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Metabolomic Profiling of Hormone-Dependent Cancers: A Bird's Eye View

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Abstract

Hormone-dependent cancers present a significant public health challenge, as they are among the most common cancers in the world. One factor associated with cancer development and progression is metabolic reprogramming. By understanding these alterations, we can identify potential markers and novel biochemical therapeutic targets. Metabolic profiling is an advanced technology that allows investigators to assess low molecular weight compounds that reflect physiological alterations. Current research in metabolomics in prostate and breast cancer has made great strides in uncovering specific metabolic pathways that are associated with cancer development, progression, and resistance. This review will highlight some of the major findings and potential therapeutic advances that have been reported utilizing this technology.

Keywords

metabolomics; prostate cancer; breast cancer; biomarkers; therapeutic targets

An Overview of Hormone-dependent Cancers and Cancer Metabolism

Decades ago the Nobel Laureate and biochemist Otto Warburg hypothesized that cancer cells were derived as a result of irreversible damage to mitochondrial respiratory function, thereby relying on glycolysis for the production of ATP. Therefore, compared to normal cells, cancer cells exhibit elevated bioenergetic and altered anaplerotic processes driven by oncogenic activation aimed at supporting tumor cell survival [1, 2]. Hormone-dependent cancers, including prostate and testis in men, and breast, ovarian, and endometrium in women, are the most commonly diagnosed cancers in the world [3]. In the US alone, the lifetime risk of developing prostate cancer (PCa) is 1:7 for men, and 1:8 and 1:76 for breast

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(BCa) and ovarian cancers in women [3–5]. Several lines of evidence suggest that metabolic reprogramming is associated with the development and progression of these tumors [6]. Current treatment options for hormone-dependent cancers include anti-hormone therapies, radiation, surgery, and chemotherapy; however, in several cases there is an elevated risk of relapse. Recent findings also attribute this therapeutic resistance to be, in part, associated with metabolic dysregulation [7]. The advent of advanced mass spectrometry (MS) and spectroscopy platforms (Box 1) have allowed researchers to globally profile these metabolic alterations, nominate potential markers, and identify novel biochemical druggable targets. In this review, we will provide a bird's eye view of the major metabolic findings in the areas of prostate and breast cancer.

TEXT Box 1

Metabolic Profiling Platforms

Metabolic profiling of complex biological systems has historically been performed using some form of mass spectrometry (MS) and/or nuclear magnetic resonance spectroscopy (NMR) owing to their unique analytical capabilities.

MS has emerged as a major platform for metabolic profiling studies due to its high resolution and sensitivity. MS-based applications are built on the concept of analyte detection based on mass-to-charge (m/z) ratios. Detection of metabolites is chemocentric and requires the additional use of coupled chromatographic platforms for their optimal separation in complex biological matrices. MS is often employed for discovery-based metabolic profiling to determine global metabolic changes, where metabolites are identified using databases containing molecular information (including chromatographic retention time, parent and product ion fragmentation patterns, etc.). However, the quality of these data is highly matrix dependent. Furthermore, the chemocentric nature of metabolites also influences the profiles in these studies [29]. These factors influence the quality and extent of analyte identification obtained in these studies. Another caveat is the lack of sufficient accuracy in quantification of the entities confounded by the presence of data missingness. Alternatively, MS-based methods can be designed to test specific hypotheses and measure a small number of metabolites in a targeted manner significantly improving quantification. These methods employ multiple reaction monitoring (MRM), where both the parent and product ions are used to quantify levels of metabolites [88]. In spite of these advantages, MS-based methods are still destructive and present significant challenges for *in vivo* imaging.

