CHAPTER 7

CAVEOLIN-1 AND PROSTATE CANCER PROGRESSION

Michael R. Freeman,*[,1-3] Wei Yang[1,2] and Dolores Di Vizio[1,2]

[1]*Urological Diseases Research Center, Children's Hospital Boston, Boston, Massachusetts, USA;*
[2]*Departments of Surgery and* [3]*Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA.*
**Corresponding Author: Michael R. Freeman—michael.freeman@childrens.harvard.edu*

Abstract: Caveolin-1 was identified in the 1990s as a marker of aggressive prostate cancer. The caveolin-1 protein localizes to vesicular structures called caveolae and has been shown to bind and regulate many signaling proteins involved in oncogenesis. Caveolin-1 also has lipid binding properties and mediates aspects of cholesterol and fatty acid metabolism and can elicit biological responses in a paracrine manner when secreted. Caveolin-1 is also present in the serum of prostate cancer patients and circulating levels correlate with extent of disease. Current evidence indicates that increased expression of caveolin-1 in prostate adenocarcinoma cells and commensurate downregulation of the protein in prostate stroma, mediate progression to the castration-resistant phase of prostate cancer through diverse pathways. This chapter summarizes the current state of our understanding of the cellular and physiologic mechanisms in which caveolin-1 participates in the evolution of prostate cancer cell phenotypes.

INTRODUCTION

Prostate cancer (PCa) is an androgen-sensitive malignancy that affects middle-aged or older men. PCa is the most common noncutaneous male cancer and a leading cause of cancer death in Western countries. At this writing, PCa claims about 28,000 lives per year in the US. Organ-confined prostate adenocarcinoma is essentially curable in the majority of cases by surgery or radiotherapy. However, there is no effective treatment for nonlocalized disease, which in the developed world generally emerges unpredictably

Caveolins and Caveolae: Roles in Signaling and Disease Mechanisms,
edited by Jean-François Jasmin, Philippe G. Frank and Michael P. Lisanti.

after a course of androgen suppression within several years after primary therapy begins. Once primary treatment has failed, there is no effective therapeutic strategy. Because hormone ablation is standard-of-care for nonlocalized disease, recurrence following therapy is characterized as the hormone-insensitive or "castrate-resistant" phase. Clinical progression leads to death within 5 years in most cases, even with aggressive therapeutic intervention. Limited advances in alternative chemotherapeutic modalities have been made in the past decade, however prolongation of survival in the context of the few reported successes against castration resistance has been extremely modest.

In the developed world, PCa is also greatly over-treated. About 70% of patients who receive therapy harbor cancer that would not be clinically threatening during their lifetimes. However, with current technology it is not possible to distinguish indolent cancers from those likely to progress. Many advances in this field are needed to identify new therapeutic strategies and targets that will improve overall survival, as well as quality of life for patients diagnosed with PCa. New biomarkers are also necessary to inform treatment decisions, especially as they relate to novel therapeutic approaches.

Caveolin-1 (Cav-1) is a 21-24 kDa multi-functional signaling protein and lipid transporter that has the distinction of being both a circulating PCa biomarker and a mediator of PCa progression. Cav-1 is the major structural protein within caveolae, small membranous organelles that reside in the cytoplasm or appear as invaginations of the plasma membrane. Cav-1 acts as a scaffold within these structures to organize numerous molecular complexes, thereby regulating a variety of cellular events. Alterations in expression of Cav-1 have been described in a number of malignancies. Increasing evidence points to a dichotomous role played by Cav-1 in cancer, with two prominent cases represented by breast and prostate cancer, in which Cav-1 seems to reduce and promote tumor growth, respectively. Therefore, the Cav-1 protein can be thought of as both a tumor suppressor and a tumor promoter, albeit in different contexts. In PCa, Cav-1 expression correlates positively with aggressive and metastatic potential and serum Cav-1 levels are elevated in patients with PCa but not those with benign prostatic hyperplasia. Several studies have also shown that Cav-1 is capable of actively promoting the metastatic and castrate-resistant phenotypes, suggesting it is not an innocent bystander during disease progression. Because of the diverse mechanistic roles played by Cav-1, its localization within plasma membrane microdomains where many oncogenic proteins also reside and because it circulates in the bloodstream at increased levels in advanced disease, the protein is an attractive focus for therapeutic intervention and biomarker development. The goal of this chapter will be to provide an overview of the current state of knowledge of cell signaling and metabolism in PCa as these processes relate to our understanding of the diverse functional roles of Cav-1.

THE ROLE OF ANDROGEN IN PROSTATE CANCER

Huggins and Hodges reported in 1941 that PCa is dependent on testicular androgens.[1] This critical insight has formed the basis for most of the progress in understanding mechanisms of PCa progression, as well as the development of therapeutic strategies up to the present day.[2-4] Androgens are sterolic hormones that work by binding with high affinity to the androgen receptor (AR), a member of the class I subgroup of the nuclear receptor superfamily of transcription factors.[5] The primary androgenic ligands for the AR are testosterone and its metabolite, 5α-dihydrotestosterone (DHT). DHT is the principal

bioactive androgen in the prostate. Following ligand binding, generally in the cytoplasm, the AR translocates to the nucleus where it binds as a homodimer to chromosomal regions containing short palindromic androgen response elements (AREs) within androgen responsive genes. Nuclear and cytoplasmic AR interacts with many positive and negative regulatory proteins (collectively termed "coregulators") to recruit RNA polymerase II and its associated cofactors to regulate gene expression.[6-9] Like other steroid hormone receptors, such as estrogen, progesterone and mineralocorticoid receptors, AR is highly networked to diverse biochemical mechanisms and is therefore functionally robust. In prostatic cells in vivo, the AR is a key mediator of cell cycle transit, cell differentiation, cell survival and apoptotic mechanisms, secretory processes and metabolism.[5]

Despite decades of study by many laboratories, the precise role of the AR in PCa is still not well understood. Androgen suppression inhibits PCa progression temporarily in humans and can cause regression of experimental prostatic adenocarcinoma in animal models. Studies on somatic AR mutations in human PCa, combined with in vitro and in vivo modeling approaches indicate that, in most instances, the AR appears to mediate pro-survival and pro-growth pathways in prostate tumor cells.[5] This conclusion is consistent with retention of AR expression by cancer cells even in hormonally suppressed conditions and frequent genomic amplification of the AR,[10-16] indicating a likely condition of selective pressure at the AR coding locus for higher expression levels during progression to hormone-refractory disease. However, clinical experience with androgen suppression and androgen replacement therapy also suggests that AR may inhibit prostate tumor progression in a minority of cases, or in certain phases of the disease. Data from animal models, cell line studies and analyses of human tissues are consistent with the concept that the AR takes on different and sometimes opposing roles, depending on the nature of the genetic and epigenetic alterations underlying tumor formation and progression.[17-19] For example, AR expressed by the prostatic stroma appears to play different physiologic roles and to be functionally distinct from AR expressed in epithelial cells.[20] The differential signaling capacities of the AR and the mechanisms underlying them, are presently an active area of research.

