

Review

Metabolic alterations in tissues and biofluids of patients with prostate cancer

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Abstract

Altered metabolism is one of the key molecular characteristics of prostate cancer (PCa) development, and a number of studies have searched for metabolic biomarkers in patient-derived samples. Reported metabolic changes in PCa tissue compared with normal tissue include reduced levels of citrate and spermine, elevated lipid synthesis and fatty acid oxidation, and higher levels of the amino acids alanine, glutamine, and glutamate. Recent studies have investigated the tumor micro-environment, such as reactive stroma, and so-called metabolic field effects. Furthermore, analysis of blood and urine samples reveals other, but significant, metabolic differences between patients with PCa and controls. Large-cohort studies show promising results for assessing future PCa risk from blood samples. In addition, metabolic profiling of extracellular vesicles from urine has gained interest as a noninvasive and more prostate-specific approach to diagnose PCa.

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Keywords

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Introduction

Metabolic reprogramming is one of the key features of cancer development [1]. Substantial efforts are being made to metabolically characterize prostate cancer (PCa) to identify novel biomarkers that can offer more accurate diagnosis and assessment of patient prognosis.

Here, we review the accumulated knowledge and recent findings of metabolic alterations in PCa, with a focus on metabolic changes detected in human-derived samples, such as tissue, blood, and urine.

The unique metabolism of the healthy prostate

Luminal epithelial cells represent the glandular structures in the prostate, which is known to exhibit a unique metabolism. These cells are responsible for producing and secreting prostatic fluid which has an unusually high concentration of citrate and spermine, along with the other polyamines putrescine and spermidine [2,3]. Secretion of citrate and spermine are suggested to function as quick energy sources for spermatozoa. The polyamine biosynthesis pathway is highly dependent on the methionine salvage pathway, which provides aminopropyl groups required to produce both spermidine and spermine [4]. Despite the accumulated knowledge on polyamine metabolism [5], the specific mechanisms in healthy prostatic epithelial cells that cause the particularly high production of polyamines are still unknown. The metabolic mechanism behind the elevated citrate levels in prostate epithelial cells is however better characterized. The prostate luminal cells have an unusually high concentration of the metal zinc [2,6], which is believed to be the cause of citrate accumulation. The elucidation of this mechanism has been conducted by Costello and Franklin over the last decades. Their research suggests that zinc inhibits the tricarboxylic acid (TCA) cycle, enzyme aconitase, responsible for converting citrate to isocitrate, thereby causing citrate to accumulate [2]. The elevated citrate production is also believed to be supported by a high import of carbon sources glucose and aspartate [7].