Conversely, NMR-based applications provide relatively low sensitivity but very accurate and reproducible quantitative measurements. NMR is based on the physical property that nuclei will produce a shift in resonance frequency when a strong magnetic field is applied. Most NMR-based metabolic profiling studies utilize proton (^1H)-based spectroscopy, however other modes such as ^{13}C -, ^{31}P , and ^{15}N - nuclei assessments are becoming increasingly relevant. One major disadvantage of NMR-based applications is its low sensitivity, typically in the milli- to micromolar range, which limits the number of detectable molecular species. In return however, NMR offers a major advantage in that NMR-based studies are non-destructive and require little to no sample preparation prior

to analysis, thus minimizing analytical variance and facilitating applications such as non-invasive, *in vivo* metabolic profiling. Clinically, NMR has recently been applied to monitor the intra-tumoral levels of 2HG in gliomas [89–92], indicating that NMR may have much broader applications in real-time monitoring of tumor progression and therapeutic response moving forward.

Metabolic Hallmarks of Prostate Cancer

PCa is the second most common cause of cancer-related death among men in the US. It is estimated that in 2015 alone there will be over 220,000 new cases of PCa and over 27,000 deaths, in the US [4]. Organ confined (or localized) PCa is dependent on androgen for growth and development and, if detected early, is curable with surgery or anti-hormonal therapies such as Androgen Deprivation Therapy (ADT, see glossary) [8, 9]. Nevertheless, even after treatment some men will experience an elevation of prostate specific antigen (PSA, which is indicative of the presence of prostate cancer), a condition known as “biochemical recurrence.” Biochemical recurrence is a significant predictor of PCa metastasis and death, and is treated with second generation ADT [10–12]. Yet, after approximately 2–3 years, the vast majority of men that initially responded to treatment will go on to exhibit resistance, and develop lethal castration resistant prostate cancer (CRPC) [13]. Given this broad spectrum of disease presentation, significant efforts in early detection and prognosis are ongoing. PSA and digital rectal exams (DRE), in conjunction with biopsy, are the clinical standards for early detection of PCa. While PSA lacks sensitivity and specificity, and biopsy is an invasive procedure, there is a need for improved measures of non-invasive detection [14, 15].

Metabolites are products of biochemical reactions, reflect the cellular phenotype, and are less complex and can be detected in non-invasive biofluids. Therefore, metabolomics provides a promising approach to study: 1) the biology of PCa development and progression; 2) define markers for detection and risk stratification; and 3) identify novel biochemical therapeutic targets to enhance the efficacy of current PCa treatment regimes.

Understanding prostate tissue metabolite changes

Metabolic studies have consistently been used as tools to help explain the differences between tumor and normal cell types. As early as the 1970’s, nuclear magnetic resonance (NMR) spectroscopy technology was used to differentiate malignant from normal tissue in various diseases [16, 17] (Box 1).

One of the earliest studies on prostate tissue metabolism using high resolution magic angle spinning NMR demonstrated that the metabolites citrate and spermine are linearly correlated with the volume percentage of histologically confirmed normal epithelium. In a separate study, lower levels of these metabolites were reported as potential biomarkers for PCa aggressiveness [16, 18, 19]. These findings are consistent with elevated levels of citrate and zinc in normal prostate, where high levels of zinc block the citrate oxidizing activity of mitochondrial aconitase (Figure 1). However, zinc transporters are down regulated in PCa, resulting in decreased levels of zinc. This relieves inhibition of mitochondrial aconitase

