently demonstrated that CAV-1 haploinsufficiency (achieved through a retrovirus-mediated gene trapping approach) is sufficient to induce the partial transformation of human breast epithelial cells (132).

In terms of breast carcinogenesis, we and another group (104, 122) have recently demonstrated that Cav-1 acts as an inhibitor of mammary tumorigenesis and metastasis in vivo. Molecularly, we observed cyclin D1 upregulation, ERK-1/2 hyperactivation, and Rb hyperphosphorylation in tumors derived from PyMT/Cav-1(−/−) mice. Taken together, these results indicate that Cav-1 normally functions to negatively regulate the growth and transformation of mammary epithelial cells, as well as to suppress the development of advanced mammary tumors and metastases.

CAVEOLIN-1, TUMOR SUPPRESSORS, AND ONCOGENES

Research on the relative relationship of Cav-1 to other cellular oncogenes and tumor suppressors has great importance for understanding the intricate workings of the cell. The multistep nature of cellular transformation involves the acquisition of genetic alterations that affect the transcription, translation, or the post-translational regulation of proteins, the functional end products of DNA. Mutations can be transmitted through...
the germline or can be acquired somatically during the lifetime of a cell.

Two important properties of neoplastic cells are immortalization and transformation. In some sense, all cells within a microenvironment undergo the process of natural selection, and those cells that are able to more quickly attain immortalization and transformation are more likely to survive and propagate. Therefore, it is important to determine the relative contribution of different genes in promoting immortalization and/or transformation.

While Cav-1 does not appear to have a direct role in immortalization, Cav-1 does synergize with other immortalizing genes. Loss of the INK4a locus, encoding both p16INK4a and p19ARF cell cycle regulators, is sufficient to allow cells to become immortalized. We have demonstrated that concomitant loss of Cav-1 and INK4a results in cells with a striking proliferative advantage, demonstrating that the loss of Cav-1 expression cooperates or synergizes with genetic mutations that abolish INK4a function (121). Furthermore, transformation of INK4a (−/−)/Cav-1(−/−) fibroblasts with various oncogenes [H-Ras (G12V) or v-Src] renders these cells more neoplastic, generating up to 40-fold larger tumors in nude mice (Fig. 3). These results suggest that mutation or downregulation of caveolin-1 expression, in combination with an INK4a mutation, would impart cells with a profound neoplastic advantage over those cells with a mutation in either gene alone.

Another well-characterized tumor suppressor, p53, appears to be directly involved in regulating Cav-1 expression. Razani et al. (88) have demonstrated that p53 is a positive transcriptional and translational regulator of Cav-1, and that inactivation of p53 through viral oncoproteins results in reduced Cav-1 expression. Cav-1 levels are also dramatically reduced in p53-deficient fibroblasts (56). In support of these cellular findings, we have demonstrated that p53 (−/−) null mice show dramatically reduced levels of Cav-1 in vivo (121). However, a molecular dissection of the transcriptional or translational regulatory aspects of this relationship awaits further studies.

Preliminary evidence also suggests that Cav-1 may have a role in regulating PTEN activity as a substantial amount of PTEN (a phosphatase possessing tumor suppressor functions)
localizes to caveolae and specifically forms molecular complexes with Cav-1 (11). Aldred et al. (1) have suggested that this relationship has implications for follicular thyroid neoplasms because Cav-1 and PTEN levels are simultaneously downregulated, suggesting perhaps that the downregulation of Cav-1 may contribute to dysregulation of PTEN function.

A whole host of cellular oncogenes have been shown to reduce Cav-1 expression in cells (Table 1). These include, but are not limited to, c-Myc, HPV E6, v-Abl, Bcr-Abl, H-RasG12V, v-Src, and Neu/ErbB2 (18, 48, 79, 113). Virtually all of these oncoproteins appear to downregulate Cav-1 expression through transcriptional mechanisms. In addition, Ras- and Raf-mediated downregulation of Cav-1 relies upon ERK-1/2 activation, as ERK-1/2 inhibition restores Cav-1 expression in Ras- and Raf-transformed cells, as well as in human fibrosarcoma cells (21, 117). Activation of other signaling molecules has also been demonstrated to downregulate Cav-1 expression, including PKA and PKC-α (21, 123).