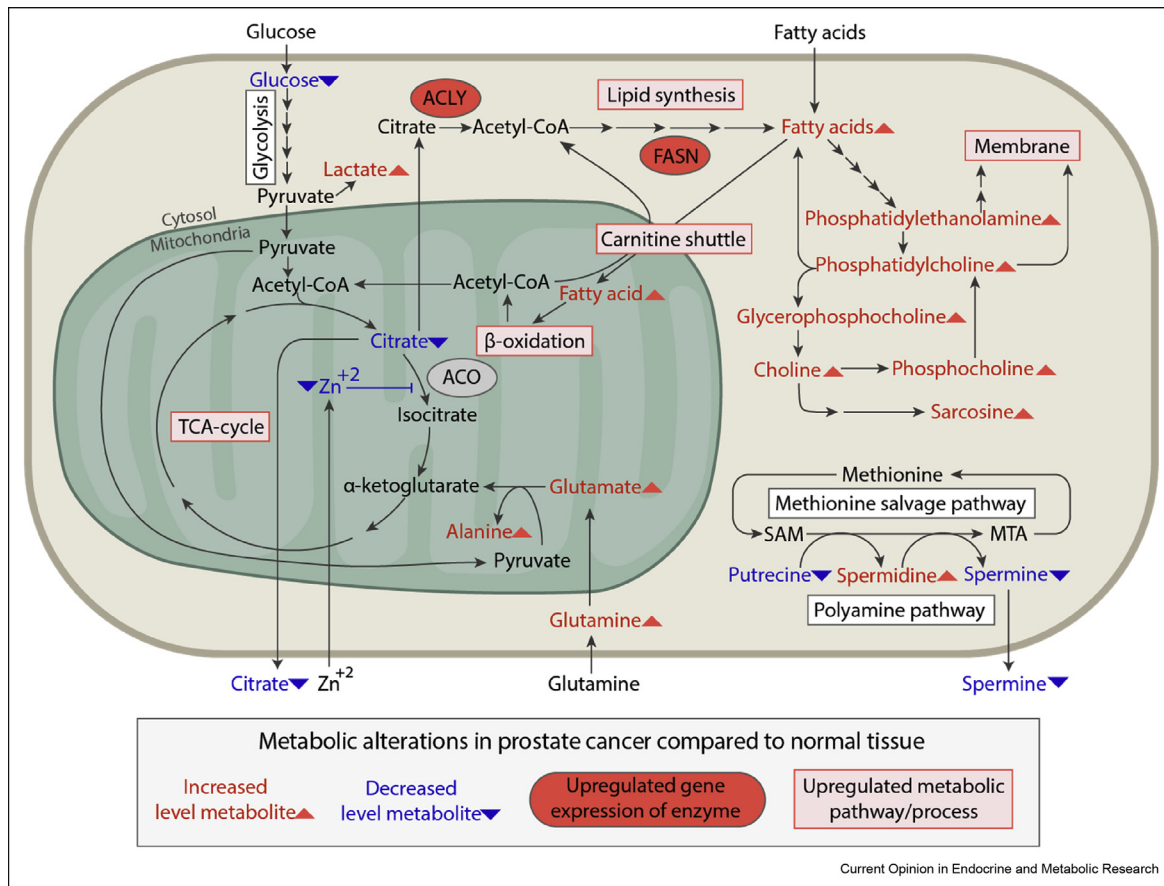
Tissue-specific metabolism of PCa

Analysis of tissue samples represent snapshots of real-life PCa metabolism, and much research has therefore been carried out measuring tissue metabolite levels. [Figure 1](#) shows an overview of the commonly reported metabolic alterations in PCa tissue compared with normal tissue.

Warburg effect and PCa

The Warburg effect represents a common metabolic change of cancer cells, namely increased import of

Figure 1



Overview of reported key metabolic alterations in prostate cancer tissue compared with normal tissue. ACLY, acetyl citrate lyase; ACO, aconitase; FASN, fatty acid synthase; MTA, 5'-methylthioadenosine; SAM, S-adenosyl methionine; Zn, zinc.

glucose for aerobic glycolysis, manifested by an increased production of lactate [8]. In contrast to most other cancers, PCa cells do not appear to have a highly increased import of glucose, showcased by the fact that positron emission tomography imaging using radiolabeled glucose is not able to accurately differentiate between healthy and malignant prostates (reviewed in the study by Bednarova et al. [9]). In fact, several studies have reported slightly lower levels of glucose in PCa tissue than in normal tissue [10–12]. Although PCa cells do not have a clearly increased glucose uptake, we have previously reported elevated lactate levels in PCa tissue compared with those in benign tissue [11,13]. This indicates a shift in prostate cells from glycolysis, producing citrate in a healthy state, to glycolysis, producing lactate during cancer progression.

Zinc and citrate metabolism

The perhaps most acknowledged and documented metabolic alteration in PCa tissue is reduced levels of citrate, which has been observed by *ex vivo* magnetic resonance spectroscopy (MRS) [11,14], *in vivo* magnetic

resonance spectroscopy [15], mass spectrometry (MS) [12,16], and more recently with MS imaging [17], [*18]. We have previously shown that citrate levels are lower not only in cancer tissue than in healthy tissue but also in high-grade PCa tissue than in low-grade PCa tissue [11]. This finding was also reported by McDunn et al. who reported citrate to be significantly inversely correlated with Gleason score [12]. Furthermore, we have showed that low citrate levels are linked to biochemical recurrence after radical prostatectomy and therefore may be a valuable prognostic biomarker [*19].

The reduced levels of citrate is suggested to be a direct consequence of lower import and hence lower levels of zinc in PCa tissue [2,6,20], which is also shown to be a predictor of biochemical recurrence [21]. Less zinc causes aconitase to regain its activity and leads to citrate being utilized further in the TCA cycle. A recent multi-omics-based publication by Shao et al. [*22] supports this hypothesis by identifying increased TCA cycle activity in PCa tissue compared with that in adjacent normal tissue, through metabolic profiling and gene

expression analysis. Citrate can also be utilized for lipid synthesis as described in the section ‘[Cholines and lipid metabolism](#).’