resulting in increased citrate oxidation [16, 20]. Alternatively, lower levels of citrate may be the result of decreased carbon flux from glycolysis to TCA, or increased citrate utilization to generate downstream products such as lipids and amino acids. Notably, Massie, *et al.* reported that androgen receptor (AR) signaling stimulates glycolysis and anabolism in PCa *in vitro*. In their study, increased glucose uptake and lactate production were accompanied by elevated levels of citrate [21]. This suggests that the decreased citrate levels observed in localized PCa may result from increased anabolic utilization and not decreased glycolytic flux. In the mitochondrion, citrate can be oxidized to carbon dioxide and oxaloacetate for the production of ATP by oxidative phosphorylation, or it can be preferentially exported to the cytosol where it is cleaved by ATP citrate lyase to produce acetyl-CoA and oxaloacetate [22]. Acetyl-CoA is essential to generate fatty acids and cholesterol, whereas oxaloacetate is an amino acid precursor. To this end, androgen responsive cell lines have been shown to have higher levels of amino acids and their methylated derivatives (Figure 1) [23, 24]. Alterations in lipid metabolism have been consistently observed in PCa development and progression, and will be discussed below in more detail. Nevertheless, amino acid accumulation in androgen-dependent PCa would provide sufficient nitrogen to fuel the urea cycle, the antecedent of polyamine synthesis. Critical to the sustained growth and survival of the normal prostate, polyamines can be synthesized *de novo* by the enzyme ornithine decarboxylase (ODC1) or taken up from the extracellular milieu [25]. Of note, polyamine levels were found to be lower in CRPC (Figure 1) [26]. It has been reported that elevated levels of polyamines induced programmed cell death in an ODC1 overexpressing mouse leukemia cell line [27], suggesting that reduction of polyamines observed in PCa may serve to protect against apoptosis. Furthermore, during metastasis polyamines are reported to indirectly contribute to the immunosuppressive tumor microenvironment, thereby permitting tumor progression (Box 2). Additionally, extracellular spermine levels have been reported to increase as a result of hypoxia, and can decrease the expression of the cell surface glycoprotein CD44 in a dose dependent manner, suggesting spermine may play a role in enabling tumor invasion [28]. Taken together, these findings suggest polyamine metabolism may be a novel therapeutic target in the treatment of PCa, however further studies are required to test this hypothesis *in vivo*.

TEXT Box 2

Outstanding Questions

1. How does the immune response affect tumor-associated metabolic profiles?
2. Can metabolism be targeted to overcome therapeutic resistance in prostate and breast cancers?
3. Can metabolic profiles be used to stratify patients into different therapeutic regimens?
4. What are the steps needed to enable translation of metabolic markers from bench to bedside?
5. Are there common metabolic profiles associated with localized and advanced tumors across various hormone-dependent cancers?

6. What makes tumors “addicted” to specific metabolites (i.e. glucose, glutamine, etc.)? How can this information be used to develop novel therapeutic and imaging modalities?

Recent data from the study of PCa metabolism demonstrated the accumulation of sarcosine, an N-methyl derivative of glycine, during tumor progression to metastasis (Figure 1). Sreekumar *et al.* observed elevated levels of sarcosine in 79% of metastatic samples and 42% of localized prostate cancer samples, a finding that was replicated in invasive PCa cell lines [29, 30]. Treatment of benign prostate cells with sarcosine induced an invasive phenotype, and when the enzyme that produces sarcosine, glycine-N-methyl-transferase (GNMT), was attenuated, the invasiveness was reduced [29]. Furthermore, high expression of GNMT and reduced expression of the sarcosine metabolizing enzymes, sarcosine dehydrogenase (SARDH) and pipecolic acid oxidase (PIPOX), were observed in metastatic tissues [30]. Sarcosine is associated with upregulation of human epidermal growth factor receptor 2 (HER2/neu), a receptor tyrosine kinase and proto-oncogene, in androgen responsive cells, and when overexpressed in androgen independent cell lines, the metastatic potential significantly increases [31, 32].

The therapeutic resistance observed in CRPC (e.g. resistance to ADT) has also been associated with alterations in the cellular lipid profile. Consistent with this, several studies have reported elevations in total cholesterol levels following ADT [33–35]. As mentioned previously, the reduced levels of citrate identified in androgen-dependent PCa could be explained by the generation of acetyl-CoA for the production of fatty acids and cholesterol (Figure 1). A recent study by Dasgupta *et al.* demonstrated increased glutamine derived lipogenesis in CRPC [36]. The increased lipid synthesis was mediated by sterol regulatory element-binding transcription factor 1 (SREBP1), a key transcription factor that activates genes for the biosynthesis of long chain fatty acids, triglycerides, and cholesterol [37, 38]. Fatostatin, a non-sterol diarylthiazole derivative, can block SREBP1 action by preventing its nuclear translocation and subsequent transcription. By inhibiting SREBP’s ability to produce lipids and cholesterol, fatostatin can inhibit PCa growth and induce apoptosis [38]. Furthermore, studies have also shown that CRPC tissue samples have increased expression of metabolic genes including *FASN*, that encodes for fatty acid synthase (FAS), as well as those involved in steroid biosynthesis and metabolism, compared to primary prostate specimens [39, 40]. In light of this, another potential therapeutic option is to block FAS enzyme activity by stimulating the production of malonyl-CoA, an important intermediate in lipid biosynthesis, that has been shown to induce apoptosis in cancer cells [41]. Taken together, these studies suggest that targeting lipid metabolism may be a viable therapeutic strategy for treating CRPC (Box 2). However, more research is warranted to understand the underlying mechanisms of lipid metabolism in CRPC.

