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Running title: From hormones to fats and back

Abstract

Metabolic dysregulation is regarded as an important driver in cancer development and progression. The impact of transcriptional changes on metabolism have been intensively studied in hormone-dependent cancers, and in particular in prostate and breast cancer. These cancers have strong similarities in the function of important transcriptional drivers, such as the estrogen and androgen receptor, at the level of dietary risk and epidemiology, genetics and therapeutically. In this review we will focus on the function of these nuclear hormone receptors and their downstream impact on metabolism, with a particular focus on lipid metabolism. We go on to discuss how lipid metabolism remains dysregulated as the cancers progress. We conclude by discussing the opportunities that this presents for drug repurposing, imaging and the development and testing of new therapeutics and treatment combinations.
Introduction: Prostate and breast cancer

Sex hormones act through nuclear hormone receptors and induce distinct transcriptional programs essential to male and female physiology. They exert their effects on target tissues such as the mammary gland, ovary and the uterus in females, and the testis and the prostate gland in males. Studies indicate that these hormones also play pivotal roles in the development of endocrine-related cancers. The highest incidence cancers affecting hormone-dependent organs are prostate cancer (PCa) and breast cancer (BCa). PCa is the second most common male malignancy in many western industrialised countries and fifth leading cause of cancer death in men worldwide (GLOBOCAN 2012). BCa is the most frequently diagnosed female malignancy and the fifth most common cause of death from cancer overall (GLOBOCAN 2012). Early stage PCa is dependent on androgens for survival and can be treated by androgen deprivation therapy; however with the advancement of cancer, it becomes refractory to hormone treatment. This later stage of PCa is known as castrate resistant prostate cancer (CRPC) or androgen-indifferent prostate cancer, meaning that the cancer thrives despite a reduction in serum androgen levels (Beltran, et al. 2011; Feldman and Feldman 2001). The prostate is also a target for estrogens among which estradiol-17β is considered the most potent inducer of prostatic proliferation and promotes epithelial-to-mesenchymal transition (EMT) in benign prostatic epithelial cells (Shi, et al. 2017). Studies also suggest that estrogen-androgen balance may be a key determinant in the development of aggressive PCa (Black, et al. 2014). Likewise androgens play important roles in development and progression of BCa subtypes such as molecular apocrine tumours (Robinson, et al. 2011). Endogenous estrogen levels have been linked to an increased risk of breast and endometrial cancers (Brown and Hankinson 2015).

The predominant BCa subtype is luminal tumours, accounting for approximately two-thirds of all diagnosed BCa cases (Ignatiadis and Sotiriou 2013). Originating from breast luminal epithelial cells, the majority of luminal tumours are estrogen receptor (ER) positive and clinical studies have highlighted a strong ovarian-derived steroid hormone driven biology, in mammary gland tumour development, particularly associated with estradiol-17β (Perou, et al. 2000; Pike, et al. 1993). ER-positive BCa patients have the most favourable BCa subtype prognosis, generally responding well to endocrine targeted therapies, however, endocrine therapy resistance does occur (Garcia-Becerra, et al. 2013). In recent years an important role for the androgen receptor (AR) in breast tumorigenesis has emerged, with between 70%-90% of tumours shown to harbour AR-positivity (Collins, et al. 2011; Moinfar, et al. 2003; Niemeier, et al. 2010). In addition, two gene expression studies have identified an AR-positive
BCa subtype; molecular apocrine BCa tumours, also characterised by ER-negativity, they constitute 8-12% of all BCa cases (Doane, et al. 2006; Farmer, et al. 2005). Despite their ER-negative status, molecular apocrine tumours express a number of genes which are usually expressed in ER-positive tumours such as XBP-1, SCUBE2, SPDEF and FOXA1 (Doane et al. 2006; Robinson et al. 2011). It is proposed that in such tumours, in the absence of ER, AR can bind to ER cis-regulatory genomic elements and transcriptionally activate canonical ER target genes (Robinson et al. 2011). Consequently, patients with molecular apocrine tumours display poor clinical response to ER antagonists, and it is believed, that the use of anti-androgens may be of increasing therapeutic benefit (Farmer et al. 2005).

**Nuclear hormone receptors- Androgen receptor and Estrogen receptor**

Nuclear hormone receptors such as the AR and ER are ligand activated transcription factors (TFs) which induce distinct transcriptional programs conducive to the development and differentiation of prostate and breast tissue respectively. The growth and maintenance of the prostate is dependent on androgens, testosterone and 5α-dihydrotestosterone (DHT), acting through their cognate receptor, AR (Heinlein and Chang 2004). Testosterone is synthesized from cholesterol, primarily by the Leydig cells in the testes and transported to the target tissues bound to serum sex hormone-binding globulin (SHBG) and albumin, where it is converted to the more active metabolite, DHT by the enzyme 5α-reductase (Eacker, et al. 2008; Heinlein and Chang 2004).

AR also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4) is a ligand dependent TF that regulates expression of genes involved in male sexual phenotype. AR gene encodes a protein of 110 KDa with three major domains, the N-terminal domain (NTD), the DNA binding domain (DBD), and the C-terminal ligand binding domain (LBD). Binding of androgens to LBD causes dissociation of heat shock proteins, its nuclear translocation through binding of importin-α, subsequent dimerization and binding to androgen response elements (AREs) in the promoter regions of target genes, such as prostate specific antigen (PSA) and trans membrane protease serine 2 (TMPRSS2) (Tan, et al. 2015). AR is expressed mostly in the secretory epithelial cells and to some extent in the stroma whereas basal cells are AR negative (Prins, et al. 1991). In a fully formed prostate, the androgens continue to function to promote the survival of secretory epithelia. Physiological testosterone and DHT levels prevented apoptosis in these secretory epithelial cells whereas castration resulted in a loss of prostate secretory epithelial cells due to apoptosis in rat models which was preceded by
degeneration of prostatic capillaries (Heinlein and Chang 2004). Hence AR signalling remains essential to the structural and functional integrity of prostate gland.