In the past, the zinc–citrate mechanism had to be analyzed by different methods. We have recently demonstrated, for the first time, simultaneous spatial detection of zinc and citrate, along with other metabolites, by MS imaging on prostate tissue sections. Zinc and citrate were positively correlated, and both had significantly lower levels in cancer glands than in noncancerous prostatic glands [*23]. In addition, substantially reducing the experimental workload, this method allows for a more accurate and coherent study of this important pathway in heterogeneous PCa tissue.

Cholines and lipid metabolism

Choline is an essential metabolite required for synthesis of many phospholipids and is widely reported as increased in many cancers as it facilitates the need for continuous cell growth and rearrangement of membranes [24]. Elevated levels of choline and choline-containing metabolites and lipids, such as phosphocholine and phosphatidylcholines, have been reported in several PCa studies [11,15,16,18,25,26]. Radiolabeled choline is also found to be a much more reliable tracer than glucose for clinical positron emission tomography imaging of PCa [9], indicating that PCa cells have an increased intake of choline rather than glucose.

PCa tissue has high lipid content [27]. Along many other cancers, PCa cells are capable of *de novo* fatty acid synthesis, a biological process normally limited to liver cells and adipocytes. A key enzyme in lipogenesis is fatty acid synthesis transferase (FASN), which is shown to have increased gene expression in PCa [10]. Another enzyme of importance is ATP citrate lyase (ACLY), which utilizes citrate for fatty acid synthesis and is also reported to have increased gene expression in PCa [10,28]. Fatty acid synthesis and elevated choline intake facilitate production of phospholipids needed for membrane synthesis.

Lipids are also an important energy source for PCa cells through fatty acid β -oxidation. The rate-limiting step of β -oxidation is the transport of fatty acids across the mitochondrial membranes, facilitated by the carnitine shuttle. Ren *et al.* [16] used an integrative metabolomics and transcriptomics approach to profile normal and cancerous prostatic tissue and found that both the carnitine shuttle metabolites and gene expression of transporter proteins were elevated in PCa.

Polyamines

Altered polyamine metabolism has been widely reported in PCa tissue compared with that in healthy tissue [11,29,30]. Lower concentrations of spermine and

putrescine are reported not only for cancer than in normal samples [10] but also in aggressive prostate tissue than in low-grade tissue [11,12]. In addition, the level of spermine is proposed to be an independent prognostic marker of biochemical recurrence [*19]. In contrast, higher levels of spermidine has been detected in cancer than in benign prostate tissue [12,30].

Altered amino acid levels

PCa tissue exhibits altered amino acid levels compared with normal tissue, which includes elevated levels of alanine, glutamine, and glutamate [11,13,26]. In addition to being important for protein synthesis, these amino acids are closely linked to both glycolysis and the TCA cycle (Figure 1). Glutamine, which is an essential amino acid for cancer cells, can be oxidized to glutamate, which can help replenishing the TCA cycle by conversion to α -ketoglutarate. This reaction in turn produces alanine as a by-product. Recently, the metabolism and uptake of glutamine has been linked to a neuroendocrine phenotype in PCa cells [31].

Metabolism and field effects

Cancer field effects describe the phenomenon in which the tumor may alter the adjacent benign tissue. Such field effects have been detected in histologically benign prostate tissue surrounding the tumor, which has a metabolic profile that can be distinguished from benign samples taken far away from the cancer [32]. Metabolic field effects could be a result of multiple mechanisms including tumor–stroma interactions [33] or possible metabolite diffusion [34]. Metabolic field effects in histologically benign tissue might be interesting tools for clinical purposes and for understanding early development of PCa.

Metabolic profiling of reactive stroma

Reactive stroma is a common tumor microenvironment characteristic present in many prostate tumors and is linked to faster clinical recurrence after radical prostatectomy [33]. We recently performed metabolic profiling of reactive stroma by comparing PCa tissue samples with low- and high-reactive stroma content and identified elevated levels of the metabolite taurine in high-reactive stroma [33]. We hypothesize that taurine, by its antioxidant effect, is linked to the inflammatory phenotype of reactive stroma.