Identifying reliable biomarkers for PCa

Biomarker studies have utilized metabolomics to delineate potential targets of PCa risk. Urinary and serum studies have consistently identified lipids, amino acids, carbohydrates, and tricarboxylic acid cycle metabolites as potential biomarkers with varying levels of accuracy [42–46]. The Area Under the Receiver Operator Characteristic curve (AUC) is a

common statistical measure used in biomarkers studies to determine how well the variables tested can differentiate between cancer vs. non-cancerous groups on a scale of 1.0 – 0.5, with 1 showing perfect discriminatory power, and 0.5 showing poor discriminatory power [47]. In fact, one article reports that the levels of nine metabolites differentiated between PCa and normal prostate tissue with an AUC of 0.94. This discriminatory power fell to only 0.83 when five of the nine metabolites were utilized in the model to distinguish between PCa and benign prostatic hyperplasia [48]. In another study of urinary sediment, sarcosine levels were higher in biopsy positive prostate cancer patients relative to biopsy negative controls with a modest AUC ranging between 0.63–0.7 across multiple studies [29, 30, 49, 50]. Currently multivariate models have demonstrated that serum sarcosine, pyruvate, alanine, and glycine were able to distinguish PCa cases from healthy controls with a sensitivity of 84.4%, specificity of 92.9%, and an overall AUC of 0.966 [51]. In addition to biomarkers for PCa identification, efforts have also been made to define markers for measuring therapeutic response. In a recent study by Huang *et al.*, cholesterol metabolites as well as tryptophan, deoxycholate, arachidonate, docosapentaenate, pyridinolone, deoxycytidine triphosphate, and glycerochenodeoxycholate were elevated in patients who developed CRPC within 1 year, but were near normal levels in patients responding well to treatment [52]. Future studies should seek to integrate these biomarkers with epidemiological data to enhance their clinic applicability. Additional studies in independent cohorts using standardized methodology are needed to ensure the clinical translation of these markers (Box 2).

The Altered Metabolism of Breast Cancer

Breast cancer (BCa) remains the deadliest cancer in women worldwide, and the expected number of new cases is predicted to increase over the next two decades [4]. Efforts to improve breast cancer diagnosis and treatment have been complicated by the heterogeneity of the disease. There now exist improved definitions for BCa heterogeneity, which has led to improvements in subtype identification and targeted therapies for some patients, ultimately resulting in better clinical outcomes [53–55]. However there are still questions and issues that remain. Relevant to this review is the knowledge that approximately 70% of all BCa tumors are hormone-dependent. These tumors are demarcated by the expression of hormone receptors for estrogen (ER+) and/or progesterone (PR+), and are typically sensitive to hormonal therapies, discussed below. One looming issue in the treatment of hormone-dependent BCa is the fact that nearly 50% of these tumors will present either inherent or acquired resistance to hormonal therapy [56]. Among the remaining 30% of BCa which do not express ER (ER–), a subset express the receptor tyrosine kinase HER2/*neu* and are treated with targeted regimens, while the rest currently lack targeted therapies. Importantly, the acquisition of this histopathological information for subtyping currently requires invasive biopsy. Additionally, non-invasive methods to monitor an individual's response to therapy are currently lacking. Thus there is a need for sensitive and specific biomarkers that can 1) predict an individual's risk of developing aggressive cancer, and 2) follow an individual's response to treatment. In addition to diagnosis/prognosis there is a need to improve the mechanistic understanding of therapeutic resistance. Here, we will highlight

recent metabolic findings that have generated insights into hormone-dependent BCa biology, and potential strategies to overcome resistance to hormonal therapy.