ER signalling is important for a number of normal cellular proliferative processes and the maintenance of lipid and carbohydrate metabolic homeostasis, primarily in reproductive-related tissues (Nilsson and Gustafsson 2011; Vrtačnik, et al. 2014). In addition, the role of the ER in both BCa and PCa development has been extensively studied. Like testosterone, estrogen is a steroid hormone, derived from cholesterol; it mediates its function through binding to one of two cognate estrogen receptors; alpha (ERα) and beta (ERβ). Two ERα isoforms ERα36 (Wang, et al. 2005) and ERα46 (Flouriot, et al. 2000) have additionally been identified. Although there are two subtypes, ERα is the main driver in ER-positive BCa and the main subtype of focus in this review. Upon ligand activation, ERα dimerises and interacts with transcriptional coregulators to bind to estrogen responsive elements (ERE) upstream of ERα target genes (Hayashi, et al. 2003; May and Westley 1988). ERα is primarily expressed in the mammary gland and is a ligand inducible TF and signal transducer that has functional roles in proliferation, differentiation, migration, and is known to transcriptionally regulate genes involved in mitochondrial biogenesis, the TCA cycle and lipid metabolism (Huss, et al. 2004; Manavathi, et al. 2013). Consequently, the overexpression of ER-α, as observed in many Luminal A BCa tumours, leads to hormone-dependent breast tumorigenesis (Barnes, et al. 2004).

AR and ER transcriptional activity is critical to the progression of PCa and BCa and are the main targets of hormonal therapies. Apart from cell-cycle regulators and signalling molecules, central metabolism and metabolic gene signatures form a core set of AR and ER target genes (Abba, et al. 2005; Massie, et al. 2011). Altered cellular metabolism is a hallmark of virtually all cancers. Both in vitro and in vivo studies support a major role for metabolic transformation in the progression of PCa and BCa. The importance of metabolism is further reinforced by the fact that both PCa and BCa are influenced by metabolic diseases like type 2 diabetes, metabolic syndrome and obesity (Hsing, et al. 2007; Vona-Davis, et al. 2007).

Metabolic reprogramming in prostate and breast cancer

Understanding the unique metabolic landscape of normal prostate glandular epithelium is imperative to better understanding the metabolic changes in PCa (Figure 1). Unlike other mammalian cells, normal prostate glandular epithelial cells are characterised by synthesis and secretion of enormously high levels of citrate, the major component of the prostatic fluid.
Citrate is produced from acetyl CoA and oxaloacetate (OAA) by the enzyme citrate synthase. In a typical mammalian cell, citrate is oxidized via Krebs cycle to regenerate OAA, where as in prostate cells, citrate is an end product of metabolism which is not further oxidized but exported to the cytosol. This is achieved through cellular accumulation of zinc that inhibits the mitochondrial aconitase activity and truncates Krebs cycle at the first step of citrate oxidation (Costello and Franklin 2006). The active transport of zinc in prostate cells is mainly mediated through SLC39A1 or ZIP1. The continued supply of acetyl CoA and OAA for citrate production are met by a high rate of aerobic glycolysis and aspartate accumulation respectively. Glycolysis converts glucose to pyruvate which enters mitochondria and gets decarboxylated to acetyl CoA by pyruvate dehydrogenase (PDH). OAA is produced from the transamination of aspartate which is actively transported into the prostate cells through the specific receptor excitatory amino acid carrier 1 (EAAC1) (Franklin, et al. 2006). Testosterone stimulates the expression/activity of EAAC1, aspartate aminotransferase and mitochondrial PDH thus facilitating citrate production by regulating both acetyl CoA and OAA levels (Costello and Franklin 1993; Franklin, et al. 1986; Franklin et al. 2006). In addition, testosterone affects citrate oxidation by differentially regulating m-aconitase gene expression in rat lateral and ventral prostate cells with an inhibitory effect on former and stimulatory effect on latter (Costello, et al. 1995; Costello, et al. 2000). Although bio-energetically less efficient, the citrate production is essential to maintain the pH of the semen, chelation of calcium ions by citrate (Ford and Harrison 1984) and may have more complex roles. Furthermore, a relatively low abundance or absence of ATP citrate lyase (ACLY) by negative regulation through miR-22 (Xin, et al. 2016) may be preventing the degradation of citrate to acetyl CoA and OAA in the cytosol thereby promoting its secretion into the prostatic fluid. Interestingly, ACLY, a key enzyme in the de novo lipogenesis has been shown to be up regulated in clinical samples from PCa, BCa osteosarcoma, cervical cancer and lung cancer with a concomitant reduction in miRNA-22 (Xin et al. 2016). To summarize normal prostate cells are characterized by a high net citrate production associated with a low level of OXPHOS and high rate of glycolysis.