AR is expressed in the majority of castrate-resistant prostate cancers and a wide variety of functional somatic mutations in the AR have been identified in human prostate tumors, suggesting that the AR continues to play a significant role under conditions of hormonal suppression, which is still the gold standard for treatment of nonlocalized disease. The manner in which the normally ligand-dependent AR continues to function under conditions of limiting androgen is poorly understood. Several possible strategies for maintenance of AR activity in the hormone-repressed state have been verified experimentally, including AR gene amplification;[10-14] somatic mutations that result in hypersensitivity to androgen or alterations in sensitivity to other steroid hormones, such as progesterone;[21,22] hormone-independent mechanisms of activation through growth factor receptor-mediated mechanisms;[23-28] and intratumoral synthesis of androgen in the castrate condition.[29-31] Some or all of these mechanisms likely operate in patients, possibly even in different phases of disease in individual patients.

CAVEOLIN-1 AND THE ANDROGEN RECEPTOR

In the absence of androgen, the AR is present largely in the cytoplasm, where it is sequestered from the transcriptional machinery. In normal males, the AR is largely present in the nucleus in hormone-sensitive tissues. The AR can form complexes with a wide variety of proteins, including classical signal transduction proteins that reside primarily

in the plasma membrane and in cytoplasmic membranes. These interactions, some of which have been shown to produce unambiguous physiologic effects, include signaling proteins in the epidermal growth factor receptor (EGFR) and insulin/insulin-like growth factor (IGF) pathways.[28,32-34] Lu et al demonstrated that in the presence of androgen, AR and Cav-1 form a complex identifiable in caveolin-enriched, low-density membrane fractions in sucrose gradients.[35] Caveolin-enriched membrane fractions isolated with non-ionic detergents in combination with sucrose gradient centrifugation are believed to represent the residue of cholesterol-rich, "lipid raft" membranes that exist in the in vivo state. Lipid raft membrane microdomains, where the metaphor of a "raft" refers to a less fluid condition than other components of the membrane, have been implicated in many signal transduction processes.[36] Association between Cav-1 and AR in caveolin-enriched fractions was found to be largely androgen-dependent.[35]

Lu et al identified the AR N-terminal and the N-terminal of Cav-1 as subdomains within the two proteins that mediate their physical association. Consistent with these observations, enforced expression of Cav-1 was found to potentiate androgen-dependent signaling, while down-regulation of Cav-1 inhibited androgen signaling. These findings suggest that Cav-1 has the potential to hypersensitize the AR to low androgen, thereby providing a Cav-1-dependent mechanism for the AR to continue to function under conditions of hormonal suppression.

Because the AR-Cav-1 interaction occurs preferentially in lipid raft membranes, these studies also suggest the possibility that Cav-1 intervenes in the androgen-AR axis in a "nongenomic" manner; that is, independently of the AR's involvement in the transcriptional machinery. Several classical steroid hormone receptors, including the AR, have been found to signal nongenomically, i.e., where hormone-dependent signals are processed by the receptors in the absence of their binding DNA.[37,38] Although this is a controversial and very active area of research, a conserved palmitoylation (S-acylation) site has been identified on ERα and ERβ, progesterone receptors A and B and the AR that appears to mediate these receptors' associations with cell membranes and Cav-1, as well as some of the nongenomic signaling effects that have been shown to arise from these associations.[39] The defined AR interaction domain on Cav-1 (residues 1-60) does not overlap with the Cav-1 scaffolding domain (residues 82-101),[35] which has been shown to bind and regulate a wide range of signaling proteins, particularly kinases.[40,41] These distinct domains provide a potential mechanism for Cav-1/AR interaction to facilitate cross-talk with an extensive signaling network without translocation of AR to nuclei. For example, in the LNCaP human PCa cell line, increased androgen promotes an interaction within minutes between AR and Src, a Cav-1 binding protein, which mediates cell proliferation and differentiation signals.[42-44] The manner in which Cav-1 might modify these types of membrane-proximal, hormone-dependent interactions in tumor cells is still not well understood. Cav-1 may act in the role of an AR cochaperone, similarly to other proteins capable of modifying or potentiating androgenic signals, such as Bag-1L, FKBP52 and hsp27.[45-47] Such cochaperone proteins are likely to cooperate to amplify hormonal signals. Hsp27, which forms a complex with AR in the presence of androgen and like Cav-1 can potentiate AR activation, has been shown to facilitate trafficking of sex steroid receptors to the plasma membrane,[48] where Cav-1 in PCa cells resides in increased amounts.[49] Cav-1, by virtue of its ability to associate with many signaling proteins in the sequestered environment of caveolae, may in theory coordinate a large array of these cooperative events.[50] Nongenomic hormonal signals evoked by steroid receptors have also been shown to cooperate and amplify genomic signals mediated by transcriptional activation of the same receptors.[35,37,51] Therefore, Cav-1 localized to

membrane-bound caveolae may still exert profound, yet indirect effects on the classical AR gene activation machinery.

Chromosomal loss at the *PTEN* (phosphatase and tensin homolog) tumor suppressor locus is common in PCa.[52] The PTEN protein is a phosphatidtyl-3,4,5-trisphosphate 3-phosphatase that negatively regulates phosphatidylinositol-3,4,5-trisphosphate, a lipid intermediate in the phosphoinositide 3′-kinase (PI3K)/Akt pathway.[53] The well-described physiologic consequence of inactivating *PTEN* mutations is PI3K/Akt pathway signaling, a potent tumor cell growth and anti-apoptotic mechanism that plays an important role in metastatic disease in many cancers. The extent to which PI3K/Akt activation affects signaling through the AR is still poorly understood. AR was shown by Cinar et al to interact with the oncogenic serine-threonine kinase Akt1 within low-density membrane fractions similar or identical to those described in the above studies of Cav-1/AR interaction.[54] These findings suggest the possibility that convergences in the AR and Akt1 signaling mechanisms may under some circumstances involve Cav-1. However, the AR and Akt1 membrane interaction described by Cinar et al was Cav-1-independent, indicating that Cav-1 is not obligatory for cross-talk between these critical signaling pathways. Significantly, however, in PCa cells Cav-1 was identified as a negative regulator of the serine-threonine phosphatases PP1 and PP2A, which normally dephosphorylate Akt and thereby attenuate signaling through the PI3K/Akt pathway.[55] This regulatory role, which also affects other oncogenic kinases, such as PDK1, has the effect of potentiating Akt signaling and thereby increasing resistance of PCa cells to pharmacologic challenge. Thus, inhibition of tumor-suppressing phosphatases is one mechanism whereby overexpression of Cav-1 in PCa cells can promote tumor cell survival. Under conditions where PP1 and PP2A activity was suppressed by Cav-1, AR localization to nuclei was enhanced in a hormone-independent manner, suggesting that Cav-1 may mediate cooperative signaling between Akt and AR signaling mechanisms at the transcriptional level. Cav-1 was also shown to coprecipitate with PTEN,[56] pointing to the possibility that Cav-1-dependent effects on the PI3K/Akt pathway may arise at multiple levels.