Successful treatment of breast cancer relies on early diagnosis and selection of appropriate treatment. To this end, several studies have applied NMR and MS-based metabolic profiling studies for the detection and identification of potential metabolic biomarkers for BCa in patient biofluids including serum and urine, respectively [57–62] (Box 1). Within these studies, several metabolites were shown to be significantly altered in patients with BCa, however review of the literature suggests the three most commonly reduced metabolites are glycine, choline, and formate (Figure 2) [57, 58]. It has been suggested that the decreased steady-state levels of these metabolites may be attributed to their increased utilization for anabolic reactions. Consistent with these findings, Jain, *et al.* used a systematic profiling technique to track the consumption and release of metabolites from each of the NCI-60 cell lines (a panel of 60 diverse human cancer cell lines) [63]. They found that glycine and choline consumption strongly correlated with proliferation rates of cancer cells, but not with rapidly proliferating untransformed cells, and further demonstrated that the dependence on glycine may represent a novel therapeutic target [63]. Interestingly, recent work has focused on the role of serine and formate metabolism for the production of NADPH. NADPH plays a critical role in maintaining cellular redox homeostasis through the regeneration of reduced glutathione, as well as the synthesis of complex molecules such as nucleotides and lipids. Utilizing isotopically-labeled tracer compounds, two recent studies have indicated that serine and formate metabolism is a major source of cellular NADPH [64, 65]. Furthermore, several reports have demonstrated the importance of *de novo* serine synthesis from glycine via the phosphoglycerate dehydrogenase (PHGDH) pathway [66–68]. Taken together, it is likely that more studies will explore the therapeutic potential of the serine, glycine, formate, and NADPH metabolic pathways, however whether this pathway is an actionable biochemical target remains to be seen (Box 2).

In addition to studies of serum, urine and cell lines, metabolic profiling studies have been carried out on tissues as well. In an effort to describe the metabolic alterations associated with BCa relative to normal tissue, Budczies *et al.* employed an unbiased gas chromatography – time-of-flight mass spectrometry (GC-TOFMS) approach to profile metabolites in 271 breast cancer tissues and 98 normal tissues [69]. Using this method they were able to identify 478 metabolites, 79% of which were significantly differential between cancer and normal tissues. Among the metabolites identified, the nucleotide cytidine-5-monophosphate was significantly elevated whereas the fatty acid pentadecanoate was significantly decreased in cancer relative to normal tissues. Using this information they developed a two-metabolite classifier comparing the ratio of cytidine-5-monophosphate to pentadecanoate to predict tumor status in patients. With this metric they were able to predict tumor status of patients with 94.8% sensitivity and 93.9% specificity [69]. Recent work by Terunuma *et al.* employed several “-omics” platforms to biochemically stratify BCa tissues [70]. In their study, the levels of 2-hydroxyglutarate (2HG) was significantly elevated in subsets of predominately ER– BCa that had poor clinical outcome [70]. These tumors exhibited a hypermethylation phenotype, an observation resembling recent findings linking 2HG to methylation in gliomas and leukemias [6]. Furthermore, 2HG accumulation in these

tumors was associated with activation of the c-Myc oncogene and glutaminolysis [70]. An independent study found that the ratio of glutamate-to-glutamine (GGR) was positively associated ($p = 8 \times 10^{-9}$) with ER- (88%) compared to ER+ (56%) tumors (Figure 2) [71]. In line with these findings, several types of cancer exhibit glutamine “addiction”, and thus glutaminase has gained attention as a rational therapeutic target [72–74]. Intriguingly, similar observations were found in studies that investigated the metabolic requirements of BCa cell lines. By characterizing glucose consumption, glutamine consumption and glutamine dependence, Timmerman *et al.* showed that only a subset of ER- cell lines are true glutamine auxotrophs, while most ER+ cell lines were highly glycolytic (*i.e.* Warburg-like) [75]. Taken together, these metabolic profiling studies indicate that most ER- tumors and only a subset of ER+ tumors may benefit from glutaminase inhibitors, while the majority of hormone-dependent ER+ BCa may benefit from metabolic therapies aimed at inhibiting glycolysis (Box 2).