A metabolic transformation from a zinc accumulating citrate secreting cell to citrate oxidizing cell is a prominent feature of prostate malignancy. Patient derived tumour metabolomics data demonstrate strikingly low citrate levels in CRPC tumours when compared to normal prostate and in androgen-dependent PCa (Shafi, et al. 2015). ZIP1 gene expression is markedly downregulated in adenocarcinomatous glands coupled with a loss of zinc accumulation (Franklin, et al. 2005). Two other zinc transporter isoforms ZIP2 and ZIP3 are
also downregulated in PCa (Desouki, et al. 2007). Cancer cells tend to evade the inefficient process of citrate production through epigenetic silencing of zinc transporters by promoter hypermethylation thereby inhibiting zinc accumulation (Costello and Franklin 2006; Makhov, et al. 2011). Interestingly, the expression of ZIP1 and ZIP2 are comparatively lower in normal prostate tissue from African-Americans patients who are at a higher risk of PCa incidence when compared to age matched Caucasian men (Rishi, et al. 2003). Epigenetic changes especially methylation is one of the most recurrent events in locally advanced and metastatic PCa with AR promoter CpG islands reported to be hotspots of aberrant methylation and consequent loss of AR expression (Kinoshita, et al. 2000; Massie, et al. 2017). DNA methylation is intimately linked to central metabolism as well as one carbon metabolism. Androgens regulate the expression of key biosynthetic enzymes involved in the polyamine synthesis in the prostate which requires one carbon metabolism and share the DNA methylation donor, S-adenosylmethionine (SAM). Therefore a decline in polyamine biosynthesis during cancer progression may enhance the availability of SAM pool for epigenetic modification (Massie et al. 2017). The absence of the Warburg effect in early stage PCa is clinically relevant as these tumours do not appear on fluorodeoxyglucose positron emission tomography (FDG-PET) scans (Liu, et al. 2001). However, in the later stages, PCa cells start exhibiting Warburg effect and FDG-PET may be useful in monitoring treatment response in advanced metastatic PCa (Eidelman, et al. 2017). Early stage PCa appears to rely on lipids for energy production and this property is exploited in visualizing tumours by $^{11}$C-acetate or $^{11}$C-choline accumulation, which seems to be a more sensitive imaging technique than FDG-PET in detection of primary and metastatic prostate cancers (Jadvar 2012). $^{11}$C-sarcosine is also gaining interest as a radiotracer for PCa imaging as sarcosine, a metabolic product of choline is associated with PCa aggressiveness and progression (Piert, et al. 2017). In BCa, most studies have focused on $^{18}$F-labeled thymidine analogs to assess cell proliferation, although promising results from using $^{11}$C-choline have been reported in BCa patients (Kenny 2016).

The metabolic characteristics of mammary epithelial cells change with tumour onset and metastatic disease progression to sustain augmented biosynthetic demand. One metabolomic study in a syngeneic mouse mammary tumour model observed metabolite alterations in a number of pathways involved in glycolysis, the pentose phosphate pathway (PPP) and a decrease in GSH-dependent antioxidative pathway, and intermediates in lipid biosynthesis like fatty acid synthase (FASN) (Lu, et al. 2010). Moreover, metabolomic analysis of 270 BCa samples and 97 normal breast samples has highlighted a switch from a positive to
negative correlation between glutamate and glutamine in tumour verses normal, indicating that breast cancer cells, particularly ER-negative tumors have a metabolic dependency on glutamine metabolism (Figure 2) (Budczies, et al. 2015).

Notably, $c$-MYC amplification is found in 15.7% of all breast cancers (Deming, et al. 2000). In ER-positive breast tumours it is known to confer response to endocrine treatment (Ellis, et al. 2012) and implicated in the development of treatment resistance (McNeil, et al. 2006; Miller, et al. 2011; Venditti, et al. 2002). Similarly, in the absence of ER, one characteristic of triple-negative breast cancer (TNBC) is increased transcriptional activity of the $c$-MYC oncogene (Alles, et al. 2009). It is established that high expression of c-MYC can augment glutamine uptake and maintain the elevated bioenergetic demands of a tumorigenic cell (Anso, et al. 2013). Interestingly, a recent study by Mishra et al reported elevated levels of the oncometabolite D-2-hydroxyglutarate (D2HG) in ER negative breast tumours driven by the c-MYC induced mitochondrial enzyme, alcohol dehydrogenase iron-containing protein 1 (ADHFE1), promoting metabolic reprogramming, reductive glutamine metabolism and disease progression (Mishra, et al. 2018). Akin to lipid metabolism in prostate cancer, glutamine metabolism requires mitochondrial function and oxidative phosphorylation.

The role of sex hormones and nuclear hormone receptors in the metabolic transformation of prostate or breast is quite complex and inadequately understood, with several caveats in existing information linking genetic and molecular changes to metabolic transformation. Genomic and metabolic studies are corroborative of the role of AR as a core regulator of an anabolic transcriptional network in PCa. Studies by Massie et al have sought to derive a detailed map of AR regulated genes in PCa cell lines representing distinct molecular subtype of the disease, by employing ChIP seq and transcript profiling (Massie et al. 2011). Stimulation of AR by androgen up-regulated genes involved in cell cycle, glucose uptake and glycolysis, lipid turnover, nucleotide and amino acid metabolism. This study identified calcium/calmodulin-dependent protein kinase 2 (CAMKK2) as a hormone-dependent modulator of anabolic metabolism in PCa in an AMP activated protein kinase (AMPK) dependent manner (Massie et al. 2011). CAMKK2 is also consistently overexpressed in clinical samples from both hormone sensitive and CRPC. AR mediated activation of cellular fuel sensor, AMPK, and downstream AMPK-PGC1α signalling axis confers distinct growth advantage to the tumour cells (Tennakoon, et al. 2014). Consequently genetic changes in AR form one of the core contributors to altered cellular metabolism in PCa. C-MYC is another TF overexpressed in PCa which partially overlaps with AR binding sites and antagonizes AR
mediated transcriptional output (Barfeld, et al. 2017). However, in molecular apocrine BCa, MYC cooperates with AR in androgen responsive gene transcription contrary to the antagonistic relationship seen in PCa (Ni, et al. 2013). *In vitro* studies provide important insights into the intricate transcriptional and gene regulatory networks, and target genes relevant to the progression of PCa and BCa. However, in order to get a comprehensive picture of the mechanisms underpinning the disease, it is important to simultaneously derive and interpret data from *in vivo* models, tissue explants and clinical specimens.

