Review

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Metabolomic profiling of hormone-dependent cancers: a bird's eye view

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Hormone-dependent cancers present a significant public health challenge, because 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 on prostate (PCa) and breast cancer (BCa) have made great strides in uncovering specific metabolic pathways that are associated with cancer development, progression, and resistance. In this review, we highlight some of the major findings and potential therapeutic advances that have been reported utilizing this technology.

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 with 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 PCa and testicular in men, and BCa, ovarian, and endometrium in women, are the most commonly diagnosed cancers in the world [3]. In the USA alone, the lifetime risk of developing PCa is 1:7 for men, and 1:8 and 1:76 for BCa and ovarian cancers, respectively, 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 antihormone 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 provide a bird's eye view of the major metabolic findings in the areas of PCa and BCa.

Metabolic hallmarks of PCa

PCa is the second most-common cause of cancer-related death among men in the USA. It is estimated that in 2015 alone there will be over 220 000 new cases of PCa and over 27 000 deaths, in the USA [4]. Organ-confined (or localized) PCa is dependent on androgen for growth and development and, if detected early, is curable with surgery or antihormonal 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 PCa), 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, most men who initially responded to treatment will go on to exhibit resistance, and develop lethal castration-resistant PCa (CRPC) [13]. Given this broad spectrum of disease presentation, significant efforts in early detection and prognosis are ongoing. PSA and digital

Glossary

Androgen deprivation therapy (ADT): antihormone therapy for PCa. ADT reduces the levels of androgens, such as testosterone and dihydrotestosterone, that are required for the growth of PCa 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 relation between sensitivity (true-positive rate) and 1-specificity (false-positive rate) of a condition; the AUC defines the capacity of a test or statistical model to distinguish a diseased state from a nondiseased state.
Castration-resistant PCa (CRPC): progression of PCa despite ADT; includes increasing PSA, progression of the pre-existing disease, and/or the development of metastasis.
Microsomal antiestrogen binding site (AEBS): an intracellular high-affinity membranous binding site for synthetic nonsteroidal antiestrogens, including Tam, which has been shown to have 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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Box 1. Metabolic-profiling platforms

Metabolic profiling of complex biological systems has historically been performed using some form of MS and/or 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: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 used 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 matrix dependent. Furthermore, the chemocentric nature of metabolites also influences the profiles in these studies [29]. These factors affect 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 used multiple reaction monitoring (MRM), where both the parent and product ions are used to quantify levels of metabolites [88]. Despite 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 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 (^{1}H)-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. However, in return NMR offers a major advantage in that NMR-based studies are nondestructive and require little to no sample preparation before 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 intratumoral levels of 2HG in gliomas [89–92], indicating that NMR may have broader application in real-time monitoring of tumor progression and therapeutic response moving forward.

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, are less complex, and can be detected in non-invasive biofluids. Therefore, metabolomics provides a promising approach to: (i) study the biology of PCa development and progression; (ii) define markers for detection and risk stratification; and (iii) 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 1970s, 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 (HRMAS) NMR demonstrated that the metabolites citrate and spermine were 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 downregulated in PCa, resulting in decreased levels of zinc. This relieves the 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 the tricarboxylic acid (TCA) cycle, or increased citrate utilization to generate downstream products, such as lipids and amino acids. Notably, Massie *et al.* reported that androgen receptor (AR) signaling stimulated 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 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-coenzyme A (CoA) and oxaloacetate [22]. Acetyl-CoA is used for the generation of 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 are 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 programed cell death in an ODC1-overexpressing mouse leukemia cell line [27], suggesting that the reduction of polyamines observed in PCa serves 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 that spermine has a role in enabling tumor invasion [28]. Taken together, these findings suggest polyamine metabolism as a novel therapeutic target in the treatment of PCa; however, further studies are required to test this hypothesis *in vivo*.

Recent data from the study of PCa metabolism demonstrated the accumulation of sarcosine, an *N*-methyl derivative of glycine, during tumor progression to metastasis