In addition to studies aimed at stratifying BCa and understanding their metabolic alterations, there is significant interest in understanding the biochemical mechanisms that accompany endocrine resistance. ER+ breast cancers are clinically treated by disrupting ER signaling using drugs such as aromatase inhibitors (AIs), which block the production of endogenous estrogens, or anti-estrogens such as tamoxifen (Tam). Tam works by competing with endogenous estrogens for ER binding, thereby preventing ER activation and downstream transcriptional programs. Despite its specificity and effectiveness in targeting hormone-dependent BCa, approximately 50% of ER+ BCa will fail Tam therapy [56]. It has long been thought that metabolism plays a role in Tam resistance, and recently several groups have started exploring this mechanism in greater detail [76]. Two stories have emerged in recent years linking Tam resistance with the activation of cholesterol and nucleotide metabolism (Figure 2) [77–79]. Using gene expression profiling, Pitroda *et al.* discovered a link between the glycoprotein Mucin 1 (MUC1) and the expression of a set of 38 cholesterol and lipid metabolism genes, including the master transcriptional regulator of lipid metabolism, *SREBP1* [80]. Elevated expression of this gene set was significantly associated with Tam treatment failure and recurrence. Another group went on to demonstrate that Tam and other ligands of the microsomal anti-estrogen binding site (AEBS) promote the accumulation of free sterols within breast cancer cells. They also found that the accumulation of these metabolites could promote cell migration and proliferation, further implicating the modulation of cholesterol metabolism as a component of Tam resistance [81–85]. While some cholesterol metabolites can promote migration and proliferation, a recent study has demonstrated the cholesterol derivative dendrogenin A inhibits tumor growth *in vitro* and *in vivo*, and is decreased in tumor tissue relative to benign adjacent tissue [86, 87]. Taken together, it would appear cholesterol metabolism plays an important and complex role in breast cancer biology. While targeting altered cholesterol and lipid metabolism may be a viable strategy, a more direct approach that successfully targeted the c-terminal subunit of *MUC1* has recently been reported. In this approach, which is currently in clinical trials, a small peptide, GO-203, was used to disrupt the interaction between the c-terminal region of *MUC1* and ER, to overcome Tam resistance [84]. More recently, two independent studies found that inhibition of the dNTP-producing enzyme ribonucleotide reductase M2 (RRM2) was associated with Tam resistance. Using gene expression analysis, elevated expression of

RRM2 was found in AKT-induced endocrine resistance breast cancer models [79]. Furthermore, genetic and pharmacological inhibition of *RRM2* significantly inhibited growth *in vitro* and *in vivo* [79]. Similar findings were reported by Putluri *et al.* that integrated metabolomic data with gene expression data in Tam resistant BCa models [78]. Importantly, in their study *RRM2* was found to be significantly associated with Tam resistance in ER+ BCa patients [78]. Although promising, it remains to be seen whether targeting lipid or nucleotide metabolic networks will prove a viable strategy in treating Tam resistant breast cancer *in vivo* (Box 2).

Concluding remarks and future perspectives

Metabolic profiling has been a vital part of our current understanding of cancer biology, and provides new opportunities to develop novel therapeutics. As this technology continues to advance, we should look forward to a fully integrated omics approach to understanding PCa and BCa. Integrating metabolomic, genomic, proteomic, transcriptomic, and epigenomic information, will provide a fully comprehensive view of the development and progression of these cancers, and identify new druggable targets. Although PCa and BCa are distinct cancers, they share many commonalities aside from their initial hormone-dependent beginnings. Metabolic studies have identified alterations in the glutamine, glycine, and lipid pathways in both cancers. Therefore, future studies may wish to focus on identifying common metabolic alterations based upon hormonal subtype and the mechanisms associated with endocrine resistance (e.g. ER+ BCa and androgen-dependent PCa; ER– BCa and androgen independent PCa, and Tam resistant BCa and castration resistant PCa) (Box 2). Looking to the future, findings from these metabolic studies should be considered and incorporated into the development of novel therapeutic drugs and strategies for the treatment of hormone-dependent cancers.