EVIDENCE OF CAV-1 INVOLVEMENT IN PROSTATE CANCER

The first suggestion that Cav-1 might be an important protein in PCa came from an unbiased gene expression screen of isogenic mouse primary vs metastatic PCa cell lines.[57] In this seminal report by the Thompson laboratory, the *cav-1* gene was identified as upregulated in the metastatic lines using differential display-PCR. The same study described elevated expression of Cav-1 protein in human PCa in comparison to normal epithelial cells. Increased frequency of Cav-1 positivity was demonstrated in tissue from lymph node metastases in comparison to primary cancer, suggesting that overexpression of Cav-1 is a relevant feature of castrate-resistant disease. This and subsequent studies have shown that normal prostate epithelia are minimally reactive with anticaveolin-1 antibodies, while endothelial cells and smooth muscle cells of the prostate stroma express the protein at substantial levels. In contrast, Cav-1 expression trends quantitatively higher in prostate adenocarcinoma cells than in normal epithelia. A series of studies, including from our group, have confirmed that elevated expression of the protein is a marker of poor prognosis in localized human PCa and correlates positively with Gleason grade (the standard PCa grading system) and other indicators of aggressive disease. One study reported that the frequency of Cav-1-positive primary prostate tumors increased from 38% in the hormonally naive patient group to 73%

in the hormone refractory group.[58] Poor prognostic features that have been reported to correlate with increased Cav-1 expression include: lymph node metastasis, positive surgical margins, extraprostatic extension, seminal vesicle involvement, tumor angiogenesis and biochemical recurrence following surgery.[49,58-62] Additional reports have demonstrated that co-expression of Cav-1 and the oncoprotein c-Myc is a significant prognostic indicator of time to disease progression following surgery[63] and that Cav-1 expression is increased in PCa in African-American in comparison to Caucasian-Americans.[64] African-American men are at higher risk for aggressive PCa.

Our group demonstrated that levels of fatty acid synthase (FASN), the enzyme responsible for most long-chain fatty acid synthesis in tumor cells and Cav-1 are coordinately upregulated in human prostate tumors and physically interact.[61] In this study, levels of FASN and Cav-1 discriminated between localized and metastatic cancers, with the two proteins occupying similar subcellular locations in a tumor subset, suggesting a functional relationship that could play a role in PCa metabolism. FASN is the sole intracellular producer of palmitate in tumor cells and Cav-1 is a palmitoylated protein. Another study from our laboratory that characterized palmitoylated (S-acylated) proteins in prostate tumor cells on a proteome scale identified Cav-1 as a central node of a novel signaling network localized to lipid raft membranes[50] (Fig. 1). These findings imply that Cav-1 may play an important role in lipid-dependent metabolic pathways relevant to oncogenesis.

Inactivation of the endogenous *cav1* gene in the mouse was able to attenuate prostate tumor progression in the TRAMP model of autochthonous PCa, which is driven by the potent T-antigen (Tag) oncogene under the control of a prostate-specific promoter (probasin).[65] Immunohistochemical analysis of the level of Tag expression and localization to nuclei with the temporal appearance of elevated Cav-1 expression in TRAMP/*cav1* ($^{+/+}$) animals indicated that the ectopic expression of Tag is not the basis of Cav-1 upregulation seen in TRAMP animals (consistent with the human data), but rather that Cav-1 overexpression is a feature of activation of endogenous processes as a result of the carcinogenic process, consistent with findings using human tissues. Although this is an artificial system with limited mechanistic relevance to human PCa, it does demonstrate the capability of the intact *cav1* gene to promote tumor progression from the native environment of the prostate in vivo. These studies also revealed that loss of only one *cav1* allele was sufficient to greatly retard prostate tumor progression in the TRAMP model, suggesting that partial loss of Cav-1 expression is sufficient to inhibit PCa progression. More recently, we found that genetic ablation of Cav-1 in TRAMP mice causes a dramatic reduction of FASN levels,[60] suggesting that Cav-1 might play a role in regulating over-expression of this enzyme, which is common in PCa and other malignancies.

The consequences of *cav1* genetic loss using this mouse PCa model are in contrast to studies in the murine mammary gland, most of which suggest that Cav-1 exerts a tumor-suppressor rather than a tumor-promoting role.[66,67] This marked disparity between two hormone-sensitive, secretory organs that are frequently compared and considered similar in many ways, is intriguing and provides striking evidence for a context-dependent physiologic role for the Cav-1 protein.

Experimental evidence supporting the involvement of Cav-1 in castrate-resistant PCa was first presented by the Thompson group using an approach where Cav-1 expression was manipulated in mouse PCa cell lines.[68] Suppression of Cav-1 expression converted androgen-insensitive cells to an androgen-sensitive phenotype and selection for androgen resistance in vivo correlated with increased Cav-1 levels. These findings provided compelling early evidence that increased Cav-1 expression is one component of the means by which PCa becomes resistant to androgen ablation therapy.

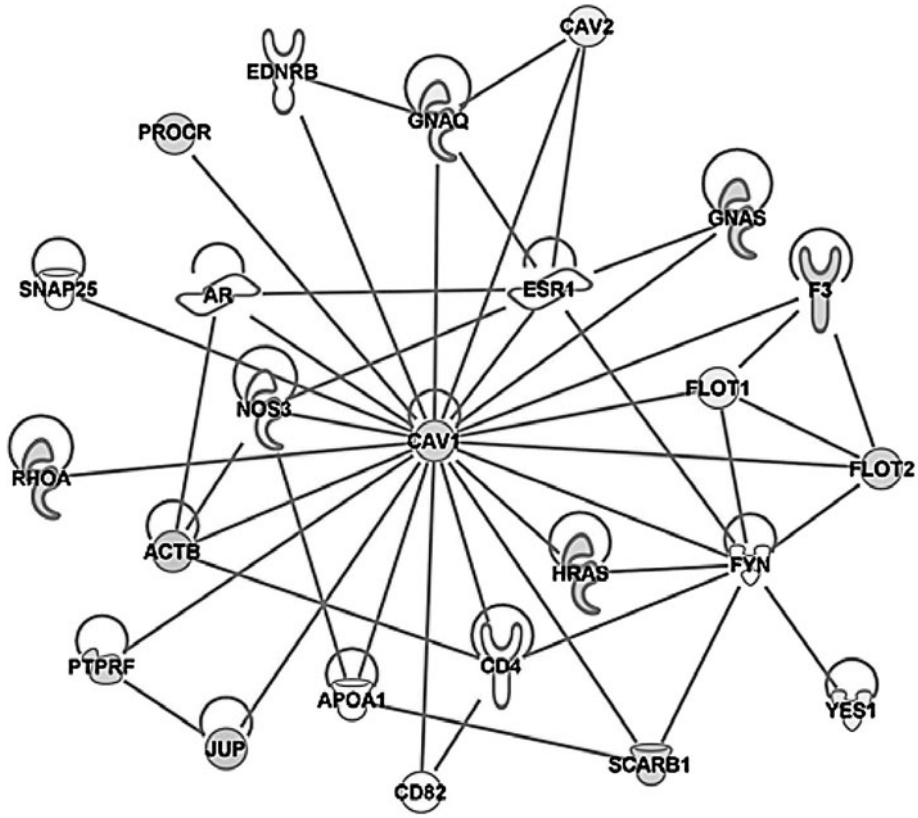

Figure 1. Caveolin-1 is the central node of a presumptive signaling network comprising multiple proteins that may be modified by dynamic and reversible S-acylation (palmitoylation). Gray nodes indicate S-acylated proteins that were identified by mass spectrometry in DU145 human prostate cancer cells. Originally published in Yang W et al. Mol Cell Proteomics 2010; 9:54-70;[50] © the American Society for Biochemistry and Molecular Biology.

