Pharm Res (2011) 28:423–437
DOI 10.1007/s11095-010-0210-y

EXPERT REVIEW

Cholesterol as a Potential Target for Castration-Resistant Prostate Cancer

Alexis L. Twiddy • Carlos G. Leon • Kishor M. Wasan

Received: 21 April 2010 / Accepted: 28 June 2010 / Published online: 4 August 2010

ABSTRACT Advanced prostate cancer (CaP) is often treated with androgen deprivation therapy (ADT). Despite high initial success rates of this therapy, recurrence of the cancer in a castration-resistant (CRPC) form is inevitable. It has been demonstrated that, despite the low levels of circulating androgens resulting from ADT, intratumoral androgen levels remain high and androgen receptor activation persists. Recently, it was discovered that *de novo* androgen synthesis is occurring within the tumor cells themselves, thus providing a potential mechanism for the high endogenous concentrations. A common upstream precursor in this steroidogenic pathway is cholesterol. For many decades, the breakdown of cholesterol homeostasis in cancer has been the focus of research, but this was largely to elucidate its involvement in maintaining membrane integrity and cell signaling. *De novo* steroidogenesis has provided a new avenue for cholesterol research and reinforces the importance of understanding the mechanisms that lead to the alterations in cholesterol regulation in the progression to CRPC. The findings to date suggest that cholesterol homeostasis is altered to support *de novo* androgen synthesis and appear to facilitate disease progression. We further propose that a better understanding of the link between cholesterol and *de novo* androgen synthesis in CaP progression may provide opportunities for novel therapeutic intervention, namely via eliminating sources of the precursor cholesterol. This review summarizes the implications of cholesterol dysregulation in CaP and particularly in the post-ADT castration-resistant state, as well as the potential implementation of novel therapies targeting these cholesterol sources.

A. L. Twiddy • C. G. Leon • K. M. Wasan (✉)
Division of Pharmaceutics and Biopharmaceutics
Faculty of Pharmaceutical Sciences
The University of British Columbia
2146 East Mall Avenue
Vancouver, British Columbia, Canada V6T 1Z3
e-mail: kwasan@interchange.ubc.ca

KEY WORDS castration resistant prostate cancer · cholesterol · novel therapeutics targets · statins · scavenger receptor class B type I

ABBREVIATIONS

ABCA1	ATP-binding cassette transporter-subfamily A1
ACAT	acyl-coenzyme A:cholesterol acyltransferase
ADT	androgen deprivation therapy
AKR1C1/2/3	aldo-keto reductase family 1 members C1/2/3
AR	androgen Receptor
C4-2	castration-resistant cell line derived from LNCaP
CaP	prostate cancer
CE	cholesteryl ester
CRPC	castration-resistant Prostate Cancer
DHT	dihydrotestosterone
DU145	castration-resistant prostate cancer carcinoma cell line derived from brain metastases
HDL	high-density lipoprotein
HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
HSD17B3	17β-Hydroxy steroid dehydrogenase 3
HSL	hormone-sensitive lipase
LDL	low-density lipoprotein
LNCaP	lymph node metastatic prostate adenocarcinoma cell line
PC-3	bone-derived castration-resistant cell line
SR-BI	scavenger Receptor Class B Type I
SRD5A1	steroid 5α-reductase Type 1
StAR	steroidogenic acute regulatory protein

INTRODUCTION

Prostate cancer (CaP) is the most prevalent cancer among North American men as well as the third leading cause of cancer-related deaths in the same cohort (1,2). In 2009,

over 215,000 men in the United States and Canada were newly diagnosed, and it is thought that incidence rates will continue to rise in the coming years due to the aging baby-boomer population (1,3). One of the traditional therapies used in the treatment of prostate cancer that has metastasized beyond the confines of the prostate is androgen deprivation therapy (ADT). This treatment serves to cut-off androgen sources in the body, namely the testes (4). Often, this therapy involves chemical or surgical castration of the testes—the major sources of androgens in males. The premise of this androgen depletion is to remove the principal driving force behind growth, proliferation and differentiation in the prostate—androgens. Although this treatment is initially very successful, it is inevitable that more than 80% of these cancers will re-emerge (5). Unfortunately, the cancer that recurs is more aggressive, evasive and deadly (4). Consequently, this form of CaP does not respond to traditional therapies, resulting in a very negative prognosis (4). This recurrent form of CaP has been termed androgen-independent, hormone-refractory and androgen-insensitive, among others. The most recent and universally accepted term is castration-resistant prostate cancer (CRPC), as it reflects the knowledge that the re-emergent CaP is not independent of androgens or their influence, as was once believed.

The recurrence of prostate cancer in the castration-resistant form arises from an array of interrelated and complex molecular changes, many of which remain incompletely understood (4,6). In the last few decades, much research has been directed toward elucidation of the many mechanisms behind this recurrent form of CaP. A number of pathogenic pathways have been proposed to date, many of which involve the continued presence of androgens and the androgen receptor (AR) in the progression to CRPC (4). Although serum androgen levels are severely stunted by ADT, it appears that this does not translate to intratumoral androgen levels (4,7,8). Thus, a greater knowledge of the processes involved in the androgen-AR signaling pathway within the tumor may lead to the development of novel therapies. One such approach, which will be discussed in this review, may be to target an upstream precursor in the androgen synthesis pathway; cholesterol.

ANDROGENS, THE ANDROGEN RECEPTOR AND PROSTATE CANCER

Androgens, including testosterone and dihydrotestosterone (DHT), are a subclass of steroid hormones that are essential for the normal growth and development of the prostate, as well as many other sex-related characteristics (9). Similarly, androgens have been implicated in the growth and proliferation of CaP (10). It is for this reason that therapeutic intervention has largely been directed towards removal of androgen sources. Androgens are primarily synthesized in the testes and the adrenal glands through a series of enzymatic bioconversions from a common precursor, cholesterol (Fig. 1-adapted from (11)). The androgen synthesis pathway is initiated in the mitochondria of these steroidogenic cells (12,13). In order for this to happen, cholesterol must be moved across the mitochondrial membrane into the intermitochondrial space where the

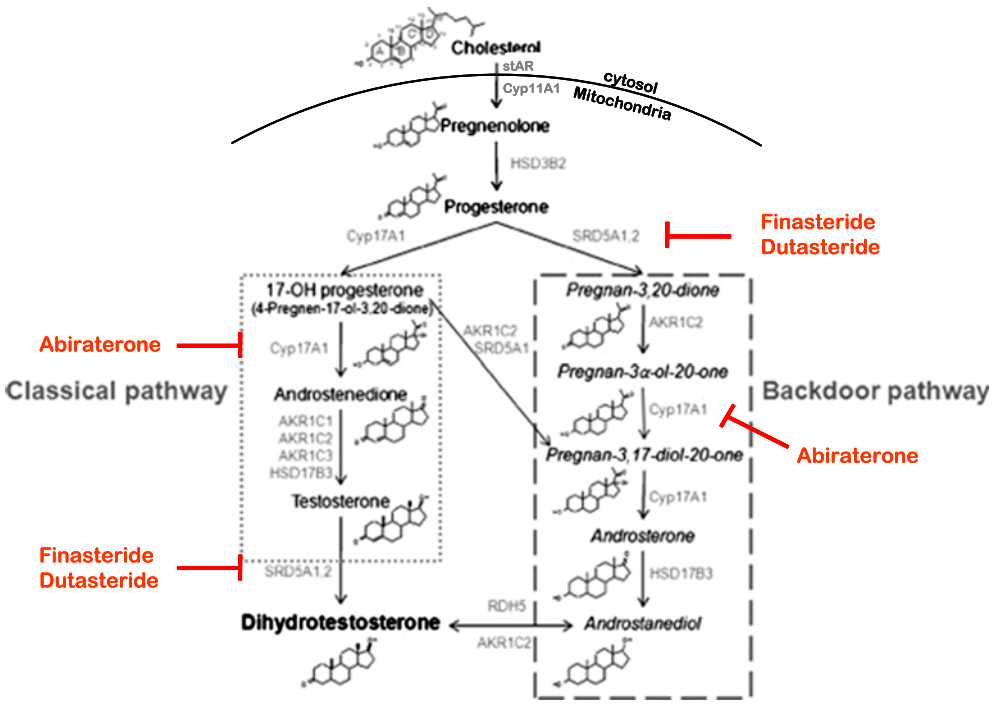

Fig. 1 Androgen synthesis via classical and backdoor pathways within mitochondria, including targets of new therapeutic intervention. Adapted from a figure in (11).

steroidogenic enzymes are present. The transport of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane is the rate-limiting step in androgen synthesis and is completed by the steroidogenic acute regulatory protein (StAR) (14,15). Once in the mitochondrial space, cholesterol is transformed by CYP11A1, also called P450 side-chain cleavage enzyme or desmolase, into the steroid precursor pregnenolone (16). CYP11A1 has been deemed the determinant enzyme for steroidogenic potential (17). The steroidogenic process continues along the classical pathway through a number of enzymatic steps terminating mainly in testosterone (Fig. 1).

Once synthesized, androgens exert their effects on the prostate and in prostate cancer by binding to the androgen receptor (AR), a ligand-activated nuclear receptor (9). Androgens, particularly DHT, bind to the AR in the cytosol, where it is generally found linked to a large group of stabilizing protein chaperones, including a large heat shock protein (HSP) (18). DHT is a more potent androgen that is the product of testosterone reduction facilitated by the action of two isoforms of steroid 5-α reductase, SRD5A1 and 5A2 (16). These enzymes are highly active in the prostate, making their reaction product, DHT, the predominant androgen in the prostate (19).

The binding of DHT to AR stimulates release of the large HSP chaperone complex, after which the DHT-AR unit homodimerizes with an additional DHT-AR complex. The homodimer then translocates to the nucleus and initiates transcription of multiple target genes that are involved in cell growth, proliferation and survival (9,20). One such target gene is prostate-specific antigen (PSA), which is a serine protease whose function is to solubilize seminal fluid. PSA is used as a biomarker for prostate cancer screening and progression and serves as a valuable indicator of response to androgens in experimental models, as discussed in the next section (21).