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Glossary

Androgen Deprivation Therapy (ADT)	anti-hormone therapy for prostate cancer. ADT reduces the levels of androgens, such as testosterone and dihydrotestosterone that are required for the growth of prostate cancer cells. Reducing androgens can slow the growth of the cancer and/or shrink the tumor
Area Under the Receiver Operator Characteristic curve (AUC)	receiver operating characteristic curves exhibit the relationship between sensitivity (true-positive rate) and 1-specificity (false-positive rate) of a condition; the area under a curve (AUC) defines the capacity of a test or statistical model to distinguish a diseased from a non-diseased

Castration resistant prostate cancer (CRPC)	progression of PCa despite ADT; includes increasing PSA, progression of the pre-existing disease, and/or the development of metastasis
Microsomal anti-estrogen binding site (AEBS)	an intracellular high-affinity membranous binding site for synthetic nonsteroidal antiestrogens, including tamoxifen, which has been shown to play an important role in cholesterol metabolism
Prostate specific antigen (PSA)	protein produced by the prostate gland; elevated blood levels of this protein have been observed in men with PCa.

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Highlights

- Hormone-dependent cancers exhibit metabolic reprogramming.
- PCa exhibits metabolic alterations in polyamines, amino acids, and fatty acids
- BCa displays reprogramming in glycine, serine and, choline metabolism
- Therapeutic resistance is associated with altered lipid metabolism.

Figure 1. Metabolic alterations of prostate cancer

Major changes in the development of hormone-dependent prostate cancer relative to normal prostate tissue include decreased levels of zinc (Zn^{2+}), enabling the utilization of citrate through the mitochondrial tricarboxylic acid (TCA) cycle, and decreased accumulation polyamines. Upon acquiring castration resistance, the prostate metabolic profile exhibits elevated levels of lipids, cholesterol and sterols. Furthermore, the level of sarcosine, a derivative of glycine, has been reported to accumulate in both hormone-dependent and castration resistant prostate cancers. Red indicates significant increase in either metabolite or enzymatic pathway activity.

Figure 2. Metabolic alterations of breast cancer

Major metabolic changes in the development of hormone-dependent breast cancer promote increased production of lipids used for the plasma membrane, nucleotides for DNA synthesis, and amino acids (AAs) for protein synthesis. This is supported through metabolic alterations, which include increased uptake of choline to synthesize lipids. Additionally, uptake of glycine and formate increase, to support production of nucleotides and AAs. It has been found that hormone-dependent cancers exhibit increased glucose consumption and aerobic glycolysis, increasing lactate production and pentose phosphate pathway (PPP) intermediates, which also fuels nucleotide production. In contrast ER-, or hormone-independent, breast cancers exhibit a shift from glycolysis towards glutamine consumption to fuel the tricarboxylic acid (TCA) cycle as well as donate nitrogen and carbon as precursors for proteinogenic AAs and nucleotides. Red indicates significant increase in either metabolite or enzymatic pathway activity.