CAV-1 IN HUMAN CANCERS: CHROMOSOMAL LOCALIZATION, GENE MUTATIONS, AND COMPLEX EXPRESSION PATTERNS

The D7S522 locus on human chromosome 7q31.1 is commonly deleted in a variety of human cancers, including carcinomas of the breast, colon, kidney, prostate, ovary, head, and neck. These findings have led to the hypothesis that this region encodes a novel tumor suppressor gene (47, 70, 102, 127–130). Recently, we mapped the human Cav-1 gene to the D7S522 region of 7q31.1 (20), suggesting that Cav-1 may indeed represent the tumor suppressor in this fragile genomic region.
Importantly, sequence analysis of Cav-1 in human tumors has also revealed sporadic mutations. In a cohort of patients with primary breast cancer, Hayashi and colleagues (36) detected a sporadic P132L mutation in up to 16% of the cases examined. Furthermore, this mutation induces cellular transformation, acts in a dominant negative fashion by causing the mislocalization and intracellular retention of wild-type Cav-1, and causes ERK-1/2 hyperactivation (Fig. 4D) (36, 52). Interestingly, this mutation is analogous to the CAV-3 (P104L) mutation that is associated with several skeletal muscle disorders, including autosomal dominant limb-girdle muscular dystrophy (LGMD-1C) (73). Independently, another group has recently identified novel Cav-1 mutations in human oral squamous cell cancers (34).

Cav-1 expression has now been assessed in a wide range of human tumors (Table 2). On the basis of the transformation suppressor activity of Cav-1 in cultured cells, we would predict that human tumors would show reductions in Cav-1 expression. However, Cav-1 expression levels are reduced, unchanged, or upregulated, depending on the tumor cell type. Interestingly, within tumor types derived from the same cell type or tissue, Cav-1 expression levels are consistently upregulated in the majority of cases. For instance, Cav-1 downregulation is typical of ovarian, lung, and mammary carcinomas, as well as mesenchymal sarcomas. On the other hand, Cav-1 is consistently upregulated in bladder, esophageal, thyroid (papillary subtype), and prostate carcinomas, with some exceptions (82). Further research will assist in...
Caveolin-1: Is it a Tumor Suppressor, an Oncogene, or Both?

Here we review three distinct mechanisms that can serve to functionally inactivate the tumor suppressor function of Cav-1: (1) tyrosine phosphorylation; (2) serine phosphorylation; and (3) a dominant-negative point mutation, P132L. These findings may explain why Cav-1 has been suggested to function both as a tumor suppressor or as an oncogene, depending on the tumor type and/or tumor stage.

Tyrosine Phosphorylation

The dually contrasting roles for Cav-1 in tumor progression may be partly explained by the observation that Cav-1 has several peptide domains with opposing functions. A molecular dissection of the Cav-1 protein has revealed distinct regions that may counteract the effects of the growth-inhibitory CSD (Fig. 5). First, Tyr14 at the extreme NH2 terminus is important for the binding and recruitment of a c-Src/Grb7 signaling complex (55). This residue is constitutively phosphorylated in v-Src- and v-Abl-transformed cells, transiently phosphorylated during growth factor stimulation in other cells, and localizes to focal adhesions, which are the predominant sites of tyrosine kinase signaling (Fig. 6A). Functionally, tyrosine 14-phosphorylated Cav-1 expression in human tumors or tumor cell lines