**Metabolic pathways in prostate and breast cancers**

**Metabolite transporters:** An increase in glucose uptake and switching to aerobic glycolysis or Warburg effect is a prominent metabolic alteration in most cancers. However, the prostate has a unique metabolic landscape that favours switching to an increased OXPHOS during cancer initiation and progression. Glucose uptake and increased glycolysis appears to be a metabolic adaptation in the late stage of the disease correlated with poor prognosis (Pertega-Gomes, et al. 2015a). Studies from our group and others have shown androgen stimulated glucose uptake and lactate production in PCa cell lines (Massie et al. 2011; Vaz, et al. 2012). The expression of ubiquitous glucose transporter, GLUT1 was elevated by androgen treatment in these cells. A recent study has further validated the critical role played by GLUT1 in PCa, the knockdown of which inhibited glycolysis, cell proliferation and induced cycle arrest at G2/M phase (Xiao, et al. 2018). Other GLUT isoforms like GLUT3 (Vaz et al. 2012), insulin sensitive isoforms GLUT4, GLUT12 (Chandler, et al. 2003) and fructose transporter GLUT5 (Reinicke, et al. 2012) are also reported in PCa cells. Similarly, GLUT1, GLUT2, GLUT3, GLUT4, GLUT5 and GLUT12, have been reported to be expressed in BCa cells (Garrido, et al. 2013; Godoy, et al. 2006; Rogers, et al. 2003). GLUT1 expression has been shown to positively correlate with ERα-positivity and confer a more aggressive disease and poorer prognosis in BCa patient tumour samples (Kang, et al. 2002). Downregulation of GLUT1 with the multi-kinase inhibitor Sorafenib has been shown to induce apoptosis through mitochondrial membrane depolarisation and AMPK-dependent inhibition of the mTORC1 pathway in BCa (Fumarola, et al. 2013). Further studies are needed to delineate the specific roles of these GLUT isoforms in PCa and BCa.

Monocarboxylate transporters (MCT) are proton symporters involved in lactate efflux to maintain the high glycolytic rate seen in solid tumours and to prevent the cytotoxicity from
acidosis (Pinheiro, et al. 2012). Androgen-responsive (LNCaP) and androgen-nonresponsive (PC3) cell lines exhibited distinct glycolytic profiles with a higher lactate production in PC3, and a concomitant increase in monocarboxylate transporter (MCT4) expression and lactate dehydrogenase activity (LDH) (Vaz et al. 2012). GLUTs and MCTs may be functioning synchronously to maintain glycolysis at a steady rate in solid tumours. However in PCa, a reciprocal metabolic relationship seems to exist between cancer associated fibroblast (CAFs) and cancer cells. When co-cultured, CAFs undergo metabolic reprogramming to a Warburg phenotype with increased GLUT1, lactate production and efflux through MCT4, whereas PCa cells are shifted to aerobic metabolism with an increase in lactate upload via MCT1. The allocation of glycolytic metabolism to CAFs and ‘lactate shuttling’ appears to be one of the strategies employed by PCa cells for growth and progression (Fiaschi, et al. 2012). MCT2 is yet another MCT isoform overexpressed in PCa through epigenetic regulation (Pertega-Gomes, et al. 2015b). These studies indicate that MCTs could be promising targets in the treatment of PCa.

Amino acid metabolism: Many cancers show increased dependency on glutamine uptake and metabolism which not only serves the protein synthesis requirements but also fuels TCA cycle (anaplerosis) and acts a source of fatty acid production through reductive carboxylation (Eidelman et al. 2017; Mullen, et al. 2011). Glutaminolysis involves conversion of glutamine to glutamate by glutaminase which is then transformed into alpha-ketoglutarate that is fed into the Krebs cycle. Glutaminase1 (GLS1) expression is augmented in PCa patients and is highly correlated with the stage of the disease (Pan, et al. 2015). The expression of major glutamine transporter in cancer cells, alanine–serine–cysteine transporter-2 (ASCT2), is also elevated in PCa patient samples (Wang, et al. 2015). Indeed, inhibition of ASCT2 limited PCa growth and metastasis in vivo and hence a putative therapeutic target.

Among the other metabolic pathways, MYC regulated purine biosynthesis has been shown to be significant in PCa with two reported biomarkers in the pathway namely, phosphoribosylaminomimidazole carboxylase and phosphoribosylaminomimidazole-succinocarboxamide synthase (PAICS) and Inosine Monophosphate Dehydrogenase 2 (IMPDH2); inhibition of IMPDH2 impaired cell proliferation in vitro by inducing nucleolar stress (Barfeld, et al. 2015). Recent studies have shown that enzymes in the hexosamine biosynthetic pathway (HBP) are androgen regulated and upregulated in PCa transcriptionally and metabolically. The enzyme involved in O-linked conjugation, O-linked β-N-acetylglucosamine transferase (OGT) is overexpressed in PCa and correlate with disease
progression. Interestingly c-MYC is a target of OGT whose OGlcNAcylation site overlaps with phosphorylation site preventing its polyubiquitination and proteosomal degradation (Itkonen, et al. 2013). Some studies support a role for one carbon metabolism in PCa, specifically choline and vitamin B12 show a positive correlation with PCa risk (Johansson, et al. 2009). Apart from these, dysregulated lipid metabolism is one of the main aspects of metabolic reprogramming in PCa as well as BCa.