Further studies revealed that testosterone stimulates *cav1* transcription in prostate cancer cells and that increased prostate tumor cell viability and clonal growth in culture arising from increases in testosterone concentration in the medium are mediated by Cav-1.[69] Because the AR continues to function in the hormone-repressed state, these findings provide direct evidence that AR signaling can modulate mechanisms controlling Cav-1 gene and protein expression and that the AR is likely to promote increased Cav-1 levels during disease progression. Cav-1 was also shown to promote metastasis in experimental models.[58,65,69] The pro-survival function of Cav-1 is not restricted to the androgen-AR axis because Cav-1 has been shown to suppress apoptotic signals from sources other than androgen withdrawal from hormone-sensitive cells, such as apoptosis induced by enforced *c-myc* expression.[70] Low levels of Cav-1 expression are also sufficient to confer a pro-survival advantage to prostate tumor cells. Experiments in animals using anti-Cav-1 antibodies and antisense approaches have demonstrated that Cav-1 is a potential therapeutic target.[68]

CELL-ASSOCIATED CAVEOLIN

Cav-1 is the primary protein constituent of the caveolar form of lipid raft microdomain and is responsible for the invaginated architecture of caveolae, recognizable as flask-shaped structures in electron micrographs. Homozygous ablation of the *cav1* and *cav3* genes in mice is sufficient to abolish caveolae formation in all organs.[71] Caveolae cannot be studied in the absence of caveolin, providing an essential challenge in determining whether a given effect of caveolar loss or gain is attributable to the membrane microdomain or to the caveolin protein itself. Consequently, the role of caveolae in PCa is still poorly understood.

"Flat" lipid rafts that do not contain caveolin proteins and consequently do not form membrane invaginations, have been described.[36] They are biochemically similar to caveolae and contain relatively high levels of glycosphingolipids and cholesterol. Normal prostate tissue has high cholesterol content, about the same concentration as the liver. Schaffner and colleagues provided the first evidence that lowering cholesterol levels alters prostate tissue homeostasis.[72] These investigators showed that prostate regression was evoked in animals by oral administration of hypocholesterolemic agents, such as the polyene macrolide candicidin.[72,73] Candicidin and structurally similar compounds bind to cholesterol and inhibit its absorption through the intestine. A similar cholesterol-targeting strategy was used in an experimental PCa study by Solomon and coworkers in which an FDA-approved drug (ezetimibe), which blocks the intestinal cholesterol transporter NPC1L1, was used to lower circulating cholesterol and the effect on growth of human PCa xenografts determined.[74] Cholesterol was also raised in another arm of the study using an isocaloric diet manipulation. Ezetimibe was found to inhibit growth of human LNCaP xenografts in mice and an unanticipated, dramatic antitumor angiogenesis effect of the drug was observed. Conversely, the high cholesterol diet, which increased circulating cholesterol, promoted tumor angiogenesis and tumor xenograft growth. These findings are significant because the LNCaP model is Cav-1-negative; consequently, the cholesterol-dependent effects on tumor growth cannot be attributed to Cav-1.

An important role for cholesterol in prostate tumor growth has recently received support from experimental and clinical observations. Older observations reported a correlation between the presence of cancer and increases in tissue cholesterol content along with evidence that irregularities in lipid metabolism underlie the basis for cholesterol accumulation in the prostate.[73,75] Several prospective observational series have now shown that long-term cholesterol-lowering therapy using HMG-CoA reductase inhibitors (aka "statins") reduces the risk of aggressive prostate cancer, suggesting that cholesterol localization in prostate tumor cells can promote either cell proliferation or other aggressive behaviors.[76-79] The prostate normally synthesizes large amounts of cholesterol, comparable to the liver. Androgens stimulate lipogenesis in human PCa cells by promoting transcription of genes, such as those encoding FASN and HMG-CoA reductase (the rate limiting step in cholesterol biosynthesis). Several unbiased studies have shown that androgens regulate lipogenesis in several cell types at the genome level.[80-82] Studies in the Cav-1-negative LNCaP cell line have demonstrated a role for membrane cholesterol in signal transduction mechanisms relevant to castrate-resistant PCa. The pathways shown to be affected by targeting membrane cholesterol in various ways include interleukin-6 to STAT-3, AR and Akt signaling mechanisms.[54,83-86] One explanation for cholesterol-dependent effects on signal transduction involves perturbations of signaling through multi-protein complexes that reside within lipid raft microdomains.[54,84,86] Another explanation involves the role of cholesterol as a metabolic precursor of androgens and the ability of tumor cells to

synthesize sufficient levels of androgen to promote tumor cell growth.[29] The relative contribution of lipid raft-dependent mechanisms, in comparison to effects on anabolic metabolism arising from perturbations in cholesterol homeostasis, remain to be assessed.

Studies in Cav-1-negative LNCaP cells are informative because they have been used to demonstrate the importance of cholesterol-dependent mechanisms employing lipid rafts, but show that Cav-1 expression is not required to elicit tumor growth or tumor cell survival effects. Whether lipid raft pathways present in Cav-1-negative cells might be enhanced by upregulation of Cav-1 is unknown. The physiologic distinction between caveolar and flat rafts is unclear.[36] Along with Cav-1 (or Cav-3 in the case of skeletal and cardiac muscle), caveolae formation requires a collaborating protein, polymerase I and transcript release factor (PTRF), also called cavin-1.[87-89] PTRF is a soluble, cytosolic protein that is recruited to the membrane to generate caveolae. Cav-1 remains associated with the plasma membrane in cells in which PTRF is silenced, despite the loss of caveolae, but lateral mobility and lysosomal degradation of Cav-1 are increased. At this writing, a single study has reported that PTRF expression is decreased in human PCa tissue.[90] If this finding is confirmed and expanded, the implications are that PCa cells in vivo may over-express Cav-1 in the absence of typical caveolar architecture. The biological implications of such a scenario are unknown. The stepwise, hierarchical assembly of Cav-1/PTRF complexes within the ER, Golgi structures and the plasma membrane suggests that caveolar membranes, distinct from flat rafts, perform specialized roles.[91]

Cav-1 regulates lipoprotein uptake and thereby affects lipoprotein and triglyceride metabolism.[92,93] Cell associated functions that involve the Cav-1 scaffolding domain, such as kinase regulation, are also unlikely to be replicated by flat lipid rafts unless the Cav-1 activity can be provided by another signaling protein. Cav-1 overexpression may feed forward onto lipid raft signaling more generally because the protein is a regulator of intracellular cholesterol level. Because Cav-1 is a mediator of LDL uptake, increases in Cav-1 in adenocarcinoma cells may promote cholesterol accumulation in cancer nodules. Increases in cholesterol within or near the cancerous tissue may then alter signal transduction mechanisms through more generic lipid raft mechanisms, or by stimulating intratumoral synthesis of androgen. One anticipated consequence of cholesterol accumulation in normal or malignant prostate tissue would be an increase in tissue inflammation, a potential effector of both benign urologic disease as well as cancer.

SECRETED CAVEOLIN

The studies described above point to an important role for increases in Cav-1 expression in the progression of PCa to castrate-resistant disease. Although over-expression of Cav-1 is common in PCa and tracks with aggressive disease, there is still considerable heterogeneity in the immunostaining patterns seen in human tissues. Cav-1-positivity, as measured by conventional immunohistochemistry, ranges from 40-60% cells in metastatic tumors.[49,57] These findings of tumor heterogeneity with respect to patterns of expression of the Cav-1 protein suggest the possibility that Cav-1 might be exported into the extracellular space and play a role in the tumor microenvironment as a component of paracrine or endocrine signaling mechanisms.