DE NOVO ANDROGEN SYNTHESIS IN CASTRATION-RESISTANT PROSTATE CANCER (CRPC)

The importance of androgens and the AR in the development of CaP was discovered in the middle of the twentieth century, when it was observed that removal of androgens decreased AR activity and expression, resulting in subsequent regression of tumors (10). In the last decade, this same relationship between androgens and the AR has been shown to remain important in the progression to and survival of CRPC. As mentioned previously, CRPC cells have the ability to synthesize androgens *de novo* (4,7,8,22). This has been demonstrated not only by following a radiolabelled precursor in the androgen synthesis pathway within *ex vivo* CRPC tumors, but also in the fact that the CRPC tumors possess the necessary enzymes to create androgens from cholesterol intracellularly (8,22). It has been demonstrated by a number of studies that many of the enzymes in the classical androgen synthesis pathway, including CYP11A1, CYP17A1, AKR1C1, AKRC1C3 (aldo-keto reductase family 1 member C1 & 3), HSD17B3 (17β-Hydroxy steroid dehydrogenase 3) and SRD5A1 (steroid 5α -reductase type 1), are not only expressed in CRPC but are also upregulated (Fig. 1). CYP11A1 is the enzyme responsible for conversion of cholesterol to pregnenolone, while CYP17A1 acts to modify pregnenolone and progesterone. HSD17B3 catalyzes the oxidation and isomerisation of steroid precursors, and AKR1C1 and 3 catalyze biosynthetic steps from androstenedione to testosterone. In addition, enzymes found in the backdoor pathway, which diverts reactants from the classical pathway and facilitates creation of DHT without the requirement of testosterone as a direct precursor, were also upregulated at CRPC—namely HSD17B3, SRD5A1 and AKR1C2 (aldo-keto reductase family 1 member C2) (7,8,22,23) (Fig. 1). In the backdoor pathway, SRD5A1 converts progesterone to pregnan-3,20-dione, which is then converted to pregnan-3α-ol-20-one by AKR1C2, while HSD17B3 is responsible for conversion of androsterone to androstanediol.

These findings are relevant because it was accepted for years that recurrent tumors were surviving in a virtually androgen-null environment as reflected by measures taken from the serum. Despite the castrate levels in the circulation, androgen levels within tumor cells persist, likely due to *de novo* synthesis, in levels sufficient to activate the AR (8,24–30). Testosterone levels within metastases of castrated men are three times higher than the levels seen within tumors of primary prostate cancers in untreated men (7). Further, it was demonstrated through the use of short-hairpin RNA that knockdown of the AR and, thus, inhibition of the androgen-AR pathway, results in lack of tumor progression after castration and even regression of CRPC cell growth (28,31). These findings indicate that the AR still requires ligand binding in order to exert its transcriptional effect on growth, proliferation, and survival of CRPC cells. Some recent studies suggest that this ligand is provided by residual sources of androgen in the body, namely the adrenal glands, rather than via *de novo* androgen synthesis within the tumor cells. This was based on the finding that enzymes converting testosterone to DHT and dihydroepiandrostenedione to androstenedione were upregulated to a greater extent than the other enzymes in the synthesis pathway (32,33). However, it has also been demonstrated that an adrenalectomy paired with ADT does not limit CaP progression, suggesting limited involvement of adrenal precursors (34,35). Overall, it appears that adrenal precursors are not the only source of ligand for AR activation, and *de novo* androgen synthesis is likely required to

provide sufficient ligand for maintaining this pathway in CRPC cells. Therefore, it becomes relevant to determine the upstream sources of cholesterol that are potentially a contributing precursor to the intratumoral creation of androgens and subsequently AR activation and CaP survival and progression.

In order to study these mechanisms effectively, it is important to have an effective experimental model. Countless cell lines have been cultured from different human CaP samples in order to capture different stages of the disease for study *in vitro*. These include the commonly used androgen-dependent CaP cell line derived from lymph node metastases, LNCaP, as well as the castration-resistant cell lines PC-3, derived from bone metastases, DU145 derived from brain metastases and C4-2 derived from a LNCaP and bone stromal subline (36–39). An animal model that has been developed to allow for study of CaP as it progresses from androgen-dependence to castration-resistance is the murine prostate xenograft model (Fig. 2) (40). The most widely used xenograft model is the LNCaP xenograft model. LNCaP cells are inoculated subcutaneously in severe combined immunodeficient (SCID) mice, and tumor growth in the normal androgen environment, termed androgen-dependent tumors (AD), is permitted until a predetermined tumor volume is attained. This tumor growth is marked by a continuous rise in PSA levels (Fig. 2). At this point, the mice are castrated and a consequent fall in PSA levels is observed. The tumors are considered to be nadir (N) when PSA drops to basal levels. Any surviving tumor cells in the mice tend to have a recurrence in growth after approximately 6 weeks. Again, this tumor growth is marked by a rebound in PSA levels, at which point the tumors are called castration-resistant prostate cancer (CRPC) (40) (Fig. 2). This model has been used for a number of studies that infer cholesterol regulation is altered between the different tumor stages, and we postulate this is to provide precursor to *de novo* androgen synthesis and facilitate cancer growth in a castration environment (41–44). Thus, the search continues to determine how this dysregulation occurs in CaP, as well

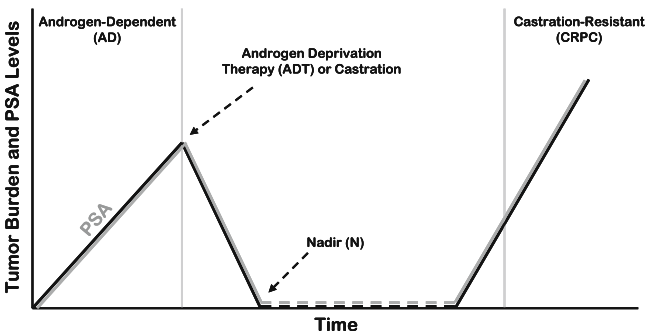

Fig. 2 Tumor burden and PSA measures in the LNCaP prostate xenograft model as tumors progress from androgen-dependence (AD) to castration-resistance (CRPC). Adapted from a figure in (87).

the implications it has on the survival and progression of the disease to a castration-resistant form.

Cholesterol in Prostate Cancer

Cholesterol is an essential molecule that has been studied extensively since it was discovered over two centuries ago. Due to its presence in virtually all the cells of the body, cholesterol predictably has many important biological roles, including maintenance of membrane structure, signal transduction and provision of precursor to bile and androgen synthesis (45). Consequently, cholesterol has been implicated in the pathogenesis of many disease states, most notably cardiovascular disease, but also in many forms of cancer, including CaP (46). Focus has been directed towards cholesterol in prostate cancer for more than 50 years following the findings of increased cholesterol content in prostatic adenomas in 1942 (46,47). This cholesterol accumulation is thought to be due to dysregulation of the complex pathways of cholesterol homeostasis. Cancer is characterized by a rapid and unregulated proliferation of cells. It has been proposed that the increased levels of cholesterol in prostate cancer may support cell proliferation by contributing cholesterol for membrane composition and signal transduction (48,49). In addition, we propose that cholesterol dysregulation may also provide a precursor to *de novo* synthesis of androgens that stimulate this cell division within castration-resistant tumors.

Cholesterol is obtained by humans from two major sources: exogenously from the diet and endogenously via *de novo* synthesis within the cells of the body, namely liver cells (45). Cholesterol in the circulation from either source is contained in soluble packages called lipoproteins. Lipoproteins, in general, consist of a neutral core of lipids surrounded by a monolayer of polarized phospholipids, often with proteins on the surface called apoproteins (50). The size and density of particles is variable, from the large and triglyceride-rich chylomicrons to the smaller high-density lipoproteins (HDL) (50). Lipoproteins are predominantly formed in the liver and the intestine and are then released into the circulation, where they undergo further enzymatic transformations, as well as interact with lipoprotein transporters that facilitate uptake of lipid contents. The most predominant lipoproteins in the circulation are low-density lipoproteins (LDL) and HDL. Both HDL and LDL contain high amounts of cholesterol and cholesteryl esters (50).

CHOLESTEROL FROM THE DIET AND PROSTATE CANCER

Cholesterol, as mentioned above, is obtained by the body from the diet and from synthesis within cells. Many

epidemiological studies over the years have examined dietary cholesterol consumption and its link to prostate cancer incidence. This research focus was stimulated by the finding that obesity and the characteristics associated with obesity, including the high fat and high calorie diet of the Western world, have been linked to many cancers, including CaP (51–53). In addition to being correlated with the overall incidence of CaP, the Western diet has also been linked to metastatic progression of CaP (54–57). One particular study associated serum cholesterol levels with grade of cancer and found that men with higher cholesterol (>240 mg/dl) were more likely than men with desirable (<200 mg/dL) or borderline levels (200–240 mg/dL) to develop high-grade or rapidly growing metastatic CaP (58). This finding was particularly prominent among men with higher body mass index. However, many other studies correlating serum cholesterol and CaP incidence have demonstrated the opposite finding (59–62), indicating a need to perhaps look directly at tumor cholesterol levels, which may not be reflective of serum levels. A few recent studies in a xenograft model demonstrate interesting results with dietary variation. Using the androgen-dependent LNCaP and the castration-resistant brain metastases-derived DU145 to grow tumor xenografts in mice, data indicate that a diet with increased fat and calories induces proliferation and growth of the tumors while inducing cholesterol accumulation in these tumors (43,44). However, the exact relationship between cholesterol, tumor growth and CaP incidence has yet to be fully elucidated.

Lipoproteins After Androgen-Deprivation Therapy

Interestingly, due to the intricate relationship that androgens have with cholesterol, they also have an impact on overall fat mass due to their anabolic action (63,64). Thus, the effects caused by androgen-deprivation therapy in men have been the subject of significant study. This research has mostly been aimed to elucidate atherogenic consequences post-therapy, but these effects are also relevant when one considers that many of these post-ADT patients will subsequently develop CRPC. Thus, any changes in cholesterol induced by reducing androgens may be significant for the progression and, ultimately, for the recurrence of the disease itself.

The loss of testosterone resulting from ADT causes significant increases in obesity, specifically truncal fat deposition, while decreasing lean body mass and causing significant changes to the lipid profile (63,65). The changes seen in the lipid profiles of post-ADT subjects include an increase in total cholesterol, triglycerides, LDL-cholesterol and oxidized LDL even in the presence of statin therapy (65,66). Mixed results have been obtained for HDL-cholesterol levels. Some groups have found a decrease in HDL-cholesterol post-ADT, while others have found no significant change (63–67).