Table 1

Reported metabolic alterations in Prostate and Breast Cancer

Methodology	Metabolites/Metabolic Classes Identified	Cancer Type	Ref
HRMAS ¹ H NMR	Spermine ↓, citrate ↓		[18]
	Choline ↑, choline derivatives ↑, lactate ↑, alanine ↑		[93]
LC GC/MS	Sarcosine ↑		[29]
GC/MS	Fatty acids ↑ Pyrimidines ↓, Creatinine ↓, Purines ↓, Glucosides ↓		[48]
LC/MS	Sarcosine ↑, Threonine ↑, Phenylalanine ↑, Alanine ↑, Nitrogen metabolites ↑, Tryptophan ↓	PCa vs. Normal	[24]
	Homocystein ↑, Polyamines ↑ S-Adenosylmethionine ↓	AR Dependent vs. AR Independent PCa	
LC/MS	Sugars ↓, Energy signaling metabolites ↓, Aminosugars ↑, Methylation metabolites ↑, Amino acids ↑	AR Dependent PCa vs CRPC	[23]
LC/HRMAS	Thymine metabolites ↓, Nitrogen metabolites ↓, Tri-peptides ↓, Tryptophan metabolites ↑	PCa vs. Normal	[44]
GC-LC/MS	Lysine degradation ↑, Fatty acids/derivatives ↑ Sugar/sugar acids ↓, Mevalonate metabolism ↓, Purines ↓		[94]
ULC/MS/MS	Amino acids ↑, Carnitines ↑, Purines ↑, Pyrimidines ↑, Choline ↑ Laurate ↓, Malate ↓, Mannose ↓, ADP ↓	Extracapsular extension PCa vs. Organ-confined PCa	[95]
	Polyamine ↓, Fatty acids ↓, Sugars ↓ NAD+ ↑, Choline derivatives ↑	Extracapsular extension PCa vs. Seminal vesical PCa	
HRMAS	Spermine ↓, Citrate ↓	High grade PCa vs. Low grade PCa	[19]
ULC/MS	Nonanedioic acid ↓, Phenylalanyl phenylalanine ↓, Lysophospholipids ↓ Hexadecanedioic acid ↑, Tryptophan ↑, Steroid hormone metabolites ↑	PCa vs. Normal	[45]
GC/LC/MS	Urea cycle ↓, TCA ↓, Amino acids ↓, Purines ↓		[43]
¹ H NMR	Sarcosine ↑, Alanine ↑, Pyruvate ↑, Glucine ↓	High grade and Low grade PCa vs. Normal	[51]
	Alanine ↓	Low grade vs. High grade PCa	[43]
HR MAS MRS	Glycine ↑, Phosphocholine (PC) ↑	Lymph node invasion vs. non-Lymph node invasion BCa	[59]
	Taurine ↓	Low grade vs. High grade PCa	
NMR and GCxGC-MS	Tyrosine ↑, Lactate ↑	Recurrent vs non-recurrent BCa	[57]
	Formate ↓, Histidine ↓, Proline ↓, Choline ↓, N-acetyl-glycine ↓, Glutamate ↓, 3-hydroxyl-2-methylbutanoic acid ↓, Nonanedioic acid ↓		
NMR	Formate ↓, Succinate ↓, Uracil ↓, Hippurate ↓, Leucine ↓, Asparagine ↓, Acetate ↓, Isoleucine ↓, Creatinine ↓, Urea ↓	BCa vs. Normal	[58]
HR MAS MRS	Glycine ↑, Glycerophosphocholine ↑, Choline ↑, Alanine ↑	ER-negative vs ER-positive BCa	[60]
	Ascorbate ↓, Creatine ↓, Taurine ↓, Phosphocholine ↓		
NOSEY1D and CPMG NMR	Phenylalanine ↑, Glucose ↑, Proline ↑, Lysine ↑, N-acetyl cysteine ↑	BCa vs Normal	[62]

Methodology	Metabolites/Metabolic Classes Identified	Cancer Type	Ref
	Lipids ↓		
NMR and LC-MS	Isoleucine ↑, Histidine ↑	Pathologic complete response vs Non-responsive BCa patients	[61]
	Threonine ↓, Glutamine ↓, Linolenic acid ↓		
GC-TOFMS	Cytidine-5-monophosphate ↑, Adenosine-5-monophosphate ↑, Phosphoethanolamine Taurine ↑, Pyrazine 2,5-dihydroxy Creatinine ↑, N-acetylaspartate Hypoxanthine ↑, Glycerol-alpha-phosphate ↑, Aminomalonate ↑, Glutamic acid ↑, Malate ↑, Oxoproline ↑	BCa vs. Normal	[69]
	Heptadecanoic acid ↓, Lignoceric acid ↓, 1-hexadecanol ↓, Pentadecanoic acid ↓, Glycolic acid ↓, Benzoic acid ↓, Hydroxylamine ↓		