Cav-1 is in fact secreted by mouse and human prostate tumor cells in culture and secretion can be stimulated by androgen in Cav-1-expressing cells expressing AR.[58] Cav-1 is also detectable in human serum, can be measured using enzyme linked absorbent

assay (ELISA) and in its circulating form has been shown to be informative clinically as a biomarker capable of predicting disease recurrence.[58,94,95] Biochemical fractionation suggests that in its soluble form the protein associates with some kind of lipoprotein particle.[96] Experiments with conditioned media indicate that Cav-1-expressing PCa cells secrete a bioactive component that can promote cell survival and clonal growth and which can be inhibited with anti-Cav-1 antibodies,[58] indicating that Cav-1 is a critical component of this paracrine mechanism. Expression of Cav-1 in Cav-1 deficient prostate tumor cells was shown to promote tumor growth in vivo and Cav-1-secreting cells were demonstrated to be targetable in vivo using anti-Cav-1 antibodies, which elicited a therapeutic effect in model systems.[58] Using a xenograft LNCaP model, Bartz et al showed that subcutaneous tumors seeded with Cav-1-deficient cells became Cav-1-positive when Cav-1 expressing tumors were grown on the contralateral side.[96] These findings suggest that Cav-1 circulating in the blood can be incorporated into tissue sites distant from the point of secretion and that circulating Cav-1 can exert autocrine, paracrine and/or endocrine effects within the tumor microenvironment and at other locations.

Di Vizio et al recently reported the identification of a novel type of bioactive vesicular particle, within the size range of 1-10 μm, secreted by prostate tumor cells in response to epidermal growth factor (EGF) and/or silencing of the actin nucleating protein, DRF3.[97] DRF3 is encoded by a gene (*DIAPH3*) that undergoes chromosomal deletion at high frequency in aggressive prostate cancer.[97] These microvesicles, termed "oncosomes," contain Cav-1, indicating that the circulating form of Cav-1 may be transported in a wide range of particle classes and sizes. Oncosome secretion elicited by EGF receptor activation coincides with dramatic changes in cell shape, cytoskeleton structure and localization of the phosphorylated form of Cav-1 (Fig. 2). Preliminary characterization by mass spectrometry of other protein cargo of oncosomes produced by LNCaP cells suggests that this large class of particle could serve as a mechanism for the widespread dissemination of cell-free signaling complexes with bioactive capability.[97] The role of Cav-1 in these signaling events is unknown, but antibodies against Cav-1 have been shown to inhibit bioactivity detectable in prostate cancer cell secretions.[58] When considered in combination with published data that Cav-1 levels in serum are potentially clinically informative, particularly when combined with other disease parameters such as preoperative PSA, these experimental findings suggest that examining the contents of Cav-1-enriched particulate fractions in serum using mass spectrometry-based proteomics methods may provide a source of new biomarkers with clinical relevance.

STROMAL CAVEOLIN

Stromal cells react to the presence of epithelial tumors by initiating a complex cascade of events that are still poorly understood. Adenocarcinomas promote a "desmoplastic" reaction in which cancer-associated fibroblasts take on the characteristics of myofibroblasts more characteristic of wound healing. Phenotypic transformations in the stromal population may conceivably arise from changes in the gene expression program of the native stromal cells, recruitment of stem cell populations, or epithelial-to-mesenchymal or endothelial-to-mesenchymal transitions. It is now well established that these "cancer-associated fibroblasts" (CAF) are active participants in tumorigenesis via mechanisms that involve remodeling of the extracellular matrix, secretion of bioactive proteins of several kinds (particularly TGFβ1) and induction of angiogenesis.[98,99] CAF

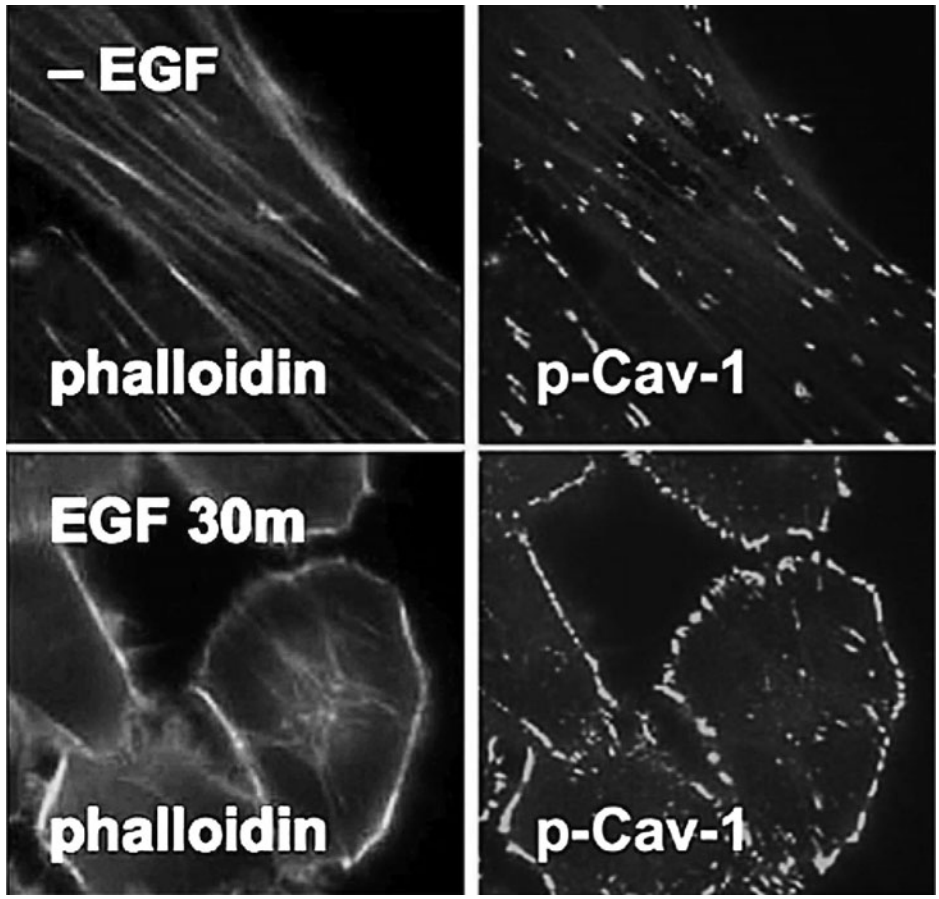

Figure 2. EGF induces rapid changes in cell shape, reordering of the cytoskeleton and redistribution of phosphorylated caveolin-1 (Tyr14) to the plasma membrane in DU145 human prostate cancer cells.

communicate with a variety of cells in the tumor microenvironment, including endothelial cells, immune cells, inflammatory cells, bone marrow-derived stem cells, adipocytes and carcinoma cells, through paracrine signaling mechanisms that can promote tumor expansion and matrix invasion. Recent studies of CAF in human tumors strongly suggest a direct role of the stroma in progression to advanced disease.[100-102]