No studies to date have looked at potential correlation between lipoprotein changes post-ADT and progression to CRPC. However, some studies have looked at the effect that lipoproteins have on androgen-dependent *versus* castration-resistant cancer cell growth. It was found that LDL, as well as remnant lipoprotein particles, which are the hydrolysis products of very low density lipoproteins and chylomicrons, induce proliferation of castration-resistant PC-3 cells, but not androgen-dependent LNCaP cells (68). Interestingly, LDL and HDL have been found to stimulate androgen production in steroidogenic tissues (69–72). Since CRPC cells have been found to be steroidogenic, it is possible that the lipoprotein-induced proliferation seen in the PC-3 cells and not the LNCaPs may be a result of lipoprotein-stimulated androgen production. Thus, the post-ADT changes may provide an environment for prostate cancer recurrence and growth. These findings provide further merit to research lipoproteins and their role in prostate cancer progression to CRPC.

Cellular Cholesterol Sources

Tumor cells can obtain cholesterol from *de novo* synthesis or from lipoproteins in the circulation (Fig. 3). Once in the cell, cholesterol is stored as cholesteryl esters in lipid droplets, but can be metabolized when the need for cholesterol arises. Cholesterol homeostasis is a very complex network of pathways, transporters and enzymes (45). As mentioned previously, cholesterol homeostasis enters into a state of dysregulation in cancer, demonstrated by the accumulation in tumors (47). Some recent discoveries by our group and many others have renewed interest in the study of cholesterol and its potential role in the progression to CRPC.

Cholesterol Synthesis

Cholesterol is synthesized from acetyl-CoA in the endoplasmic reticulum (ER) of virtually all cells of the body through the multi-step mevalonate pathway. The rate-limiting step in this process is 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR), which converts 3-hydroxy-3-methyl-glutaryl-Coenzyme A into mevalonate (45,73). Cholesterol synthesis is controlled at the transcriptional level by sterol regulatory element binding protein-2 (SREBP-2). SREBP-2 reacts to low cellular cholesterol levels and, thus, cellular need, by up-regulating expression of HMGCR as well as many other factors, including influx transporters, to increase cholesterol levels within the cell (45). Once the level of cholesterol is restored, the transcriptional factor is returned to the ER. This pathway also responds to androgens because their biomolecular structure is similar to their precursor, cholesterol (41).

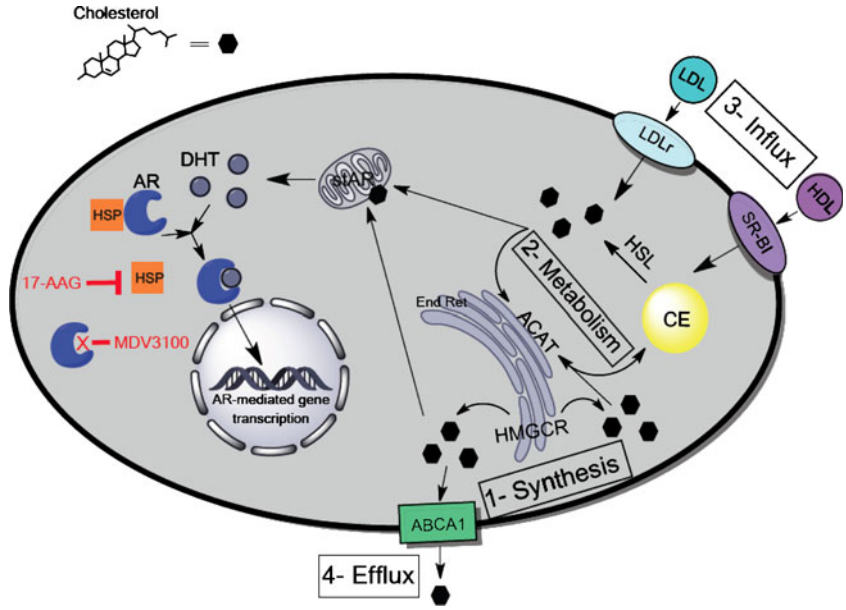

Fig. 3 Cholesterol 1-Synthesis, 2-Metabolism, 3-Influx and 4-Efflux. Cholesterol moves to mitchondria, where stAR initiates androgen synthesis by shuttling cholesterol into the intermitochondrial space. Androgens (DHT) bind to the AR and cause gene transcription. 17-AAG is a new therapy that inhibits AR stabilization by binding to the heat-shock protein, while MDV3100 is an AR inhibitor that blocks ligand binding and translocation of AR to the nucleus. Adapted from figure in (42).

Our group and others have found that HMGCR activity is increased in a CRPC cell-line (PC-3) when treated with a synthetic androgen, as well as in *ex vivo* tumor samples in the progression to the castration-resistant state in a LNCaP xenograft model (42,74). Many other groups have found increases in protein expression of HMGCR in the progression to CRPC (70,75–78); however, our group did not find a significant change in HMGCR protein expression between tumor stages in a LNCaP xenograft model (42). The transcriptional regulation of HMGCR via SREBP-2 in CRPC has been explored *in vitro* and in a LNCaP xenograft model. It was shown at the cellular level in PC-3 and DU145, both castration-resistant cell lines, that the presence of cholesterol no longer initiates down-regulation of this key transcriptional factor. Thus, the downstream effectors of SREBP-2, including HMGCR, are not down-regulated, and thus cholesterol levels remain high. However, a normal response to sterols was seen in androgen-dependent LNCaP cells and non-carcinogenic prostatic epithelium (70,75–78). These findings were mirrored in AD and CRPC cell lines and LNCaP xenograft tumor samples (41,79,80). These findings put forth an explanation for the increased levels of cholesterol in CRPC tissue specimens and brought to light a specific point of dysregulation in cholesterol homeostasis (41,50,81).

Metabolism of Intracellular Cholesterol

Cholesterol in its free form is toxic to the cell; thus, it is quickly converted to the nontoxic storage form, cholesteryl esters (CE), after synthesis or influx (45). This conversion from free cholesterol to CE is completed by acetyl coA:acyl tranferase (ACAT), which adds a fatty acid to the cholesterol (73) (Fig. 3). ACAT exists as two isoforms in humans: ACAT1 and ACAT2. Both are integral membrane proteins of approximately 50 kDa (82). In general, the ACAT enzymes are most active in times of cholesterol excess within cells (73). While ACAT1 is thought to be ubiquitously expressed in the body, ACAT2 is predominantly found in the brain and intestine (83,84). However, protein expression of both has been demonstrated in CaP (42,74).

When cholesterol levels are low or free cholesterol is required for cellular processes, hormone-sensitive lipase (HSL), a neutral cholesteryl ester hydrolase hydrolyzes CE to free cholesterol (85). As its name suggests, HSL is regulated in part by hormone levels, but appears to also be controlled by cholesterol feedback mechanisms similar to HMGCR (86). Furthermore, it has been found in murine steroidogenic tissues that HSL selectively hydrolyzes CE from HDL to create androgens, and this enzyme is present in the CRPC tumor cell (70,77,87).

Changes in the expression of ACAT1, ACAT2 and HSL have been demonstrated in CaP. Among the changes observed was a significant decrease in ACAT2 expression from AD to the CRPC state in a LNCaP xenograft model, as well as an increase in HSL from AD to N (42,87). These findings, paired with the fact that testosterone levels also increased from N to CRPC in the same model, indirectly imply that the alterations in cholesterol processes, such as decreased packaging of cholesterol to its storage form, are perhaps to provide substrate for *de novo* androgen synthesis (42).

Cholesterol Transport: Efflux and Influx

In addition to being synthesized and metabolized within the cell, cholesterol is fluxed in and out of the cell through the plasma membrane in a dynamic fashion (45). Since

cholesterol exists in the circulation within lipoproteins, it is transported to and from these lipoproteins at cell membranes (50). Lipoprotein profiles naturally change with age and obesity, two factors that have been correlated to CaP incidence, perhaps inferring provision of lipoprotein-derived cholesterol to cells also changes in CaP (51,57).

ATP-Binding Cassette Transporter Subfamily A1

As mentioned, when the cell has ample free cholesterol, the conversion to the storage form is activated. Additionally, efflux pathways are upregulated in order to rid the cell of the toxic molecule. The major cholesterol efflux transporters in cells are from the ATP-binding cassette transporter superfamily (ABCs). The major families involved in the efflux of cholesterol are the ABCAs and ABCGs, but most namely, ABCA1. This is a large, 220 kDa protein that is located on the cytoplasmic side of the plasma membrane (88). It is found in many tissues of the body because of its important cholesterol efflux function, including prostate cancer and, specifically, CRPC (42,89,90). Preliminary data suggest that ABCA1 protein expression is increased in castration-resistant tumor cells when compared to androgen-dependent counterparts, further implicating altered cholesterol regulation in CaP recurrence (42,90). Limited work has been put forth to determine whether or not this regulation exists in CRPC cells, thus we hope to take the next step in determining the role of cholesterol efflux via this pathway.

Low-Density Lipoprotein Receptor

Cholesterol in the circulation is predominantly found in lipoproteins and can be taken up by lipoprotein transporters into the cell. One of the major lipoprotein transporters involved in this cholesterol influx is the low-density lipoprotein (LDL) receptor. This transporter interacts with a number of different donor lipoproteins in order to take up cholesterol (91). The LDL receptor predominantly binds to apoB100 and apoE-containing lipoproteins, such as LDL, VLDL and chylomicrons (91). Lipoproteins bound to the LDL receptor are then taken into cells via an endosomal pathway. This involves uptake of the entire lipoprotein particle into clathrin-coated endosomal compartment where acidic catabolism occurs, and the lipid contents of the lipoprotein particles can then be used or metabolized by the cell into storage form (91). The receptors are recycled back to the cell surface in the endosome. The LDL receptor is controlled at the transcriptional level by SREBP-2, much like HMGCR and HSL. As mentioned previously, SREBP-2-mediated cholesterol regulation appears to be dysfunctional in CRPC. These findings have stimulated some research exploring the relationship between the SREBP-2 dysregulation and its implication for LDL receptor expression and activity. The few studies that have examined LDL receptor in CRPC have found that the transporter is present in CRPC and is upregulated, perhaps due to the lack of SREBP-2-mediated control (36,42,79,80).