**Lipid metabolism**

Aberrant lipogenesis is a prominent feature of cancer cells and increasingly recognized as an important factor in tumour growth and metastases (Figure 3). Most mammalian cells meet their lipid requirements through diet and lipid biosynthesis is restricted to tissues like adipose, liver and lactating breast tissue. Studies by Medes *et al* in the early 1950’s demonstrated that *de novo* lipogenesis is induced in neoplastic tissues (Medes, *et al*. 1953). It is not surprising that lipid synthesis is induced in proliferating cancer cells in order to meet the increased demand for membrane biogenesis. In addition, *de novo* lipogenesis also serves to provide building blocks for lipid signalling molecules such as phosphatidylinositol-3,4,5-trisphosphate (PIP3), ceramide-1-phosphate, phosphatidic acid (PA), diacylglycerol (DAG), and lysophosphatidic acid (LPA) all of which fuel cancer cell growth (Ray and Roy 2017). Another advantage of lipogenesis is availability of substrates for posttranslational modification of proteins like prenylation, palmitoylation, GPI modification and lipids also serve as an energy reserve during times of nutrient limitation (Zhang and Du 2012). *De novo* lipogenesis ensures a constant supply of fatty acids in poorly vascularized tumour tissues, thereby maintaining a high proliferative rate.

Several key enzymes involved in the lipid biosynthetic pathway are frequently upregulated in PCa. Kadhi *et al* made an interesting observation that the peripheral zone of prostate are naturally endowed with a higher capacity for *de novo* lipogenesis and fatty acid oxidation which makes it susceptible to oncogenesis and explains the frequent occurrence of cancer in this zone (Al Kadhi, *et al*. 2017). Androgens through coordinating the expression of enzymes involved in lipogenesis, play a central role in mediating the lipogenic switch in PCa cells. One of the mechanisms by which androgens exert this lipogenic switch is through activation of sterol regulatory element-binding proteins (SREBPs), master regulators of lipid and cholesterol biosynthesis. SREBP family of TFs encompasses SREBP-1a, SREBP-1c and SREBP-2 which share similar structure and mechanism of activation. In brief, SREBP
precursors are anchored to endoplasmic reticulum (ER) membrane where it forms a complex with SREBP cleavage-activating protein (SCAP) which in turn interacts with a retention protein complex. The loss of interaction between SCAP and retention complex leads to the migration of SREBP/SCAP complex to the golgi where it is subjected to proteolytic cleavage by resident proteases and subsequent release of N-terminal SREBP segment (nSREBP). NSREBP further translocates to the nucleus and binds to SREs in the promoter region of key lipogenic genes involved in both the synthesis of fatty acids and cholesterol (Heemers, et al. 2006). SREBP dependent de novo lipogenesis promotes PCa growth and metastases. Huang et al has shown a positive correlation between SREBP1 protein expression and clinical Gleason score in PCa (Huang, et al. 2012). Androgen induced SREBP activation is proposed to occur through increased AR induced expression of SCAP which favours SREBP/SCAP translocation to the golgi and activation (Heemers et al. 2006). SREBP1 in turn regulates the expression of AR by binding to its promoter region (Huang et al. 2012) thereby maintaining the lipogenic phenotype. Two recent studies have identified novel genomic drivers of lipid metabolism in PCa with implications for subtyping and treatment of the disease. Both studies employed PTEN-null transgenic mouse model of PCa which gives high-grade intraepithelial prostate tumours at an early age and invasive PCa at a late stage. The first study focused on pyruvate dehydrogenase complex (PDC), a complex with a gatekeeper function in converting pyruvate into acetyl-CoA for entry into the TCA cycle in mitochondria, demonstrates how cellular metabolism is intimately linked to the regulation of gene expression. By inactivating pyruvate dehydrogenase A1 (PDHA1), prostate tumour growth was restrained at an early stage mainly through suppressing lipid biosynthesis. Mechanistically, this is due to reduced histone acetylation at regulatory regions bound by SREBP owing to a reduction in the activity nuclear compartmentalized PDHA1 (nDHA1) (Chen, et al. 2018b). Furthermore, PDHA1 is frequently amplified and overexpressed in these tumours signifying its potential as a therapeutic target. The second study utilizes the same genetic background and found that co-deletion of Pml leads to hyperactivation of an SREBP dependent lipogenic program in a MAPK dependent manner and can be blocked by SREBP inhibitor, fatostatin. Intriguingly a high fat diet was sufficient to drive metastasis in PTEN null model highlighting the importance of dietary intervention in regulating PCa progression (Chen, et al. 2018a). SREBP pathways are also activated by mutant p53, often seen in metastatic PCa. In fact fatostatin alone or in combination with docetaxel displayed significant antitumor effects in PCa xenograft models (Li, et al. 2015). Studies in an LNCaP xenograft model of human PCa indicate increased lipogenesis as an adaptive mechanism during progression to androgen independence (Ettinger, et al. 2004). These studies
significantly enhance our understanding of the mechanistic basis for dysregulated lipid metabolism in PCa and could open doors to new approaches for patient stratification and molecular subtyping.

FASN, an AR target gene and a key enzyme in the lipid biosynthetic pathway is frequently upregulated in human PCa and BCa and has been widely studied. The recent development of Fasnall, a FASN inhibitor with anti-tumour activity (Alwarawrah, et al. 2016), provides an interesting therapeutic opportunity. FASN expression is an early event in the development of PCa with a gradual increase with the stage of cancer and a higher expression correlates with poor prognosis and patient survival, suggesting the use of FASN as a general PCa marker (Rossi, et al. 2003; Swinnen, et al. 2002). It is noteworthy that many of the oncogenic signalling pathways are involved in the regulation of lipid metabolism. Growth factor associated signalling pathways like PI3K/Akt and MAPK regulates FASN expression through SREBP1 (Zhang and Du 2012). Other TFs such as Sp1, members of p53 family and the lipogenesis-related nuclear protein S14 are also known to modulate FASN expression (Zhang and Du 2012). FASN is also regulated post-translationally in PCa by androgen regulated ubiquitin-specific protease-2a (USP2a) that removes ubiquitin residues from FASN and stabilizes the protein. USP2a is overexpressed in PCa and represents another therapeutic target (Graner, et al. 2004). Other enzymes involved in fatty acid synthesis which are frequently upregulated in PCa are ACLY (Xin et al. 2016), acetyl-CoA carboxylase (ACC) that catalyses the conversion of acetyl CoA to malonyl CoA and stearoyl-CoA desaturases (SCD), rate limiting enzyme in the biosynthesis of monounsaturated fatty acids (Kim, et al. 2011). It is interesting to note that SCD peptides derived from proteolytic cleavage of SCD can transactivate AR and facilitate cell proliferation (Kim et al. 2011).