In contrast to the upregulation of Cav-1 seen in aggressive PCa cells, studies of NIH3T3 fibroblasts transformed with several oncogenes demonstrated that Cav-1 levels were down-regulated and caveolae formation was ablated, by these manipulations.[103-105] Consistent with these experimental data, CAF isolated from breast cancer patients showed down-regulation of Cav-1.[100,106] Tumor implantation into the mammary fat pad of Cav-1($^{-/-}$) mice resulted in a significant enhancement of tumor growth, suggesting that Cav-1-negative stroma is tumor promoting. RNA profiling of human breast CAF indicated that Cav-1 downregulation corresponded with a gene signature resembling that seen with functional repression of the RB1 tumor suppressor gene. Additional data suggest that loss of stromal Cav-1 can be used clinically as a novel biomarker of poor

clinical outcome in breast cancer patients, with Cav-1 loss predictive of recurrence-free survival and progression to invasive disease or metastases.[107] Similar to breast cancer, limited studies in PCa have shown that loss of stromal Cav-1 is positively correlated with increased Gleason score and progression to metastatic disease.[108] Additional unpublished data from our group indicate that silencing of Cav-1 in prostate stromal cells promotes PCa tumor cell migration and predicts disease-free survival. Collectively, these and other reports of the stroma reaction to adjacent tumor, the so-called "reactive stroma," are consistent with the hypothesis that carcinoma growth, survival and progression are mediated in some cases by Cav-1 downregulation in the stromal compartment.[98,99,109]

CONCLUSION

Cav-1 is a multi-functional protein that promotes PCa progression when expressed at elevated levels by adenocarcinoma cells and when downregulated in prostate stromal cells. The specific mechanisms where by Cav-1 takes on oncogenic cellular activities are not well understood, but its role as a binding partner of many signal transduction proteins and as a mediator of cholesterol and fatty acid metabolism, are likely to be critical aspects of its oncogenic function. The evidence we have described indicates that increased Cav-1 expression promotes phenotypic transformation from an androgen-sensitive phenotype to an androgen-insensitive phenotype. Although the biochemical data described in this chapter strongly support a mediating role for Cav-1 in signaling through the androgen-AR axis, it is nevertheless remarkable that a single protein that resides primarily at extranuclear membrane sites and primarily at the plasma membrane when expressed at high levels, is capable of conferring androgen-insensitivity to PCa cells. Because Cav-1 is found in both cell-associated and secreted forms, it is important to consider how the protein may operate in several distinct tissue compartments during disease progression. Future studies will employ new technologies and approaches to dissect these diverse functions. Because of its role in a wide variety of pathways and processes, studies of Cav-1 and its signaling partners are likely to provide new avenues toward the treatment of castrate-resistant PCa.

REFERENCES

1. Huggins C, Hodges CV. Studies on prostatic cancer: I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatc carinoma of the prostate. J Urol 1941; 168:9-12.
2. Attard G, Reid AH, Yap TA et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. J Clin Oncol 2008; 26:4563-4571.
3. Attar RM, Takimoto CH, Gottardis MM. Castration-resistant prostate cancer: locking up the molecular escape routes. Clin Cancer Res 2009; 15:3251-3255.
4. Miyamoto H, Messing EM, Chang C. Androgen deprivation therapy for prostate cancer: current status and future prospects. Prostate 2004; 61:332-353.
5. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev 2004; 25:276-308.
6. Chmelar R, Buchanan G, Need EF et al. Androgen receptor coregulators and their involvement in the development and progression of prostate cancer. Int J Cancer 2007; 120:719-733.
7. Fu M, Rao M, Wang C et al. Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. Mol Cell Biol 2003; 23:8563-8575.
8. Hu YC, Yeh S, Yeh SD et al. Functional domain and motif analyses of androgen receptor coregulator ARA70 and its differential expression in prostate cancer. J Biol Chem 2004; 279:33438-33446.
9. Linja MJ, Porkka KP, Kang Z et al. Expression of androgen receptor coregulators in prostate cancer. Clin Cancer Res 2004; 10:1032-1040.

10. Linja MJ, Savinainen KJ, Saramaki OR et al. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001; 61:3550-3555.
11. Koivisto PA, Rantala I. Amplification of the androgen receptor gene is associated with P53 mutation in hormone-refractory recurrent prostate cancer. J Pathol 1999; 187:237-241.
12. Haapala K, Kuukasjarvi T, Hyytinen E et al. Androgen receptor amplification is associated with increased cell proliferation in prostate cancer. Hum Pathol 2007; 38:474-478.
13. Edwards J, Krishna NS, Grigor KM et al. Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. Br J Cancer 2003; 89:552-556.
14. Ford OH, 3rd, Gregory CW, Kim D et al. Androgen receptor gene amplification and protein expression in recurrent prostate cancer. J Urol 2003; 170:1817-1821.
15. Koivisto P, Kononen J, Palmberg C et al. Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res 1997; 57:314-319.
16. Visakorpi T, Hyytinen E, Koivisto P et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995; 9:401-406.
17. Zhau HY, Chang SM, Chen BQ et al. Androgen-repressed phenotype in human prostate cancer. Proc Natl Acad Sci USA 1996; 93:15152-15157.
18. Magi-Galluzzi C, Xu X, Hlatky L et al. Heterogeneity of androgen receptor content in advanced prostate cancer. Mod Pathol 1997; 10:839-845.
19. Niu Y, Altuwaijri S, Lai KP et al. Androgen receptor is a tumor suppressor and proliferator in prostate cancer. Proc Natl Acad Sci USA 2008; 105:12182-12187.
20. Niu Y, Altuwaijri S, Yeh S et al. Targeting the stromal androgen receptor in primary prostate tumors at earlier stages. Proc Natl Acad Sci USA 2008; 105:12188-12193.
21. Bergerat JP, Ceraline J. Pleiotropic functional properties of androgen receptor mutants in prostate cancer. Hum Mutat 2009; 30:145-157.
22. Li W, Cavasotto CN, Cardozo T et al. Androgen receptor mutations identified in prostate cancer and androgen insensitivity syndrome display aberrant ART-27 coactivator function. Mol Endocrinol 2005; 19:2273-2282.
23. Yeh S, Lin HK, Kang HY et al. From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci USA 1999; 96:5458-5463.
24. Kang HY, Lin HK, Hu YC et al. From transforming growth factor-beta signaling to androgen action: identification of Smad3 as an androgen receptor coregulator in prostate cancer cells. Proc Natl Acad Sci USA 2001; 98:3018-3023.
25. Nickerson T, Chang F, Lorimer D et al. In vivo progression of LAPC-9 and LNCaP prostate cancer models to androgen independence is associated with increased expression of insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR). Cancer Res 2001; 61:6276-6280.
26. Yang L, Xie S, Jamaluddin MS et al. Induction of androgen receptor expression by phosphatidylinositol 3-kinase/Akt downstream substrate, FOXO3a and their roles in apoptosis of LNCaP prostate cancer cells. J Biol Chem 2005; 280:33558-33565.
27. Yang L, Wang L, Lin HK et al. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3 and MAPK, three distinct signal pathways in prostate cancer cells. Biochem Biophys Res Commun 2003; 305:462-469.
28. Plymate SR, Haugk K, Coleman I et al. An antibody targeting the type I insulin-like growth factor receptor enhances the castration-induced response in androgen-dependent prostate cancer. Clin Cancer Res 2007; 13:6429-6439.
29. Montgomery RB, Mostaghel EA, Vessella R et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008; 68:4447-4454.
30. Locke JA, Fazli L, Adomat H et al. A novel communication role for CYP17A1 in the progression of castration-resistant prostate cancer. Prostate 2009; 69:928-937.
31. Locke JA, Guns ES, Lubik AA et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res 2008; 68:6407-6415.
32. Lee MS, Igawa T, Lin MF. Tyrosine-317 of p52(Shc) mediates androgen-stimulated proliferation signals in human prostate cancer cells. Oncogene 2004; 23:3048-3058.
33. Mellinghoff IK, Vivanco I, Kwon A et al. HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell 2004; 6:517-527.
34. Wu JD, Haugk K, Woodke L et al. Interaction of IGF signaling and the androgen receptor in prostate cancer progression. J Cell Biochem 2006; 99:392-401.
35. Lu ML, Schneider MC, Zheng Y et al. Caveolin-1 interacts with androgen receptor. A positive modulator of androgen receptor mediated transactivation. J Biol Chem 2001; 276:13442-13451.
36. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science 2010; 327:46-50.
37. Freeman MR, Cinar B, Lu ML. Membrane rafts as potential sites of nongenomic hormonal signaling in prostate cancer. Trends Endocrinol Metab 2005; 16:273-279.