Scavenger Receptor Class B Type I

The second major cholesterol influx transporter is scavenger receptor class B type I (SR-BI), or CLA-1 (CD36 and LIMPII analogous-1) as it is sometimes called in humans. This 82 kDa protein is a ubiquitous protein involved in reverse cholesterol transport (85,92), but it is more densely expressed in cells involved in cholesterol metabolism, such as the liver, and steroidogenic tissues (93). Morphologically, SR-BI has a horseshoe-like membrane topology with two transmembrane domains that anchor a large extracellular domain (93). It has become colloquially termed the 'HDL transporter' because it is one of the few receptors through which HDL-cholesterol can be taken into cells and due to its apparent preference for selective uptake of HDL-CE in steroidogenic and hepatic tissues of mice (85,93–97). However, there is some debate whether this holds true for human tissues, as rodent models predominantly rely on HDL rather than LDL, which is the most abundant lipoprotein in humans (98,99). Furthermore, research of the last decade has suggested that SR-BI is also capable of bidirectional free cholesterol flux, as well as uptake of cholesteryl esters, triglycerides and phospholipids from LDL, VLDL and modified lipoproteins (85,93,100,101).

The exact mechanism by which SR-BI takes in cholesterol from lipoproteins is not completely understood. However, the mechanism of selective HDL-CE uptake is thought to involve a collision-mediated transfer of only the CE content after the lipoprotein docks onto the receptor surface (95,102,103). The docking is thought to occur because of interaction with both the lipid and apoprotein of the lipoprotein (101,102,104). SR-BI is thought to be regulated in a feedback-type fashion by both androgens and cholesterol, although inversely, in that decreased androgen levels and increased cholesterol curtail the expression of SR-BI (85,105).

The presence of SR-BI in CRPC has been confirmed by protein expression in PC-3 cells and in the CRPC tumors in a LNCaP xenograft model by our group and others (42,90,106,107). Furthermore, our group found that protein expression of SR-BI was significantly increased from N to CRPC (42). Interestingly, it has also been discovered that males have significantly higher mRNA levels of *SCARB1*, the SR-BI gene (108–110). The natural predominance of *SCARB1* in males paired with the preliminary protein results our group has obtained in the LNCaP xenograft model provides more impetus to pursue an explanation for

the potential role of SR-BI in changing androgen and cholesterol levels in the progression to CRPC.

Inferences From Steroidogenic Tissues

As mentioned, many groups have demonstrated that *de novo* androgen synthesis is occurring intratumorally in CRPC cells, indicating that they have become steroidogenic. Our data indicate that cholesterol pathways are altered, and this is perhaps to facilitate provision of the substrate, free cholesterol, to this intratumoral steroidogenesis. Other steroidogenic tissues in the body, namely the adrenals, have been studied extensively and have demonstrated some interesting preferences when it comes to cholesterol sources for androgen synthesis. Since CRPC is steroidogenic, it may adapt the same behaviours as the other steroidogenic tissues of the body and may aid us in understanding altered cholesterol regulation in CRPC. Although it is thought that CRPC cells create androgens from a backdoor pathway that diverts reactants from the classical pathway and facilitates creation of DHT without the requirement of testosterone as a direct precursor, in addition to the major pathway used by other tissues, the common precursor, cholesterol, is constant (74) (Fig. 1).

Adrenal cells seem to preferentially rely on lipoprotein sources for cholesterol precursor, namely through SR-BI, rather than from *de novo* synthesis via HMGCR (70,77,104). This was demonstrated by the fact that LDLr knockout mice had unaffected steroid production, suggesting that either synthesis of cholesterol or a different lipoprotein transporter was compensating for the loss of LDLr-mediated cholesterol influx (77). Furthermore, SR-BI knockout mice have severely stunted steroidogenic capabilities (111). This decline in steroid production occurs despite a significant compensatory increase in HMGCR activity (104). This indicates that *de novo* synthesis of cholesterol is not able to compensate for the loss of cholesterol uptake via SR-BI. These studies suggest that steroidogenic tissues preferentially use HDL-CE from SR-BI-mediated influx for *de novo* androgen synthesis, and perhaps by inference, CRPC cells might have the same bias. This bias has also been demonstrated in breast cancer cells that are thought to possess a similar steroidogenic potential as their male counterpart, prostate cancer (112,113). Also, adrenal cells over-expressing SR-BI have demonstrated induction of steroidogenesis, and, therefore, SR-BI changes following castration may serve a similar function in CaP (114). Interestingly, the work completed to date by our group in CRPC complements the inferences made from steroidogenic tissues. Namely, SR-BI expression is significantly increased in progression to CRPC, paired with an unchanged HMGCR expression and an increased HMGCR activity (42). However, as mentioned, caution must be taken when attempting to translate murine findings to humans, particularly from a lipoprotein prospective. Thus, it is important in the future to complete more work in human models to determine if CRPC has, in fact, adapted a similar preference to other steroidogenic tissues for SR-BI-derived cholesterol for the creation of intratumoral *de novo* androgens and, ultimately, their survival and persistence in the absence of exogenous androgens.

FUTURE CONSIDERATIONS FOR THERAPY

It has become very apparent with the research of the last few decades that the aggressive, complex and heterogeneous nature of castration-resistant prostate cancer has made it a very challenging and, thus far, impossible disease to treat effectively. The focus in the last few decades in CRPC has been towards identifying potential new therapeutic targets, as the therapies currently implemented—namely chemotherapy—achieve limited and often short-lived success. Considering that average survival of men with CRPC is less than 2 years, the need for more successful therapies is evident (115). The acknowledgment that the androgen receptor and androgen-response axis is still an integral part of CRPC has resulted in the development of a number of new therapies that are focused on this relationship between androgens and the androgen receptor at different points in the pathway. Such therapies include abiraterone acetate, an orally bioavailable specific CYP17A inhibitor (Fig. 1), MDV3100, a small androgen receptor antagonist, heat-shock protein-90 targeting via 17-allylamino-17-demethoxygeldanamycin (17-AAG) (Fig. 3) and SRD5A inhibitors-finasteride and dutasteride (Fig. 1), all of which are under development in a clinical setting (116–120).

Abiraterone acetate acts in a similar manner to ketoconazole, in that it is a cytochrome P450 inhibitor. However, it has greater specificity for CYP17A1 and displays more potent inhibition thereof. Abiraterone inhibits its two key reactions in androgen biosynthesis via 17α hydroxylase and 17,20 lyase, preventing modification of pregnenolone and progesterone. The drug is currently in Phase III trials after Phase II trials elicited PSA decreases of more than 50% from baseline in 20% of chemotherapy-naïve patients and 36% of docetaxel-treated patients when treated with abiraterone acetate (121,122). Despite the success in lowering PSA, the side effects that have limited the utility of ketoconazole, primarily due to secondary mineralocorticoid excess, are still present in patients taking abiraterone acetate. However, administration of eplerenone, a mineralocorticoid inhibitor, eliminated a large portion of these side effects, including hypertension, hypokalemia and edema (120,121). MDV3100 is an orally

administered AR inhibitor that is more potent than its AR antagonist predecessors, flutamide and bicalutamide, which have low affinity for the AR. Like abiraterone acetate, MDV3100 is entering Phase III trials after 56% of chemotherapy-naïve patients experienced more than a 50% decrease in PSA levels (123). However, the initial success of this AR inhibitor is shadowed by the reversal of function of bicalutamide, from antagonist to agonist, seen in the presence of increased AR after long-term androgen withdrawal, such as in CRPC (124–127). This paradigm is thought to occur in part because these AR inhibitors bind to the ligand binding pocket of the receptor causing a subsequent change in the ligand binding domain. The change in the ligand binding domain induced by these anti-androgens, although effective at first, increases the rate of AR mutations, AR expression and cofactor expression thought to lead to the CRPC state and the paradoxical agonist effect of bicalutamide seen at this state (6,124,128,129). Although MDV3100 acts directly to block androgen binding and prevents translocation to the nucleus, it is possible that the same reversal of function may occur as seen with bicalutamide (123). Thus far, MDV3100 has not elicited significant side effects in subjects other than fatigue (123). 17-AAG is a benzoquinone ansamycin antibiotic that acts to inhibit the binding of heat shock protein-90 to the AR and subsequently disrupts AR stabilization. This drug is currently in Phase II trials and has shown limited reduction in PSA (119). Dutasteride, an inhibitor of SRD5A1 and 2 that convert testosterone to DHT, has been explored in Phase II trials in men with CRPC, but despite excellent safety in humans, it has limited effect (130,131). Finasteride, an inhibitor of SRD5A1, has been implemented in combination with the anti-androgen flutamide in Phase II trials with PSA decreases compared to flutamide alone, as well as fewer patients experiencing tumor progression with combined treatment (132).

Although numerous novel therapeutics are being tested in clinical trials, this small selection was mentioned to demonstrate the many potential points of intervention in the androgen receptor signaling pathway. The recent focus on cholesterol regulation upstream of this pathway may give further insight into the progression and transformation of cells into the castration-resistant phenotype and, thus, provide possible new targets for therapy. One such target area may be the sources of cholesterol to the cell, in particular, endogenous synthesis and exogenous influx from lipoproteins. These sources of cholesterol to tumor cells may be involved in provision of substrate for *de novo* steroidogenesis. Thus, it may be possible to implement novel therapies to cut off the supply of cholesterol for the generation of intratumoral androgens and, subsequently, may inhibit androgen-mediated growth and survival pathways.