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Subtype</th>
<th>Expression Levels Compared with Normal Tissue</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Carcinoma</td>
<td>↑↑↑</td>
<td>24, 87, 96</td>
</tr>
<tr>
<td>Brain</td>
<td>Astrocytoma</td>
<td>NC</td>
<td>25</td>
</tr>
<tr>
<td>Breast</td>
<td>Adenocarcinoma</td>
<td>↓↓↓</td>
<td>18, 23, 56, 94, 95, 104, 122, 123, 132</td>
</tr>
<tr>
<td>Cervix</td>
<td>Carcinoma</td>
<td>↓</td>
<td>88</td>
</tr>
<tr>
<td>Colon</td>
<td>Carcinoma</td>
<td>♦</td>
<td>5, 23, 81</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Squamous cell</td>
<td>↑</td>
<td>39, 46</td>
</tr>
<tr>
<td>Kidney</td>
<td>Carcinoma</td>
<td>♦</td>
<td>7, 8, 10, 38, 43</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>T cell leukemia</td>
<td>↑</td>
<td>35</td>
</tr>
<tr>
<td>Lung</td>
<td>Small cell carcinoma</td>
<td>VE</td>
<td>83</td>
</tr>
<tr>
<td>Mesenchyme</td>
<td>Sarcoma (various)</td>
<td>↓↓</td>
<td>117</td>
</tr>
<tr>
<td>Oral</td>
<td>Carcinoma</td>
<td>VE</td>
<td>34, 41</td>
</tr>
<tr>
<td>Ovary</td>
<td>Carcinoma</td>
<td>↓↓↓</td>
<td>3, 15, 116</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Carcinoma</td>
<td>VE</td>
<td>109, 112</td>
</tr>
<tr>
<td>Prostate</td>
<td>Carcinoma</td>
<td>↑↑↑</td>
<td>58, 76, 98, 110, 114, 124, 125</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Papillary carcinoma</td>
<td>↑</td>
<td>42</td>
</tr>
<tr>
<td>Vascular</td>
<td>Angiosarcoma</td>
<td>♦</td>
<td>49</td>
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NC, no correlation; VE, variable expression or conflicting reports; ↑, overexpression of Cav-1; ↓, downregulation of Cav-1. The number of arrows corresponds to the extent of research performed on the various tissues; the more arrows, the more the evidence suggests a particular trend.
changes its topology, thereby converting Cav-1 from an integral membrane protein to a secreted protein product. Normally, the majority of Cav-1 is associated with the plasma membrane. However, Cav-1 also appears to be secreted, especially in exocrine cell types, where it is packaged into secretory vesicles (66, 101). Phosphorylation of Cav-1 at Ser80 directly regulates its conversion to a secreted protein. For example, mutation of Ser80 to glutamate (S80E, which mimics chronic phosphorylation) results in preferential targeting of Cav-1 to the ER membrane and directs its subsequent packaging for secretion (Fig. 7) (101). Furthermore, phosphorylation at Ser80 is required for proper secretion because its mutation to alanine (S80A; which abrogates phosphorylation) results in no detectable secretion of Cav-1 (101), concomitant with its intracellular accumulation (Fig. 7A, bottom). Mechanistically, in terms of cellular transformation, shunting Cav-1 for secretion to the extracellular environment would subvert its normal intracellular tumor suppressive functions. Essentially, these changes in Cav-1 membrane topology have the same consequences as a loss of Cav-1 expression.

In addition, the secretion of Cav-1 results in a protein product that possesses autocrine or paracrine tumor-promoting functions. Thompson and colleagues (110) have demonstrated that Cav-1 is secreted in androgen-insensitive human prostate cancer cells and that secreted Cav-1 acts in an autocrine/paracrine fashion, directly stimulating prostate tumor cell growth and survival.

**Dominant-Negative Point Mutations**

Finally, the previously mentioned identification of Cav-1 (P132L) mutations in up to 16% of human breast cancers provides a third distinct mechanism to inactivate the tumor suppressor function of caveolin-1. This mutation drives cellular transformation in NIH 3T3 cells. Briefly, NIH 3T3 cells expressing Cav-1 (P132L) show augmented growth in soft agar, as well as increased invasiveness, and increased chemotaxis (36).

Thus these three defined mechanisms provide a clear explanation for how the growth-inhibitory functions of the CSD may be subverted.

**OTHER CAVEOLIN GENES: ROLE OF CAVEOLIN-2 AND CAVEOLIN-3**

Caveolin-2 (Cav-2) and caveolin-3 (Cav-3/M-caveolin) are the two remaining members of the caveolin gene family. Whereas caveolin-2 is co-localized, co-expressed, and requires caveolin-1 for proper membrane targeting (80, 100), caveolin-3 is expressed mainly in skeletal muscle fibers and cardiac myocytes (107, 111, 115).

Despite the fact that the CAV-2 gene is co-localized with Cav-1 at the 7q31.1/D7S522 locus (20), the bulk of caveola-related cancer research has focused on Cav-1. This is primarily due to the early observation that Cav-2 levels are not down-regulated in response to oncogenic transformation, in contrast to Cav-1 (18, 100).

Recent research, however, is beginning to implicate the other caveolin family members in human cancers. Cav-2 expression has been detected in various lung cancers using a cDNA microarray approach, and its expression correlates with...
shorter survival in stage I adenocarcinomas (119). In addition, Cav-2 upregulation is also observed in esophageal and bladder carcinomas (24, 39). In another study on germ cell tumors of the testis, Cav-3 overexpression was detected in seminomas (45). Future research will undoubtedly uncover altered Cav-2 and Cav-3 expression patterns within other human tumors, therefore necessitating studies to address their functional importance in tumor growth and metastasis.