Altered metabolite levels in biofluids

Because sampling of biofluids such as blood or urine is minimally invasive, it is highly suitable for screening and patient monitoring. Unlike metabolism measured in tumor tissue, the metabolome of biofluids will reflect the metabolism of the entire organism, and the correlation between tumor and serum metabolites have been shown to be limited [35]. Still, several studies have shown significant metabolic differences in serum from

patients with PCa and controls [36–40]. Similarly, studies analyzing urine have detected metabolic differences between patients with PCa and controls, both with [41] and without [39,42–46] prior prostate massage. Analysis of volatile organic compounds in urine has also shown significant differences between patients with PCa and controls [47–49].

Perhaps the metabolite that has gained most attention as a biofluid PCa biomarker is sarcosine, which Sreekumar et al. [46] suggested as a diagnostic marker in tissue, urine, and serum samples. Since then, many have tried to validate sarcosine as a biofluid biomarker for PCa, both supporting [37,50] and contradicting [39,41,51,52] the results of Sreekumar et al. The case of sarcosine exemplifies the challenges with finding a single metabolic biomarker with consistent and reliable clinical discriminatory power across different methods and patient cohorts.

Metabolic profiling of serum metabolites to assess future PCa risk

Recent studies on large patient cohorts have been performed to assess the risk of future PCa diagnosis from serum metabolomics. de Vogel et al. [53] performed targeted metabolic measurements of six metabolites in serum samples from a group of 6000 men, of which half were diagnosed with PCa after inclusion. They found that high levels of sarcosine and glycine were associated with a modestly decreased PCa risk. In a more recent study, Schmidt et al. [54] investigated the possibility to detect future or undetected PCa from serum metabolites in a group of more than 2000 men. Metabolites related to lipid metabolism, such as acylcarnitines, glycerophospholipids, and sphingolipids, were associated with future PCa diagnosis. Sarcosine and glycine were measured, but were not among the significant metabolites. Furthermore, seven metabolites were associated with lethal PCa before, but not after, multiple testing correction. In a similar study, Huang et al. [*55] identified 34 serum metabolites, including amino acids, lipids, nucleotides, and peptides, associated with lethal PCa in a cohort of more than 1000 cases and controls. Only two of the metabolites associated with lethal cancer in the study by Schmidt et al. [54] were measured, and these were not found to be significant.

Metabolic profiling of extracellular vesicles in urine

Extracellular vesicles (EVs) in urine are secreted from cells surrounding the urinary tract and are interesting because they potentially contain metabolic information directly from the PCa tissue. Puhka et al. [56] were the first to demonstrate the possibility to perform metabolic profiling of urinary EVs in patients with PCa and detected four metabolites (adenosine, glucuronate, isobutyryl-L-carnitine, and D-ribose 5-phosphate) with

lower levels in patients with PCa. These metabolic alterations in EVs were different from the metabolic changes in the remaining urine. Skotland et al. [57] identified a panel of lipids that could separate patients (n = 15) and controls (n = 13) with high accuracy. In the most recent study, Clos-Garcia et al. [*58] found 76 EV metabolites to have different levels between patients with PCa and benign hyperplasia controls, including reduced levels of several phosphatidylcholines and increased levels of acylcarnitines and sterols in PCa. Despite the small study cohorts, these studies demonstrate the possibility to detect PCa noninvasively by metabolic profiling of urinary EVs, which may be more prostate specific than analyzing urine.

Future perspectives and concluding remarks

Metabolic profiling remains a hot topic in PCa research. There are still knowledge gaps concerning metabolism and PCa development. Although there are several recent publications suggesting metabolic biomarkers for PCa diagnosis and monitoring, none of the findings are currently ready to be used in the clinic. There is a need for better methodological standardization and large, randomized clinical trials before results can be brought into clinical use. We anticipate that any future metabolomics-based tests for PCa will consist of a signature of several metabolites or a metabolic pathway rather than a single metabolite biomarker. Furthermore, new methodical developments within MS imaging, which shows the spatial distribution of metabolites on heterogeneous tissue, along with multi-omics analysis and EV profiling offer new insight into both established metabolic alterations and facilitate findings of novel metabolic biomarkers to aid future PCa diagnostics and prognostics.

Conflict of interest statement

Nothing declared.

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