Statins as Prostate Cancer Therapeutics

Statins are a large class of drugs that are used extensively in cardiovascular conditions because these drugs act to lower cholesterol levels by inhibiting HMGCR, which is, as mentioned, the rate-limiting enzyme in cholesterol synthesis. Not only do statins cause a decrease in total cholesterol, they also decrease LDL-cholesterol and provide a modest increase in HDL-cholesterol (133). Interestingly, this reduction in plasma cholesterol and change in lipoprotein profile is accompanied by a reduction in PSA in non-cancer patients, which may indicate implications for interruption in the normal steroidogenic processes in these men (58,134,135). These lipoprotein effects may be corrective for the changes induced post-ADT, namely increased LDL and decreased HDL; thus, statins could potentially be implemented to prevent progression to CRPC. Although no studies have looked specifically at statins in CRPC patients or the direct effects on tumor cholesterol, many studies have explored the potential of using statins as a preventative therapy. The results of epidemiologic studies assessing the association between statin use and incident prostate cancer risk have been mixed (134,136). These mixed findings may be due to the heterogeneity of study protocols or may be a result of a statin-induced SRD5A2 expression. As mentioned, this enzyme catalyzes the conversion of testosterone to the potent metabolite, DHT (137). More recent studies have explored the specific association between statin use and more advanced prostate cancer cases that have metastasized. These studies have found a reduction in the onset of the aggressive, late-stage disease state in statin users (58,134,138). The apparent benefit of statin use in the later stages of cancer provides support for the importance of cholesterol, but more research is necessary, particularly in a CRPC cohort. An obstacle encountered when attempting to make inferences about the specific intratumoral effects rather than indirect effects induced by systemic cholesterol-lowering is that none of the studies cited above have determined the amount of drug entering the tumor tissues themselves or the tumor-specific cholesterol-lowering effect, if any. Despite the increased permeability of tumor tissues, in order to deliver sufficient statin to tumors, the dose may have to be increased to an amount that elicits toxic levels in normal tissues. Drug delivery is an obstacle faced when designing and implementing any type of cancer therapeutic, particularly due to the requirement for systemic administration. Thus, if statins continue to be explored as a CaP therapeutic, particularly as a CRPC therapeutic, a different delivery system that will target, permeate and accumulate in tumor tissues, such as a nano-molecular formulation, should be considered (139).

In vitro studies bypass the obstacle of delivery. A few studies have looked at statin effectiveness, specifically in

tumor cells. Interestingly, the inhibition of HMGCR by statins has a greater effect in androgen-dependent LNCaP cells compared to castration-resistant PC-3 cells (80). Although both cell lines were responsive to changing levels of sterols, the LNCaP cells appeared to alter synthesis to a greater extent than the PC-3, demonstrating that cholesterol dysregulation may be playing a role in the effectiveness of statins. Furthermore, some *in vitro* research has shown that statins, namely lovastatin, simvastatin and atorvastatin, cause growth inhibition in LNCaP cells, as well as in a few CRPC cell lines, but to a lesser extent (140). However, in a recent study exposing castration-resistant cells to statins combined with a potent anti-inflammatory, significant cell death was observed (141). Because these results are preliminary, it cannot be ascertained whether the effects observed are as a result of decreased steroid synthesis, rather than reflecting anti-proliferative or autophagic effects (142). However, the different responses observed between statin-treated androgen-dependent and castration-resistant cells warrant further study to properly assess the effect of statins on intratumoral steroidogenesis and the utility of statins as a viable therapeutic option alone or in combination.

Lipoprotein Transporters as Potential Therapeutic Targets

The role of lipoprotein transporters in castration-resistant prostate cancer has yet to be elucidated, but it is apparent that cholesterol regulation is altered in CRPC. The finding that both the LDL receptor and SR-BI are upregulated in a castration-resistant state indicates that the cells may be taking in more cholesterol for cellular processes, including *de novo* androgen synthesis (42,80). If it is found that these cholesterol sources, along with synthesis via HMGCR, are necessary for survival of cells, targeted inhibition thereof could serve as a potential novel therapeutic target. A SR-BI inhibitor, block-lipid transport (BLT), has been synthesized and is being tested *in vitro* by a group at Harvard University (143). Thus far, the *in vitro* data has been promising, demonstrating an increased HDL binding to the transporter followed by significantly decreased dissociation rendering SR-BI inactive to further lipid flux (143). As was demonstrated in mice, removal of SR-BI function or substrate severely stunted steroid production despite a functional HMGCR (104,111). Implementation of SR-BI blockade or knockdown could potentially serve as an effective therapy alone or in combination with a tumor-targeted statin to remove sources of cholesterol to the cell and, thus, inhibit *de novo* androgen synthesis. One study has explored altering the functionality of the SR-BI in breast cancer. Breast cancer and prostate cancer are thought to be analogous diseases between the sexes due to the similarities in their hormone-dependent development, pathophysiology and treatment strategies (113). Akin to castration-resistant prostate cancer, recurrent breast cancer has the ability to synthesize intratumoral steroids, namely estradiol (113). The breast cancer cell-line, MCF-7 (144), was transfected with a plasmid that induced expression of a mutant SR-BI. The expression of the mutant receptor caused inhibition of cell proliferation (145). Because of the similarities between these diseases, the anti-proliferative effects in breast cancer cells will likely translate to prostate cancer cells. However, attempts to implement DNA-based therapies, such as plasmid transfection, have been limited by the risk of adverse effects on genomic DNA.

A method that would eliminate adverse effects on genomic DNA while simultaneously removing the function of SR-BI is gene silencing. Gene silencing or gene knockdown via RNA interference has recently been explored as a potential therapeutic modality in cancer. RNA interference (RNAi) exploits an existing intracellular process and cell machinery to silence the expression of a specific gene. Small segments of double-stranded RNA called small interfering RNA (siRNA) that have been chemically synthesized to be homologous to target gene messenger RNA sequences can be introduced to tumor cells. The siRNA is incorporated into an RNA-induced silencing complex (RISC) that exists within cells where it is unwound. Argonaute 2 protein then facilitates binding of the single-stranded anti-sense product to its complementary sequence on the target gene mRNA and subsequently cleaves the mRNA (146,147). This ultimately results in post-transcriptional disruption of target gene expression without interfering with genomic DNA.

RNAi is a new modality for cancer therapy and is not without its own obstacles, particularly targeting and delivery. siRNAs are negatively charged, hydrophilic molecules that cannot enter cells by passive diffusion, are extensively degraded by plasma enzymes and are rapidly excreted by the kidneys (148). As a result, the use of siRNA directed against SR-BI in castration-resistant tumor tissues must have a delivery medium that facilitates efficient targeting when systemically administered. Many promising delivery mediums have been developed and are being tested in prostate cancer models, including lipid-based, polymer-mediated and aptamer-targeted formulations. Lipid and polymeric formulations generally act by complexing cationic moieties, either lipid or polymeric, with the anionic siRNAs (147). Aptamer-targeted delivery methods involve conjugating a nucleic acid sequence that selectively binds to a particular target, namely those that are overexpressed or exclusive to a particular tissue type (147,149). Some of the most promising siRNA delivery methods that have been tested in prostate cancer xenograft models are cationic liposomes and prostate-specific membrane antigen

(PSMA)-targeted siRNA conjugates delivering siRNA targeted to a number of different genes (150–154). These methods have resulted in good transfection efficiency and tumor specificity, as well as low toxicity and immunogenicity in animal applications (150–154). PSMA is a membrane protein that is constantly recycled from the cell surface via endocytosis, making it a good target for siRNA delivery methods (147,149,155,156). In addition, PSMA is over-expressed in prostate cancer, particularly at castration resistance (157,158), which may further help to prevent any off-target responses elicited by siRNA. It is possible that implementing a targeted siRNA delivery modality towards prostate-specific surface receptors and markers, such as PSMA, may facilitate SR-BI gene silencing within tumor cells, thus eliminating a source of cholesterol for the cells and inhibiting AR activation by reducing synthesis of its ligands.

Additionally, it may be possible to alter transporter expression and function via targeting their regulatory pathways. Although cholesterol regulation is altered in CRPC, it has been shown that androgen-dependent LNCaP cells respond to intervention at the level of liver X receptor alpha (LXRα), a transcriptional regulator. The implementation of an LXRα agonist induced apoptosis in LNCaP cells and xenografts, simultaneously with decreased tumoral cholesterol (159). This effect was associated with an increased efflux transporter expression and subsequent apoptosis of cells, but influx transporters were not explored. Interestingly, cell survival was maintained when cholesterol was replenished, suggesting that the cell death observed was due specifically to lack of cholesterol. Since LXRα is responsible for regulation of many molecules (45), it may not be a viable therapeutic option, but it reinforces the validity of targeting cholesterol pathways in the cell.

One group has already tested, *in vitro*, the application of delivering drug in reconstituted HDL particles. Paclitaxel, a chemotherapeutic agent in the taxane family, was introduced into the neutral core of HDL particles and was traced to its point of delivery, SR-BI, in the castration-resistant cell-line, PC-3 (107). Furthermore, the same group found that drug delivered within HDL had a half maximal inhibitory concentration (IC50) that was almost 20 times lower than that of the free drug in the same cell-line (107). In addition, over-expression of SR-BI in lung cancer cells facilitated efficient delivery of α-tocopheryl succinate, a drug used to inhibit tumor cell growth in lung carcinomas. The drug was delivered to cells within HDL particles and, in the presence of cells over-expressing SR-BI, caused a significant reduction in tumor burden (160). Thus, if blockade or silencing of this cholesterol influx transporter is found, it might be possible to exploit its presence, as can be inferred from these studies, for lipoprotein-mediated drug delivery. In any drug targeting strategy there are inherent challenges, especially when implementing delivery or blockade using targets that are systemic. Thus, the need for more knowledge about cholesterol influx, efflux, metabolism and synthesis in CRPC is critical.

CONCLUSION

The emergent evidence of the last few years has implicated androgens and the androgen signaling pathway as important players in the development, progression and continued survival of castration-resistant prostate cancer. It has also become apparent that *de novo* androgen synthesis is occurring intratumorally, as many steroidogenic enzymes in both the classical and backdoor pathways are upregulated. Furthermore, proteins involved in cholesterol homeostasis are altered in a manner that appears to be generating free cholesterol that may be used to provide precursor to this steroidogenic pathway. A greater understanding of cholesterol metabolism, transport and synthesis within the CRPC tumor environment may not only help elucidate the major source of precursor to *de novo* androgen synthesis, but might also serve as a valuable potential therapeutic pathway in an insofar untreatable disease.