38. Haas D, White SN, Lutz LB et al. The modulator of nongenomic actions of the estrogen receptor (MNAR) regulates transcription-independent androgen receptor-mediated signaling: evidence that MNAR participates in G protein-regulated meiosis in Xenopus laevis oocytes. Mol Endocrinol 2005; 19:2035-2046.
39. Pedram A, Razandi M, Sainson RC et al. A conserved mechanism for steroid receptor translocation to the plasma membrane. J Biol Chem 2007; 282:22278-22288.
40. Couet J, Sargiacomo M, Lisanti MP. Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. J Biol Chem 1997; 272:30429-30438.
41. Vihanto MM, Vindis C, Djonov V et al. Caveolin-1 is required for signaling and membrane targeting of EphB1 receptor tyrosine kinase. J Cell Sci 2006; 119:2299-2309.
42. Sen A, O'Malley K, Wang Z et al. Paxillin regulates androgen- and epidermal growth factor- induced MAPK signaling and cell proliferation in prostate cancer cells. J Biol Chem 2010; 285(37):28787-95.
43. Migliaccio A, Varricchio L, De Falco A et al. Inhibition of the SH3 domain-mediated binding of Src to the androgen receptor and its effect on tumor growth. Oncogene 2007; 26:6619-6629.
44. Migliaccio A, Castoria G, Di Domenico M et al. Steroid-induced androgen receptor-oestradiol receptor beta-Src complex triggers prostate cancer cell proliferation. EMBO J 2000; 19:5406-5417.
45. Shatkina L, Mink S, Rogatsch H et al. The cochaperone Bag-1L enhances androgen receptor action via interaction with the NH2-terminal region of the receptor. Mol Cell Biol 2003; 23:7189-7197.
46. Cheung-Flynn J, Prapapanich V, Cox MB et al. Physiological role for the cochaperone FKBP52 in androgen receptor signaling. Mol Endocrinol 2005; 19:1654-1666.
47. Zoubeidi A, Zardan A, Beraldi E et al. Cooperative interactions between androgen receptor (AR) and heat-shock protein 27 facilitate AR transcriptional activity. Cancer Res 2007; 67:10455-10465.
48. Razandi M, Pedram A, Levin ER. Heat shock protein 27 is required for sex steroid receptor trafficking to and functioning at the plasma membrane. Mol Cell Biol 2010; 30:3249-3261.
49. Yang G, Truong LD, Wheeler TM et al. Caveolin-1 expression in clinically confined human prostate cancer: a novel prognostic marker. Cancer Res 1999; 59:5719-5723.
50. Yang W, Di Vizio D, Kirchner M et al. Proteome scale characterization of human S-acylated proteins in lipid raft-enriched and nonraft membranes. Mol Cell Proteomics 2010; 9:54-70.
51. Aronica SM, Kraus WL, Katzenellenbogen BS. Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. Proc Natl Acad Sci USA 1994; 91:8517-8521.
52. Whang YE, Wu X, Suzuki H et al. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc Natl Acad Sci USA 1998; 95:5246-5250.
53. Wu X, Senechal K, Neshat MS et al. The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci USA 1998; 95:15587-15591.
54. Cinar B, Mukhopadhyay NK, Meng G et al. Phosphoinositide 3-kinase-independent nongenomic signals transit from the androgen receptor to Akt1 in membrane raft microdomains. J Biol Chem 2007; 282:29584-29593.
55. Li L, Ren CH, Tahir SA et al. Caveolin-1 maintains activated Akt in prostate cancer cells through scaffolding domain binding site interactions with and inhibition of serine/threonine protein phosphatases PP1 and PP2A. Mol Cell Biol 2003; 23:9389-9404.
56. Caselli A, Mazzinghi B, Camici G et al. Some protein tyrosine phosphatases target in part to lipid rafts and interact with caveolin-1. Biochem Biophys Res Commun 2002; 296:692-697.
57. Yang G, Truong LD, Timme TL et al. Elevated expression of caveolin is associated with prostate and breast cancer. Clin Cancer Res 1998; 4:1873-1880.
58. Tahir SA, Yang G, Ebara S et al. Secreted caveolin-1 stimulates cell survival/clonal growth and contributes to metastasis in androgen-insensitive prostate cancer. Cancer Res 2001; 61:3882-3885.
59. Tahir SA, Frolov A, Hayes TG et al. Preoperative serum caveolin-1 as a prognostic marker for recurrence in a radical prostatectomy cohort. Clin Cancer Res 2006; 12:4872-4875.
60. Di Vizio D, Sotgia F, Williams TM et al. Caveolin-1 is required for the upregulation of fatty acid synthase (FASN), a tumor promoter, during prostate cancer progression. Cancer Biol Ther 2007; 6:1263-1268.
61. Di Vizio D, Adam RM, Kim J et al. Caveolin-1 interacts with a lipid raft-associated population of fatty acid synthase. Cell Cycle 2008; 7:2257-2267.
62. Yang G, Addai J, Wheeler TM et al. Correlative evidence that prostate cancer cell-derived caveolin-1 mediates angiogenesis. Hum Pathol 2007; 38:1688-1695.
63. Yang G, Timme TL, Frolov A et al. Combined c-Myc and caveolin-1 expression in human prostate carcinoma predicts prostate carcinoma progression. Cancer 2005; 103:1186-1194.
64. Yang G, Addai J, Ittmann M et al. Elevated caveolin-1 levels in African-American versus white-American prostate cancer. Clin Cancer Res 2000; 6:3430-3433.
65. Williams TM, Hassan GS, Li J et al. Caveolin-1 promotes tumor progression in an autochthonous mouse model of prostate cancer: genetic ablation of Cav-1 delays advanced prostate tumor development in tramp mice. J Biol Chem 2005; 280:25134-25145.