Fig. 6. Tyrosine phosphorylation of Cav-1 enhances anchorage-independent growth. A: immunolocalization. Cos-7 cells were transiently co-transfected with Cav-1 and a constitutively activated form of the human c-Src-tyrosine kinase (Y529F). Cells were then double labeled with anti-phospho-Cav-1 (pY14) IgG (mAb) and anti-Cav-1 IgG (pAb; N-20). Note that tyrosine phosphorylated Cav-1 is preferentially localized at focal adhesions, while the distribution of total Cav-1 is clearly distinct. B: foci formation. Human embryonic kidney (HEK)-293 cells were transiently transfected with Cav-1 WT alone, or in combination with c-Src and Grb7. Similar experiments were carried out with a nonphosphorylatable Cav-1 mutant in parallel (Cav-1 (Y14A)). Note that co-expression of either Cav-1 WT plus c-Src or Cav-1 WT plus c-Src and Grb7 significantly increases foci formation. However, this effect is blocked by the use of Cav-1 (Y14A). See boxed area. Figure data adapted from Lee et al. (53).

Fig. 7. Serine phosphorylation of Cav-1 converts it to a secreted protein. A: Cav-1 secretion (WT, S80A, and S80E). Note that Cav-1 (S80A) is not secreted by AR42J pancreatic adenocarcinoma cells even in the presence of dexamethasone, an agent that induces the secretory phenotype. Conversely, Cav-1 (S80E) is secreted to a greater extent than wild-type Cav-1 after dexamethasone treatment. Thus Cav-1 phosphorylation on invariant Ser80 is required for ER retention and entry into the regulated secretory pathway. Top, cell lysates; Bottom, cell media. B: serine phosphorylation and the membrane topology of Cav-1. Schematic diagram summarizing the idea that phosphorylation at Ser80 converts Cav-1 from a cytoplasmically oriented integral membrane protein to a luminal secreted protein at the level of the ER membrane. Figure adapted from Schlegel et al. (101).
FUTURE DIRECTIONS: POTENTIAL CAVEOLIN-BASED ANTI-CANCER THERAPIES

Additional avenues of caveolin-related cancer research are still uncovering novel roles for the caveolins. Recently, Hunter and colleagues (69) showed that Cav-1 is important for the endocytosis of E-cadherin and that downregulation of Cav-1 decreases E-cadherin expression, increases β-catenin transcriptional activation, and results in increased invasiveness in neoplastic cells. Because invasiveness and the loss of E-cadherin expression are common features of metastatic cells, these results have important implications for metastasis.

Likewise, a novel association among caveolae, caveolin-1, and matrix metalloproteinases (MMPs), a family of matrix-degrading enzymes that facilitate invasion/metastasis, is being established. Several groups have shown that MT1-MMP and MMP-2 are localized to caveolae and that caveolin are required for proper MT1-MMP localization and function (2, 28, 85). Furthermore, we (122) have recently shown that recombinant expression of Cav-1 in mammary epithelial cells suppresses their metastatic capacity, inhibits their invasiveness, and prevents their ability to secrete MMPs (MMP-2/9). These results were obtained using a cell-permeable peptide containing the CSD (Cav-1; residues 82–101). Thus it may be fruitful to develop novel anticancer therapies that mimic the activity of the CSD.

Because of the heterogeneity of Cav-1 expression in different tumors, therapies targeting Cav-1 will need to consider the specific roles or functions of this protein within that particular type of tumor cell. In one interesting gene therapy strategy, the Cav-1 promoter was used to specifically target prostate cancer cells in vitro and in vivo with minimal toxicity (84).

Another type of anti-tumor therapy is based on the observation that Cav-1 is highly expressed in endothelium. Therefore, targeting a tumor’s blood supply provides an interesting opportunity for Cav-1-directed therapies. In exciting confirmation of this hypothesis, Gratton et al. (33) have demonstrated that a cell-permeable peptide derived from the CSD markedly reduced tumor progression in mice by regulating microvascular permeability.

In summary, current research has clearly established a role for Cav-1 as a “tumor and metastasis-modifying gene.” Future studies will undoubtedly reveal novel relationships between Cav-1 and a variety of signaling pathways, and offer exciting opportunities to develop anti-cancer therapies that target Cav-1 and caveolae, both in primary tumors and in metastatic disease.

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