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009;59:225–49.
2. Prostate Cancer Statistics, Canadian Cancer Society. http://www.cancer.ca/canada-wide/about%20cancer/cancer%20statistics/stats%20at%20a%20glance/prostate%20cancer.aspx.
3. Marrett LD, De P, Airia P, Dryer D. Cancer in Canada in 2008. CMAJ. 2008;179:1163–70.
4. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001;1:34–45.
5. Goldenberg SL, Gleave ME, Taylor D, Bruchovsky N. Clinical experience with intermittent androgen suppression in prostate cancer: minimum of 3 years' follow-up. Mol Urol. 1999;3:287–92.
6. Schroder FH. Progress in understanding androgen-independent prostate cancer (AIPC): a review of potential endocrine-mediated mechanisms. Eur Urol. 2008;53:1129–37.
7. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, *et al.* Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res. 2008;68:4447–54.
8. Mostaghel EA, Nelson PS. Intracrine androgen metabolism in prostate cancer progression: mechanisms of castration resistance and therapeutic implications. Best Pract Res Clin Endocrinol Metab. 2008;22:243–58.
9. Srinivasan G, Campbell E, Bashirelahi N. Androgen, estrogen, and progesterone receptors in normal and aging prostates. Microsc Res Tech. 1995;30:293–304.
10. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941. J Urol. 2002;167:948–51. discussion 952.
11. Locke JA, Nelson CC, Adomat HH, Hendy SC, Gleave ME, Guns ES. Steroidogenesis inhibitors alter but do not eliminate

androgen synthesis mechanisms during progression to castration-resistance in LNCaP prostate xenografts. J Steroid Biochem Mol Biol. 2009;115:126–36.
12. Bartsch W, Klein H, Schiemann U, Bauer HW, Voigt KD. Enzymes of androgen formation and degradation in the human prostate. Ann NY Acad Sci. 1990;595:53–66.
13. Hales DB, Allen JA, Shankara T, Janus P, Buck S, Diemer T, *et al.* Mitochondrial function in Leydig cell steroidogenesis. Ann NY Acad Sci. 2005;1061:120–34.
14. Miller WL. Steroidogenic acute regulatory protein (StAR), a novel mitochondrial cholesterol transporter. Biochim Biophys Acta. 2007;1771:663–76.
15. Manna PR, Dyson MT, Stocco DM. Regulation of the steroidogenic acute regulatory protein gene expression: present and future perspectives. Mol Hum Reprod. 2009;15:321–33.
16. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev. 2004;25:947–70.
17. Miller WL. Molecular biology of steroid hormone synthesis. Endocr Rev. 1988;9:295–318.
18. Edwards J, Bartlett JM. The androgen receptor and signal-transduction pathways in hormone-refractory prostate cancer. Part 2: androgen-receptor cofactors and bypass pathways. BJU Int. 2005;95:1327–35.
19. Wright AS, Douglas RC, Thomas LN, Lazier CB, Rittmaster RS. Androgen-induced regrowth in the castrated rat ventral prostate: role of 5alpha-reductase. Endocrinology. 1999;140:4509–15.
20. Rennie PS, Bruchovsky N, Goldenberg SL. Relationship of androgen receptors to the growth and regression of the prostate. Am J Clin Oncol. 1988;11 Suppl 2:S13–7.
21. Kuriyama M. Prostate-specific antigen in prostate cancer. Int J Biol Markers. 1986;1:67–76.
22. Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, *et al.* Androgen levels increase by intratumoral *de novo* steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res. 2008;68:6407–15.
23. Wako K, Kawasaki T, Yamana K, Suzuki K, Jiang S, Umezu H, *et al.* Expression of androgen receptor through androgen-converting enzymes is associated with biological aggressiveness in prostate cancer. J Clin Pathol. 2008;61:448–54.
24. Ryan CJ, Smith A, Lal P, Satagopan J, Reuter V, Scardino P, *et al.* Persistent prostate-specific antigen expression after neoadjuvant androgen depletion: an early predictor of relapse or incomplete androgen suppression. Urology. 2006;68:834–9.
25. Shiraishi S, Lee PW, Leung A, Goh VH, Swerdloff RS, Wang C. Simultaneous measurement of serum testosterone and dihydrotestosterone by liquid chromatography-tandem mass spectrometry. Clin Chem. 2008;54:1855–63.
26. So A, Gleave M, Hurtado-Col A, Nelson C. Mechanisms of the development of androgen independence in prostate cancer. World J Urol. 2005;23:1–9.
27. Gregory CW, Johnson Jr RT, Mohler JL, French FS, Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res. 2001;61:2892–8.
28. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, *et al.* Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004;10:33–9.
29. van Weerden WM, van Steenbrugge GJ, van Kreuningen A, Moerings EP, De Jong FH, Schroder FH. Effects of low testosterone levels and of adrenal androgens on growth of prostate tumor models in nude mice. J Steroid Biochem Mol Biol. 1990;37:903–7.
30. Mohler JL, Gregory CW, Ford 3rd OH, Kim D, Weaver CM, Petrusz P, *et al.* The androgen axis in recurrent prostate cancer. Clin Cancer Res. 2004;10:440–8.
31. Snoek R, Cheng H, Margiotti K, Wafa LA, Wong CA, Wong EC, *et al. In vivo* knockdown of the androgen receptor results in growth inhibition and regression of well-established, castration-resistant prostate tumors. Clin Cancer Res. 2009;15:39–47.
32. Hofland J, van Weerden WM, Dits NF, Steenbergen J, van Leenders GJ, Jenster G, *et al.* Evidence of limited contributions for intratumoral steroidogenesis in prostate cancer. Cancer Res. 2010;70:1256–64.
33. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, *et al.* Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 2006;66:2815–25.
34. Seftel AD, Spirnak JP, Resnick MI. Hormonal therapy for advanced prostatic carcinoma. J Surg Oncol Suppl. 1989;1:14–20.
35. Bhanalaph T, Varkarakis MJ, Murphy GP. Current status of bilateral adrenalectomy or advanced prostatic carcinoma. Ann Surg. 1974;179:17–23.
36. Waltering KK, Helenius MA, Sahu B, Manni V, Linja MJ, Janne OA, *et al.* Increased expression of androgen receptor sensitizes prostate cancer cells to low levels of androgens. Cancer Res. 2009;69:8141–9.
37. Wu HC, Hsieh JT, Gleave ME, Brown NM, Pathak S, Chung LW. Derivation of androgen-independent human LNCaP prostatic cancer cell sublines: role of bone stromal cells. Int J Cancer. 1994;57:406–12.
38. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol. 1979;17:16–23.
39. Mickey DD, Stone KR, Wunderli H, Mickey GH, Vollmer RT, Paulson DF. Heterotransplantation of a human prostatic adenocarcinoma cell line in nude mice. Cancer Res. 1977;37:4049–58.
40. Sato N, Gleave ME, Bruchovsky N, Rennie PS, Beraldi E, Sullivan LD. A metastatic and androgen-sensitive human prostate cancer model using intraprostatic inoculation of LNCaP cells in SCID mice. Cancer Res. 1997;57:1584–9.
41. Ettinger SL, Sobel R, Whitmore TG, Akbari M, Bradley DR, Gleave ME, *et al.* Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence. Cancer Res. 2004;64:2212–21.
42. Leon CG, Locke JA, Adomat HH, Etinger SL, Twiddy AL, Neumann RD, *et al.* Alterations in cholesterol regulation contribute to the production of intratumoral androgens during progression to castration-resistant prostate cancer in a mouse xenograft model. Prostate. 2010;70:390–400.
43. Connolly JM, Coleman M, Rose DP. Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice. Nutr Cancer. 1997;29:114–9.
44. Wang Y, Corr JG, Thaler HT, Tao Y, Fair WR, Heston WD. Decreased growth of established human prostate LNCaP tumors in nude mice fed a low-fat diet. J Natl Cancer Inst. 1995;87:1456–62.
45. Ikonen E. Cellular cholesterol trafficking and compartmentalization. Nat Rev Mol Cell Biol. 2008;9:125–38.
46. Hager MH, Solomon KR, Freeman MR. The role of cholesterol in prostate cancer. Curr Opin Clin Nutr Metab Care. 2006;9:379–85.
47. Swyer G. The cholesterol content of normal and enlarged prostates. Cancer Res. 1942;2:372–5.
48. Di Vizio D, Solomon KR, Freeman MR. Cholesterol and cholesterol-rich membranes in prostate cancer: an update. Tumori. 2008;94:633–9.
49. Freeman MR, Solomon KR. Cholesterol and prostate cancer. J Cell Biochem. 2004;91:54–69.
50. Jonas A. In: Vance DE, Vance JE, editors. Biochemistry of lipids, lipoproteins and membranes. 4th ed. Elsevier; 2002. pp. 484–490.