SREBP-1 has additionally been shown to confer poor clinical prognosis in BCa and promote invasive and metastatic potential, correlating with a greater tumour-node-metastasis and lymph node metastasis stage in BCa patients (Bao, et al. 2016). SREBP-1 transcriptionally activates a number of enzymes involved in lipid metabolism and lipogenesis, including; FASN, ACC and SCD-1 (Bao et al. 2016; Lee, et al. 2013; Song, et al. 2012). In normal breast cells, circulating lipids are used to synthesize lipids, whilst in breast tumour cells FASN is utilised in lipid generation and FASN upregulation is frequently observed in BCa (Mashima, et al. 2009; Zhang and Du 2012).
In addition to an increase in lipogenesis, PCa cells heavily rely on lipid oxidation for their growth and survival. A recent study by Itkonen et al has demonstrated Enoyl-CoA delta isomerase 2 (ECI2), an enzyme involved in lipid degradation, as a direct AR target and overexpressed in clinical PCa. Consequently PCa cells are vulnerable to inhibitors of lipid degradation like perhexiline or by ECI2 knockdown, and respond by activating incomplete autophagy followed by cell death response. Moreover, the clinically approved drug perhexiline exhibited potent anti-tumour activity in combination with anti-androgen, enzalutamide and abiraterone acetate (Itkonen, et al. 2017). Fatty acid oxidation is also an important bioenergetic pathway in MYC-overexpressing triple negative breast cancer (Camarda, et al. 2016). Hence targeting lipid metabolism would be a promising approach in PCa and BCa treatment.

The biosynthesis of cholesterol, an important component of the lipid bilayer, occurs through the mevalonate pathway using the same precursor, acetyl CoA, used in fatty acid synthesis. Enhanced cholesterol biosynthesis regulated by SREBP-2 is a major player in the initiation and progression of PCa with an increase in PCa stem cell population with SREBP-2 overexpression (Li, et al. 2016). Aberrant cholesteryl ester accumulation in lipid droplets aggravates the cancer invasiveness and is found in high grade PCa with PTEN loss and PI3K/Akt activation (Yue, et al. 2014). Squalene monooxygenase (SQLE), the second rate-limiting enzyme of cholesterol synthesis is highly expressed in lethal PCa and may be a therapeutic target in high risk patients (Stopsack, et al. 2017). SQLE is also considered a bonafide oncogene in BCa (Brown, et al. 2016). Cholesterol forms the structural backbone for steroid hormone biosynthesis (Figure 4). Intratumoral steroid biosynthesis has been recognized as crucial factor in the progression of both PCa and BCa. Although serum androgen levels are diminished following hormone ablation therapy, the intraprostatic androgen levels remain moderately high as the tumour cells start synthesizing their own androgens and this could be the driving force in CRPC progression (Locke, et al. 2008). This finding has paved way for the development of novel therapeutic approaches to treat PCa such as abiraterone acetate, an inhibitor of 17α-hydroxylase and C17,20 lyase (CYP17A1) for the treatment of men with advanced CRPC (Ryan, et al. 2013). In fact genetic polymorphisms of CYP17A1 are associated with higher aggressiveness and risk of progression to CRPC in patients receiving androgen deprivation therapy (Robles-Fernandez, et al. 2017). The importance of cholesterol in PCa progression is further established by a clinical study by Platz et al where the use of statins (cholesterol lowering drugs) in patients is found to be associated with reduced risk of metastatic PCa (Platz, et al. 2006). The cholesterol metabolite, 27-hydroxyl-cholesterol (27-
OHC) is a known selective estrogen receptor modulator (SERM) which promotes tumourigenesis in ER-positive BCa (Figure 2) (Warner and Gustafsson 2014). Higher levels of 27-OHC have been reported in ERα-positive breast tumours in comparison to normal breast tissue, along with an observed reduction in the 27-OHC metabolising enzyme CYP7B1 (Wu, et al. 2013). *In vivo* xenograft experiments have additionally shown that 27-OHC alone is sufficient to support estrogenic activity in ER-dependent breast cancer models, providing rationale for 27-OHC targeting (Wu et al. 2013). Similar to the Chen *et al* study in PCa, a high-cholesterol diet alone increased the growth and metastasis of ER-positive tumours in a mouse BCa model (Nelson, et al. 2013).

Obesity is major challenge faced by the modern society and associated with pathogenesis of cardiovascular diseases and type 2 Diabetes. Obesity is also a driving force in incidence and increased recurrence of PCa and BCa. Besides *de novo* adipogenesis induced in these cancers, adipocyte derived fatty acids also accelerate cancer progression. Although the tumour microenvironment of PCa and BCa differ, emerging evidence suggests a similar relationship between local adipocytes and PCa and BCa cells. The lipid content of peri-prostatic and mammary adipocytes is proportionately increased during obesity. An *in vitro* co-culture of obese adipocytes with breast or prostate cancer cell lines, showed a higher rate of transfer of FA from adipocytes to the cancer cells correlated with increased proliferation and survival (Balaban, et al. 2017). Interestingly, it is the peripheral zone of the prostate which is more susceptible to becoming cancerous and is enriched in genes regulating lipid metabolism like FASN, ACACA, ACSL1 and ACSL3. Together these studies underscore the importance of targeting intermediates in the lipid/cholesterol biosynthetic pathway in the treatment of PCa. *De novo* lipid biosynthesis requires the substrates acetyl CoA and NADPH. Therefore, lipogenesis is coupled to other metabolic pathways like glucose and glutamine to derive these substrates (Zhang and Du 2012).