66. Williams TM, Medina F, Badano I et al. Caveolin-1 gene disruption promotes mammary tumorigenesis and dramatically enhances lung metastasis in vivo. Role of Cav-1 in cell invasiveness and matrix metalloproteinase (MMP-2/9) secretion. J Biol Chem 2004; 279:51630-51646.
67. Williams TM, Cheung MW, Park DS et al. Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. Mol Biol Cell 2003; 14:1027-1042.
68. Nasu Y, Timme TL, Yang G et al. Suppression of caveolin expression induces androgen sensitivity in metastatic androgen-insensitive mouse prostate cancer cells. Nat Med 1998; 4:1062-1064.
69. Li L, Yang G, Ebara S et al. Caveolin-1 mediates testosterone-stimulated survival/clonal growth and promotes metastatic activities in prostate cancer cells. Cancer Res 2001; 61:4386-4392.
70. Timme TL, Goltsov A, Tahir S et al. Caveolin-1 is regulated by c-myc and suppresses c-myc-induced apoptosis. Oncogene 2000; 19:3256-3265.
71. Park DS, Woodman SE, Schubert W et al. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and nonmuscle caveolae and develop a severe cardiomyopathic phenotype. Am J Pathol 2002; 160:2207-2217.
72. Schaffner CP, Gordon HW. The hypocholesterolemic activity of orally administered polyene macrolides. Proc Natl Acad Sci USA 1968; 61:36-41.
73. Schaffner CP. Prostatic cholesterol metabolism: regulation and alteration. Prog Clin Biol Res 1981; 75A:279-324.
74. Solomon KR, Pelton K, Boucher K et al. Ezetimibe is an inhibitor of tumor angiogenesis. Am J Pathol 2009; 174:1017-1026.
75. Freeman MR, Solomon KR. Cholesterol and prostate cancer. J Cell Biochem 2004; 91:54-69.
76. Murtola TJ, Tammela TL, Maattanen L et al. Prostate cancer and PSA among statin users in the Finnish prostate cancer screening trial. Int J Cancer 2010; 127(7):1650-9.
77. Platz EA, Till C, Goodman PJ et al. Men with low serum cholesterol have a lower risk of high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev 2009; 18:2807-2813.
78. Platz EA, Clinton SK, Giovannucci E. Association between plasma cholesterol and prostate cancer in the PSA era. Int J Cancer 2008; 123:1693-1698.
79. Platz EA, Leitzmann MF, Visvanathan K et al. Statin drugs and risk of advanced prostate cancer. J Natl Cancer Inst 2006; 98:1819-1825.
80. Zhang Y, Calvo E, Martel C et al. Response of the adipose tissue transcriptome to dihydrotestosterone in mice. Physiol Genomics 2008; 35:254-261.
81. Chauvin TR, Griswold MD. Androgen-regulated genes in the murine epididymis. Biol Reprod 2004; 71:560-569.
82. Swinnen JV, Heemers H, van de Sande T et al. Androgens, lipogenesis and prostate cancer. J Steroid Biochem Mol Biol 2004; 92:273-279.
83. Kim J, Adam RM, Solomon KR et al. Involvement of cholesterol-rich lipid rafts in interleukin-6-induced neuroendocrine differentiation of LNCaP prostate cancer cells. Endocrinology 2004; 145:613-619.
84. Adam RM, Mukhopadhyay NK, Kim J et al. Cholesterol sensitivity of endogenous and myristoylated Akt. Cancer Res 2007; 67:6238-6246.
85. Zhuang L, Lin J, Lu ML et al. Cholesterol-rich lipid rafts mediate akt-regulated survival in prostate cancer cells. Cancer Res 2002; 62:2227-2231.
86. Zhuang L, Kim J, Adam RM et al. Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. J Clin Invest 2005; 115:959-968.
87. Liu L, Brown D, McKee M et al. Deletion of Cavin/PTRF causes global loss of caveolae, dyslipidemia and glucose intolerance. Cell Metab 2008; 8:310-317.
88. Hill MM, Bastiani M, Luetterforst R et al. PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. Cell 2008; 132:113-124.
89. Liu L, Pilch PF. A critical role of cavin (polymerase I and transcript release factor) in caveolae formation and organization. J Biol Chem 2008; 283:4314-4322.
90. Gould ML, Williams G, Nicholson HD. Changes in caveolae, caveolin and polymerase 1 and transcript release factor (PTRF) expression in prostate cancer progression. Prostate 2010; 70(15):1609-21.
91. Hayer A, Stoeber M, Bissig C et al. Biogenesis of caveolae: stepwise assembly of large caveolin and cavin complexes. Traffic 2010; 11:361-382.
92. Frank PG, Pavlides S, Cheung MW et al. Role of caveolin-1 in the regulation of lipoprotein metabolism. Am J Physiol Cell Physiol 2008; 295:C242-248.
93. Razani B, Combs TP, Wang XB et al. Caveolin-1-deficient mice are lean, resistant to diet-induced obesity and show hypertriglyceridemia with adipocyte abnormalities. J Biol Chem 2002; 277:8635-8647.
94. Tahir SA, Ren C, Timme TL et al. Development of an immunoassay for serum caveolin-1: a novel biomarker for prostate cancer. Clin Cancer Res 2003; 9:3653-3659.
95. Tahir SA, Yang G, Goltsov AA et al. Tumor cell-secreted caveolin-1 has proangiogenic activities in prostate cancer. Cancer Res 2008; 68:731-739.

96. Bartz R, Zhou J, Hsieh JT et al. Caveolin-1 secreting LNCaP cells induce tumor growth of caveolin-1 negative LNCaP cells in vivo. Int J Cancer 2008; 122:520-525.
97. Di Vizio D, Kim J, Hager MH et al. Oncosome formation in prostate cancer: association with a region of frequent chromosomal deletion in metastatic disease. Cancer Res 2009; 69:5601-5609.
98. Dakhova O, Ozen M, Creighton CJ et al. Global gene expression analysis of reactive stroma in prostate cancer. Clin Cancer Res 2009; 15:3979-3989.
99. Tuxhorn JA, Ayala GE, Smith MJ et al. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res 2002; 8:2912-2923.
100. Mercier I, Casimiro MC, Wang C et al. Human breast cancer-associated fibroblasts (CAFs) show caveolin-1 downregulation and RB tumor suppressor functional inactivation: Implications for the response to hormonal therapy. Cancer Biol Ther 2008; 7:1212-1225.
101. Josson S, Matsuoka Y, Chung LW et al. Tumor-stroma co-evolution in prostate cancer progression and metastasis. Semin Cell Dev Biol 2010; 21:26-32.
102. Vanpoucke G, Orr B, Grace OC et al. Transcriptional profiling of inductive mesenchyme to identify molecules involved in prostate development and disease. Genome Biol 2007; 8:R213.
103. Galbiati F, Volonte D, Engelman JA et al. Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. EMBO J 1998; 17:6633-6648.
104. Engelman JA, Wykoff CC, Yasuhara S et al. Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. J Biol Chem 1997; 272:16374-16381.
105. Koleske AJ, Baltimore D, Lisanti MP. Reduction of caveolin and caveolae in oncogenically transformed cells. Proc Natl Acad Sci USA 1995; 92:1381-1385.
106. Sotgia F, Del Galdo F, Casimiro MC et al. Caveolin-$1^{-/-}$ null mammary stromal fibroblasts share characteristics with human breast cancer-associated fibroblasts. Am J Pathol 2009; 174:746-761.
107. Witkiewicz AK, Dasgupta A, Sotgia F et al. An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers. Am J Pathol 2009; 174:2023-2034.
108. Di Vizio D, Morello M, Sotgia F et al. An absence of stromal caveolin-1 is associated with advanced prostate cancer, metastatic disease and epithelial Akt activation. Cell Cycle 2009; 8:2420-2424.
109. Yang F, Tuxhorn JA, Ressler SJ et al. Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. Cancer Res 2005; 65:8887-8895.