51. Grosman H, Fabre B, Mesch V, Lopez MA, Schreier L, Mazza O, *et al.* Lipoproteins, sex hormones and inflammatory markers in association with prostate cancer. Aging Male. 2009;13:87–92.
52. Laukkanen JA, Laaksonen DE, Niskanen L, Pukkala E, Hakkarainen A, Salonen JT. Metabolic syndrome and the risk of prostate cancer in Finnish men: a population-based study. Cancer Epidemiol Biomark Prev. 2004;13:1646–50.
53. Hsing AW, Gao YT, Chua Jr S, Deng J, Stanczyk FZ. Insulin resistance and prostate cancer risk. J Natl Cancer Inst. 2003;95:67–71.
54. Hsing AW, Devesa SS. Trends and patterns of prostate cancer: what do they suggest? Epidemiol Rev. 2001;23:3–13.
55. Kolonel LN, Yoshizawa CN, Hankin JH. Diet and prostatic cancer: a case-control study in Hawaii. Am J Epidemiol. 1988;127:999–1012.
56. Mettlin C, Selenskas S, Natarajan N, Huben R. Beta-carotene and animal fats and their relationship to prostate cancer risk. A case-control study. Cancer. 1989;64:605–12.
57. Gronberg H. Prostate cancer epidemiology. Lancet. 2003;361:859–64.
58. Mondul AM, Clipp SL, Helzlsouer KJ, Platz EA. Association between plasma total cholesterol concentration and incident prostate cancer in the CLUE II cohort. Cancer Causes Control. 2009;21:61–8.
59. Williams RR, Sorlie PD, Feinleib M, McNamara PM, Kannel WB, Dawber TR. Cancer incidence by levels of cholesterol. JAMA. 1981;245:247–52.
60. Chyou PH, Nomura AM, Stemmermann GN, Kato I. Prospective study of serum cholesterol and site-specific cancers. J Clin Epidemiol. 1992;45:287–92.
61. Hiatt RA, Fireman BH. Serum cholesterol and the incidence of cancer in a large cohort. J Chronic Dis. 1986;39:861–70.
62. Fiorenza AM, Branchi A, Cardena A, Molgora M, Rovellini A, Sommariva D. Serum cholesterol levels in patients with cancer. Relationship with nutritional status. Int J Clin Lab Res. 1996;26:37–42.
63. Derby CA, Zilber S, Brambilla D, Morales KH, McKinlay JB. Body mass index, waist circumference and waist to hip ratio and change in sex steroid hormones: the Massachusetts Male Ageing Study. Clin Endocrinol (Oxf). 2006;65:125–31.
64. Traish AM, Abdou R, Kypreos KE. Androgen deficiency and atherosclerosis: the lipid link. Vasc Pharmacol. 2009;51:303–13.
65. Yannucci J, Manola J, Garnick MB, Bhat G, Bubley GJ. The effect of androgen deprivation therapy on fasting serum lipid and glucose parameters. J Urol. 2006;176:520–5.
66. Usui S, Suzuki K, Yamanaka H, Nakano T, Nakajima K, Hara Y, *et al.* Estrogen treatment of prostate cancer increases triglycerides in lipoproteins as demonstrated by HPLC and immunoseparation techniques. Clin Chim Acta. 2002;317:133–43.
67. Ahn J, Lim U, Weinstein SJ, Schatzkin A, Hayes RB, Virtamo J, *et al.* Prediagnostic total and high-density lipoprotein cholesterol and risk of cancer. Cancer Epidemiol Biomark Prev. 2009;18:2814–21.
68. Sekine Y, Koike H, Nakano T, Nakajima K, Takahashi S, Suzuki K. Remnant lipoproteins induced proliferation of human prostate cancer cell, PC-3 but not LNCaP, via low density lipoprotein receptor. Cancer Epidemiol. 2009;33:16–23.
69. Parinaud J, Perret B, Ribbes H, Chap H, Pontonnier G, Douste-Blazy L. High density lipoprotein and low density lipoprotein utilization by human granulosa cells for progesterone synthesis in serum-free culture: respective contributions of free and esterified cholesterol. J Clin Endocrinol Metab. 1987;64:409–17.
70. Azhar S, Reaven E. Scavenger receptor class BI and selective cholesteryl ester uptake: partners in the regulation of steroidogenesis. Mol Cell Endocrinol. 2002;195:1–26.
71. Enk L, Crona N, Hillensjo T. High- and low-density lipoproteins stimulate progesterone production in cultured human granulosa cells. Hum Reprod. 1987;2:291–5.
72. Richardson MC, Davies DW, Watson RH, Dunsford ML, Inman CB, Masson GM. Cultured human granulosa cells as a model for corpus luteum function: relative roles of gonadotrophin and low density lipoprotein studied under defined culture conditions. Hum Reprod. 1992;7:12–8.
73. Chang TY, Chang CC, Ohgami N, Yamauchi Y. Cholesterol sensing, trafficking, and esterification. Annu Rev Cell Dev Biol. 2006;22:129–57.
74. Locke JA, Wasan KM, Nelson CC, Guns ES, Leon CG. Androgen-mediated cholesterol metabolism in LNCaP and PC-3 cell lines is regulated through two different isoforms of acyl-coenzyme A:Cholesterol Acyltransferase (ACAT). Prostate. 2008;68:20–33.
75. Holzbeierlein J, Lal P, LaTulippe E, Smith A, Satagopan J, Zhang L, *et al.* Gene expression analysis of human prostate carcinoma during hormonal therapy identifies androgen-responsive genes and mechanisms of therapy resistance. Am J Pathol. 2004;164:217–27.
76. Swinnen JV, Heemers H, van de Sande T, de Schrijver E, Brusselmans K, Heyns W, *et al.* Androgens, lipogenesis and prostate cancer. J Steroid Biochem Mol Biol. 2004;92:273–9.
77. Kraemer FB, Shen WJ, Patel S, Osuga J, Ishibashi S, Azhar S. The LDL receptor is not necessary for acute adrenal steroidogenesis in mouse adrenocortical cells. Am J Physiol Endocrinol Metab. 2007;292:E408–12.
78. Lee MY, Moon JS, Park SW, Koh YK, Ahn YH, Kim KS. KLF5 enhances SREBP-1 action in androgen-dependent induction of fatty acid synthase in prostate cancer cells. Biochem J. 2009;417:313–22.
79. Chen Y, Hughes-Fulford M. Human prostate cancer cells lack feedback regulation of low-density lipoprotein receptor and its regulator, SREBP2. Int J Cancer. 2001;91:41–5.
80. Krycer JR, Kristiana I, Brown AJ. Cholesterol homeostasis in two commonly used human prostate cancer cell-lines, LNCaP and PC-3. PLoS ONE. 2009;4:e8496.
81. Solomon KR, Freeman MR. Do the cholesterol-lowering properties of statins affect cancer risk? Trends Endocrinol Metab. 2008;19:113–21.
82. Chang TY, Li BL, Chang CC, Urano Y. Acyl-coenzyme A: cholesterol acyltransferases. Am J Physiol Endocrinol Metab. 2009;297:E1–9.
83. Accad M, Smith SJ, Newland DL, Sanan DA, King Jr LE, Linton MF, *et al.* Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. J Clin Invest. 2000;105:711–9.
84. Repa JJ, Buhman KK, Farese Jr RV, Dietschy JM, Turley SD. ACAT2 deficiency limits cholesterol absorption in the cholesterol-fed mouse: impact on hepatic cholesterol homeostasis. Hepatology. 2004;40:1088–97.
85. Connelly MA. SR-BI-mediated HDL cholesteryl ester delivery in the adrenal gland. Mol Cell Endocrinol. 2009;300:83–8.
86. Kraemer FB, Shen WJ, Harada K, Patel S, Osuga J, Ishibashi S, *et al.* Hormone-sensitive lipase is required for high-density lipoprotein cholesteryl ester-supported adrenal steroidogenesis. Mol Endocrinol. 2004;18:549–57.
87. Locke JA, Guns ES, Lehman ML, Ettinger S, Zoubeidi A, Lubik A, *et al.* Arachidonic acid activation of intratumoral steroid synthesis during prostate cancer progression to castration resistance. Prostate. 2009;70:239–51.
88. Klappe K, Hummel I, Hoekstra D, Kok JW. Lipid dependence of ABC transporter localization and function. Chem Phys Lipids. 2009;161:57–64.

89. Fukuchi J, Hiipakka RA, Kokontis JM, Hsu S, Ko AL, Fitzgerald ML, *et al.* Androgenic suppression of ATP-binding cassette transporter A1 expression in LNCaP human prostate cancer cells. Cancer Res. 2004;64:7682–5.
90. Ni J, Pang ST, Yeh S. Differential retention of alpha-vitamin E is correlated with its transporter gene expression and growth inhibition efficacy in prostate cancer cells. Prostate. 2007;67:463–71.
91. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science. 1986;232:34–47.
92. Calvo D, Vega MA. Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. J Biol Chem. 1993;268:18929–35.
93. Rhainds D, Brissette L. The role of scavenger receptor class B type I (SR-BI) in lipid trafficking. defining the rules for lipid traders. Int J Biochem Cell Biol. 2004;36:39–77.
94. Saddar S, Mineo C, Shaul PW. Signaling by the high-affinity HDL receptor scavenger receptor B type I. Arterioscler Thromb Vasc Biol. 2010;30:144–50.
95. Krieger M. Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. Annu Rev Biochem. 1999;68:523–58.
96. Reaven E, Lua Y, Nomoto A, Temel R, Williams DL, van der Westhuyzen DR, *et al.* The selective pathway and a high-density lipoprotein receptor (SR-BI) in ovarian granulosa cells of the mouse. Biochim Biophys Acta. 1999;1436:565–76.
97. Zimetti F, Weibel GK, Duong M, Rothblat GH. Measurement of cholesterol bidirectional flux between cells and lipoproteins. J Lipid Res. 2006;47:605–13.
98. Dietschy JM, Turley SD. Control of cholesterol turnover in the mouse. J Biol Chem. 2002;277:3801–4.
99. Galman C, Angelin B, Rudling M. Prolonged stimulation of the adrenals by corticotropin suppresses hepatic low-density lipoprotein and high-density lipoprotein receptors and increases plasma cholesterol. Endocrinology. 2002;143:1809–16.
100. Connelly MA, Williams DL. SR-BI and HDL cholesteryl ester metabolism. Endocr Res. 2004;30:697–703.
101. Trigatti BL, Krieger M, Rigotti A. Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol. 2003;23:1732–8.
102. Rhainds D, Bourgeois P, Bourret G, Huard K, Falstrault L, Brissette L. Localization and regulation of SR-BI in membrane rafts of HepG2 cells. J Cell Sci. 2004;117:3095–105.
103. Gu X, Kozarsky K, Krieger M. Scavenger receptor class B, type I-mediated [3H]cholesterol efflux to high and low density lipoproteins is dependent on lipoprotein binding to the receptor. J Biol Chem. 2000;275:29993–30001.
104. Plump AS, Erickson SK, Weng W, Partin JS, Breslow JL, Williams DL. Apolipoprotein A-I is required for cholesteryl ester accumulation in steroidogenic cells and for normal adrenal steroid production. J Clin Invest. 1996;97:2660–71.
105. Hersberger M, von Eckardstein A. Low high-density lipoprotein cholesterol: physiological background, clinical importance and drug treatment. Drugs. 2003;63:1907–45.
106. McConathy WJ, Nair MP, Paranjape S, Mooberry L, Lacko AG. Evaluation of synthetic/reconstituted high-density lipoproteins as delivery vehicles for paclitaxel. Anticancer Drugs. 2008;19:183–8.
107. Mooberry LK, Nair M, Paranjape S, McConathy WJ, Lacko AG. Receptor mediated uptake of paclitaxel from a synthetic high density lipoprotein nanocarrier. J Drug Target. 2009;18:53–8.
108. Lopez D, McLean MP. Estrogen regulation of the scavenger receptor class B gene: Anti-atherogenic or steroidogenic, is there a priority? Mol Cell Endocrinol. 2006;247:22–33.
109. Chiba-Falek O, Nichols M, Suchindran S, Guyton J, Ginsburg GS, Barrett-Connor E, *et al.* Impact of gene variants on sex-specific regulation of human Scavenger receptor class B type 1 (SR-BI) expression in liver and association with lipid levels in a population-based study. BMC Med Genet. 2010;11:9.
110. Bush TL, Fried LP, Barrett-Connor E. Cholesterol, lipoproteins, and coronary heart disease in women. Clin Chem. 1988;34:B60–70.
111. Hoekstra M, Ye D, Hildebrand RB, Zhao Y, Lammers B, Stitzinger M, *et al.* Scavenger receptor class B type I-mediated uptake of serum cholesterol is essential for optimal adrenal glucocorticoid production. J Lipid Res. 2009;50:1039–46.
112. Pussinen PJ, Karten B, Wintersperger A, Reicher H, McLean M, Malle E, *et al.* The human breast carcinoma cell line HBL-100 acquires exogenous cholesterol from high-density lipoprotein via CLA-1 (CD-36 and LIMPII analogous 1)-mediated selective cholesteryl ester uptake. Biochem J. 2000;349:559–66.
113. Risbridger GP, Davis ID, Birrell SN, Tilley WD. Breast and prostate cancer: more similar than different. Nat Rev Cancer. 2010;10:205–12.
114. Reaven E, Nomoto A, Cortez Y, Azhar S. Consequences of over-expression of rat Scavenger Receptor, SR-BI, in an adrenal cell model. Nutr Metab (Lond). 2006;3:43.
115. Cho D, Di Blasio CJ, Rhee AC, Kattan MW. Prognostic factors for survival in patients with hormone-refractory prostate cancer (HRPC) after initial androgen deprivation therapy (ADT). Urol Oncol. 2003;21:282–91.
116. Lattouf JB, Srinivasan R, Pinto PA, Linehan WM, Neckers L. Mechanisms of disease: the role of heat-shock protein 90 in genitourinary malignancy. Nat Clin Pract Urol. 2006;3:590–601.
117. Mostaghel EA, Geng L, Holcomb I, Coleman IM, Lucas J, True LD, *et al.* Variability in the androgen response of prostate epithelium to 5alpha-reductase inhibition: implications for prostate cancer chemoprevention. Cancer Res. 2010;70:1286–95.
118. Taichman RS, Loberg RD, Mehra R, Pienta KJ. The evolving biology and treatment of prostate cancer. J Clin Invest. 2007;117:2351–61.
119. Heath EI, Hillman DW, Vaishampayan U, Sheng S, Sarkar F, Harper F, *et al.* A phase II trial of 17-allylamino-17-demethoxygeldanamycin in patients with hormone-refractory metastatic prostate cancer. Clin Cancer Res. 2008;14:7940–6.
120. Attard G, Reid AH, Yap TA, Raynaud F, Dowsett M, Settatree S, *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;26:4563–71.
121. Reid AH, Attard G, Danila DC, Oommen NB, Olmos D, Fong PC, *et al.* Significant and sustained antitumor activity in post-docetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abiraterone acetate. J Clin Oncol. 2010;28(9):1489–95.
122. Danila DC, Morris MJ, de Bono JS, Ryan CJ, Denmeade SR, Smith MR, *et al.* Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. J Clin Oncol. 2010;28(9):1496–501.
123. Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, *et al.* Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1–2 study. Lancet. 2010;375:1437–46.
124. Culig Z, Comuzzi B, Steiner H, Bartsch G, Hobisch A. Expression and function of androgen receptor coactivators in prostate cancer. J Steroid Biochem Mol Biol. 2004;92:265–71.
125. Culig Z, Hoffmann J, Erdel M, Eder IE, Hobisch A, Hittmair A, *et al.* Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. Br J Cancer. 1999;81:242–51.
126. Culig Z, Klocker H, Bartsch G, Hobisch A. Androgen receptor mutations in carcinoma of the prostate: significance for endocrine therapy. Am J Pharmacogenomics. 2001;1:241–9.