**Treatment resistance in AR-positive and ER-positive cancers**

AR plays a central role in the pathogenesis of PCa and is the main therapeutic target. Several studies indicate that polymorphisms/mutations in the AR predispose men to PCa (Gottlieb, et al. 2012). For instance, polymorphic variation in the length of CAG repeats in the amino terminus of AR is found to be inversely correlated to AR transcriptional activity, with short repeats associated with an increased risk of PCa (Heinlein and Chang 2004). Although disputed this association may partly explain the ethnic differences seen in PCa incidence, where
African-American men have short CAG repeat length and higher incidence, and the vice versa with Asian men (Heinlein and Chang 2004). The initiation of PCa may involve gene rearrangements that activate common growth promoting pathways (Hermans, et al. 2006; Tomlins, et al. 2005). Dysregulation of PI3K/Akt and and RAS/RAF pathways are also implicated in PCa initiation and progression. Early stage PCa can be treated by castration through surgical (orchiectomy) or chemical means. Chemical castration involves use of gonadotropin releasing hormone (GnRH) analogues such as leuprolide and goserelin or GnRH antagonist such as degarelix. Patients are also subjected to androgen deprivation therapy (ADT) by treatment with anti-androgens which are AR ligands that compete for AR binding sites. The steroidal antiandrogens are now replaced with non-steroidal ones such as flutamide, bicalutamide, nilutamide (Tan et al. 2015). The second-generation AR antagonist enzalutamide is a more potent antagonist with higher affinity than the first generation counterparts. Abiraterone acetate that blocks androgen biosynthesis is clinically approved and used in combination with prednisone as a treatment for metastatic CRPC (Auchus, et al. 2014).

Although ADT or chemical castration remains first line of treatment of PCa, disease eventually progresses to castration resistant mode which is ultimately fatal. AR is expressed throughout cancer progression and remains a major driving force for CRPC. Clinical and experimental evidences suggest a positive correlation between AR expression and lower recurrence-free survival and disease progression. Different mechanisms have been proposed to explain the progression to CRPC like 1) AR gene amplifications enabling increased sensitivity of the AR to its agonists under conditions of androgen deprivation 2) Over expression of AR coregulators such as SRC1, SRC-3, cdc25B, Tip60 and nmt55, allowing AR sensitization at low levels of androgens 3) AR mutations that render the receptor responsive to alternate, non-androgen ligands like estrogen, progesterone, cortisol and anti-androgens 4) ligand-independent AR activation such as mediated by IL6, the circulating levels of which is often higher in metastatic PCa (Hobisch, et al. 1998) 5) Interaction with tumour suppressors like PTEN, the loss of which in PCa may increase AR turnover and hence transcriptional activity, and 6) AR-independent mechanisms (Heinlein and Chang 2004; Tan et al. 2015). The acquisition of some AR mutations that renders AR transcriptional activity by allowing antiandrogens like bicalutamide (Hara, et al. 2003) and adrenal androgens dehydroepiandrosterone (DHEA) and androstenediol to function as transcriptional agonists is a frequent cause of failure of ADT. Consequently, antiandrogen withdrawal syndrome is observed in a proportion of patients with failure of ADT, with tumour regression after
discontinuation of antiandrogen treatment. For a detailed description of various mechanisms leading to failure of ADT, audience is directed to reviews by Heinlein et al (Heinlein and Chang 2004) and Karantanos et al (Karantanos, et al. 2013).

In BCa, endocrine therapies such as tamoxifen, aromatase inhibitors (AIs), including Letrozole and Anastrozole and the ER antagonists Faslodex (fulvestran) target circulating oestrogen and consequently, deprive ER-dependent tumour cells of ligand activation. However, both intrinsic and acquired de novo resistance to endocrine therapies occurs in a subset of patients. Breast tumour resistance to ER targeted therapies has clinically been observed to occur via a plethora of mechanisms, with such tumours, reverting to low expression of ERα and thus, losing their ER-driven phenotype (Osborne and Schiff 2011). ER-positive breast cancers have clinically been shown to revert to a HER2 driven dependency following endocrine treatment, predominately, via HER2 gene amplification or overexpression (Gutierrez, et al. 2005; Lipton, et al. 2005; Meng, et al. 2004). Several pre-clinical studies have highlighted that breast tumours can alternate between an ER and HER2 dependency following prolonged targeted therapy exposure (Gutierrez et al. 2005; Lipton et al. 2005; Massarweh, et al. 2008; Meng et al. 2004). Growth factor receptor overexpression is one method by which tumours can circumvent endocrine therapy responsiveness, in addition to HER2; IGF, FGF, VEGF and Src have also been identified as ER-independent drivers of ER-positive breast cancer in response to hormone targeted therapy (Arpino, et al. 2008; Chakraborty, et al. 2010; Morgan, et al. 2009). Similar to HER2, the pathways regulated by such receptors and their corresponding ligands are frequently overexpressed or genetically amplified in a number of clinical cases of endocrine therapy resistance. Consequently, a number of clinical trials combining such therapies with those that target HER2 such as gefitinib have been undertaken, yet unpublished to date (Baselga, et al. 2005; ClinicalTrials.gov 2009a, b) The growth factors and signalling pathways driven by these tumours despite their growth factor dependency switch, regulate BCa tumour metabolism.

Alterations in both tumour epigenomic and transcriptomic profiles post-endocrine treatment have been observed in vitro and in vivo by the Magnani group following the generation of endocrine therapy resistance breast cancer cell lines and NOD-SCID mouse metastatic model generation (Nguyen, et al. 2015). The epigenetic reprogramming of ERα-positive breast cancers, in particular H3K27ac modifications in promoter-proximal regions has been shown to occur and potentiate endocrine therapy resistance mechanisms following treatment and particularly augment metastatic development. A number of H3K27ac alterations
in close proximity to the promoters of several genes which are already known to be involved in endocrine therapy resistance were observed (Magnani, et al. 2013). In addition, the upregulation of the cholesterol biosynthesis (CB) pathway in AI-resistant breast cancer cells was also reported, including the upregulation of a number of genes which are known to regulate the synthesis of 27-OHC (Nguyen et al. 2015). It is known that 27-OHC facilitates the oestrogen-independent binding of ERα to a plethora of genomic regulatory loci and hence can drive ER-dependent transcription when endogenous levels of oestrogen are low following endocrine therapy (Nelson et al. 2013). Furthermore, endocrine therapy can directly regulate cellular cholesterol metabolism via targeting the cholesterol epoxide hydrolase (ChEH) enzymatic complex and the generation of the oncometabolite oxysterol 6-oxo-cholestan-3β,5α-diol, otherwise referred to as OCDO (Leignadier, et al. 2017; Voisin, et al. 2017). OCDO drives breast cancer progression through facilitating the nuclear translocation of the glucocorticoid receptor (GR) and consequently, leads to the transcriptional activation of a number of GR target genes involved in endocrine therapy resistance (Voisin et al. 2017). The Magnani group has developed a CB signature which could be used in ERα-positive BCa patients to stratify before adjuvant treatment, to determine whether they are likely to develop endocrine therapy tumour resistance via the upregulation of the CB pathway and potentially benefit from adjuvant metabolic inhibitor treatment (Nguyen et al. 2015).

Conclusion and future directions: Stratification of prostate and breast cancer

Studies have highlighted the importance of studying metabolic characteristics of PCa and BCa in order to subtype the diseases and accelerate the discovery of novel metabolic biomarkers in the diagnosis of these cancers. PSA is a widely used biomarker in PCa but is also elevated in men with benign prostatic hyperplasia and prostatitis which limits its usefulness (Nicholson and Ricke 2011). A combinatorial approach involving transcriptomic, ChIP, proteomic and metabolomic data are currently employed to study novel biomarkers for early detection of these cancers. Novel approaches to quantify lipids like vibrational Raman Microspectroscopy are being developed which could be a sensitive technique for tumour diagnosis and staging (O’Malley, et al. 2017). Further stratification incorporating metabolite and lipogeneic gene markers might prove beneficial in the accurate diagnosis and treatment of the disease. The overexpression of SREBP and FASN in PCa and BCa makes them attractive therapeutic targets. Other potential therapeutic targets include ACLY, PDH, ACC and SCD. A more detailed understanding of dysregulated lipid metabolism will help in designing better drugs or repurposing those that are already developed (Table 1). It would also be beneficial to
incorporate a spectrum of biochemical measures such as insulin, IGF, leptin and adipokine concentrations, assessment of obesity and BMI of the patient along with etiologic heterogeneity. The use of metabolic inhibitors would warrant a careful examination of patient metabolic history as these cancers are influenced by metabolic disorders. Therefore it is important to consider the medical history of patients, existing drugs used like metformin and statins and the presented side effects. Thus, the successful clinical application of metabolic drugs lies in clinical trial patient stratification and combination therapy with existing drugs.

**Declaration of interest**

The authors declare that they have no conflicts of interest of any type in relation to this work.

**Funding**

The article does not present primary research however the authors would like to acknowledge the support of Prostate Cancer UK/Movember (RES and IGM), the Norwegian Research Council (NP) and the John Black Foundation (IGM).

**Author contributions statement**

NP and RES wrote the text and made the figures and table. IGM conceived the structure of the review and contributed to the writing of the text.

**Acknowledgements**

The authors would like to acknowledge the support of all within the Centre for Cancer Research and Cell Biology and particularly Dr. Paul Mullan and Professor David Waugh.
Figure and Table Legends

Figure 1. Schematic diagram showing the metabolic landscape in A) the normal prostate gland and B) prostate cancer.

EAAC1- Excitatory amino acid carrier 1; MAAT- Aspartate aminotransferase; CS- Citrate synthase; PDH- Pyruvate dehydrogenase; ACON- Aconitase; ACLY- ATP citrate lyase. Pathways are shown in black arrows and inhibitory steps in red lines.

Figure 2. A) Key metabolic differences between ER positive and ER negative breast tumours. B) Schematic diagram showing role of glutamine and cholesterol metabolite, 27-OHC in supporting tumour growth.

SLC1A5- Solute Carrier Family 1 Member 5; SLC7A5- SLC Family 7 Member 5; GLS- Glutaminase; GLUD- Glutamate dehydrogenase; 27-OHC- 27-hydroxyl-cholesterol ; CYP27A1- cytochrome P450 family 27 subfamily A member 1; CYP7B1- Cytochrome P450 Family 7 Subfamily B Member 1; AI- Aromatase inhibition

Figure 3. Schematic diagram showing SREBP mediated lipogenic program and pathways involved in lipid and cholesterol biosynthesis.

The pathways are shown in blue arrows, key metabolic enzymes upregulated in PCa and BCa in blue boxes, inhibitors of the enzymes are in red

Figure 4. Steroid Hormone Biosynthesis Pathway

A schematic representation highlighting the structural similarities of Cholesterol, Testosterone, Estradiol and Dihydrotestosterone (DHEA) in the biosynthesis of cholesterol-derived steroid hormones and the key enzymes involved. CYP11A1, Cytochrome P450 Family 11 Subfamily A. CYP17A1, Cytochrome P450 Family 17α-hydroxylase/17,20-lyase. 3β-HSD, 3β-Hydroxysteroid dehydrogenase.

Table 1. Metabolic Inhibitors in pre-clinical development and clinical trials
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