127. Hobisch A, Hoffmann J, Lambrinidis L, Eder IE, Bartsch G, Klocker H, *et al.* Antagonist/agonist balance of the nonsteroidal antiandrogen bicalutamide (Casodex) in a new prostate cancer model. Urol Int. 2000;65:73–9.
128. Taplin ME, Bubley GJ, Ko YJ, Small EJ, Upton M, Rajeshkumar B, *et al.* Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res. 1999;59:2511–5.
129. Taplin ME, Rajeshkumar B, Halabi S, Werner CP, Woda BA, Picus J, *et al.* Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol. 2003;21:2673–8.
130. Shah SK, Trump DL, Sartor O, Tan W, Wilding GE, Mohler JL. Phase II study of Dutasteride for recurrent prostate cancer during androgen deprivation therapy. J Urol. 2009;181:621–6.
131. Vis AN, Schroder FH. Key targets of hormonal treatment of prostate cancer. Part 2: the androgen receptor and 5alpha-reductase. BJU Int. 2009;104:1191–7.
132. Banez LL, Blake GW, McLeod DG, Crawford ED, Moul JW. Combined low-dose flutamide plus finasteride *vs.* low-dose flutamide monotherapy for recurrent prostate cancer: a comparative analysis of two phase II trials with a long-term follow-up. BJU Int. 2009;104:310–4.
133. Gotto Jr AM. Management of dyslipidemia. Am J Med. 2002;112(Suppl 8A):10S–8.
134. Murtola TJ, Visakorpi T, Lahtela J, Syvala H, Tammela T. Statins and prostate cancer prevention: where we are now, and future directions. Nat Clin Pract Urol. 2008;5:376–87.
135. Mener DJ, Cambio A, Stoddard DG, Martin BA, Palapattu GS. The impact of HMG-CoA reductase therapy on serum PSA. Prostate. 2009;70:608–15.
136. Graaf MR, Beiderbeck AB, Egberts AC, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. J Clin Oncol. 2004;22:2388–94.
137. Seo YK, Zhu B, Jeon TI, Osborne TF. Regulation of steroid 5-alpha reductase type 2 (Srd5a2) by sterol regulatory element binding proteins and statin. Exp Cell Res. 2009;315:3133–9.
138. Platz EA, Till C, Goodman PJ, Parnes HL, Figg WD, Albanes D, *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 Biomark Prev. 2009;18:2807–13.
139. Torchilin VP. Passive and active drug targeting: drug delivery to tumors as an example. Handb Exp Pharmacol. 2010;197:3–53.
140. Hoque A, Chen H, Xu XC. Statin induces apoptosis and cell growth arrest in prostate cancer cells. Cancer Epidemiol Biomark Prev. 2008;17:88–94.
141. Zheng X, Cui XX, Avila GE, Huang MT, Liu Y, Patel J, *et al.* Atorvastatin and celecoxib inhibit prostate PC-3 tumors in immunodeficient mice. Clin Cancer Res. 2007;13:5480–7.
142. Parikh A, Childress C, Deitrick K, Lin Q, Rukstalis D, Yang W. Statin-induced autophagy by inhibition of geranylgeranyl biosynthesis in prostate cancer PC3 cells. Prostate. 2010;70(9):971–81.
143. Nieland TJ, Penman M, Dori L, Krieger M, Kirchhausen T. Discovery of chemical inhibitors of the selective transfer of lipids mediated by the HDL receptor SR-BI. Proc Natl Acad Sci USA. 2002;99:15422–7.
144. Soule HD, Vazguez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst. 1973;51:1409–16.
145. Cao WM, Murao K, Imachi H, Yu X, Abe H, Yamauchi A, *et al.* A mutant high-density lipoprotein receptor inhibits proliferation of human breast cancer cells. Cancer Res. 2004;64:1515–21.
146. Hannon GJ. RNA interference. Nature. 2002;418:244–51.
147. Oh YK, Park TG. siRNA delivery systems for cancer treatment. Adv Drug Deliv Rev. 2009;61:850–62.
148. Bumcrot D, Manoharan M, Koteliansky V, Sah DW. RNAi therapeutics: a potential new class of pharmaceutical drugs. Nat Chem Biol. 2006;2:711–9.
149. Zhou J, Rossi JJ. Aptamer-targeted cell-specific RNA interference. Silence. 2010;1(1):4.
150. Kim HR, Kim IK, Bae KH, Lee SH, Lee Y, Park TG. Cationic solid lipid nanoparticles reconstituted from low density lipoprotein components for delivery of siRNA. Mol Pharm. 2008;5:622–31.
151. Aleku M, Schulz P, Keil O, Santel A, Schaeper U, Dieckhoff B, *et al.* Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression. Cancer Res. 2008;68:9788–98.
152. Bisanz K, Yu J, Edlund M, Spohn B, Hung MC, Chung LW, *et al.* Targeting ECM-integrin interaction with liposome-encapsulated small interfering RNAs inhibits the growth of human prostate cancer in a bone xenograft imaging model. Mol Ther. 2005;12:634–43.
153. Pal A, Ahmad A, Khan S, Sakabe I, Zhang C, Kasid UN, *et al.* Systemic delivery of RafsiRNA using cationic cardiolipin liposomes silences Raf-1 expression and inhibits tumor growth in xenograft model of human prostate cancer. Int J Oncol. 2005;26:1087–91.
154. Thomas M, Kularatne SA, Qi L, Kleindl P, Leamon CP, Hansen MJ, *et al.* Ligand-targeted delivery of small interfering RNAs to malignant cells and tissues. Ann NY Acad Sci. 2009;1175:32–9.
155. Chu TC, Twu KY, Ellington AD, Levy M. Aptamer mediated siRNA delivery. Nucleic Acids Res. 2006;34:e73.
156. Dassie JP, Liu XY, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, *et al.* Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. Nat Biotechnol. 2009;27:839–49.
157. Murphy GP, Su S, Jarisch J, Kenny GM. Serum levels of PSMA. Prostate. 2000;42:318–9.
158. Kawakami M, Nakayama J. Enhanced expression of prostate-specific membrane antigen gene in prostate cancer as revealed by *in situ* hybridization. Cancer Res. 1997;57:2321–4.
159. Pommier AJ, Alves G, Viennois E, Bernard S, Communal Y, Sion B, *et al.* Liver X Receptor activation downregulates AKT survival signaling in lipid rafts and induces apoptosis of prostate cancer cells. Oncogene. 2010;29:2712–23.
160. Hrzenjak A, Reicher H, Wintersperger A, Steinecker-Frohnwieser B, Sedlmayr P, Schmidt H, *et al.* Inhibition of lung carcinoma cell growth by high density lipoprotein-associated alpha-tocopheryl-succinate. Cell Mol Life Sci. 2004;61:1520–31.