



Metabolomics study in diagnosis of prostate cancer with metastases

Pradeep Kumar, Sujeet Kumar Mewar*, and Pawan Kumar

Department of NMR, AIIMS, New Delhi, India

Abstract: Metastasis to the bone is one clinically significant feature of prostate cancer (PCa). Present diagnostic approaches cannot predict metastases of PCa at a treatable stage of the disease. PCa is rarely present with symptoms in the initial course of the disease and appears during the advanced stage of cancer. The technical investigation of all the metabolites in tissues and biofluids is known as metabolomics. Information on the dynamic changes that take place during the onset and course of cancer can be obtained using metabolomics. Sophisticated metabolomics approaches will identify metabolites that will lead to the discovery of biomarkers for cancer therapy, diagnosis, and early detection. The metabolic alterations in cancer are briefly discussed in this overview, with an emphasis on metastasized prostate cancer.

Index Terms: Prostate Cancer, Metastases cascades, Metabolomic, Biomarker, Biofluids

1. Introduction:

PCa is the most frequently diagnosed non-cutaneous malignancy in men in the world [1]. Progression of cancer can present with symptoms such as obstructive, irritative micturition, etc. The mainstream of PCa screening is a serum prostate-specific antigen (PSA) test along with digital rectal examination (DRE). The early diagnosis of PCa is based on the combination of abnormal DRE and elevated serum PSA followed by transrectal ultrasound scan (TRUS) guided prostate biopsy and bone scan. The diagnosis of metastases PCa is difficult due to the lack of sensitive and specific tumor markers. PCa incidence increases rapidly with age, particularly over the age of 50 years [2]. Also, other multiple factors including genetic differences, environmental factors, changing diet habitats, increasing obesity, and increasing adoption of PCa screening have been proposed to be responsible for the rise in incidence rates [2, 3]. The exact cause of developing PCa is not known though aging, ethnicity, and heredity are important factors involved in the initiation and development of this cancer. Aging is considered the most prominent risk factor, with the majority of cases being diagnosed in men between 60 and 70 years of age. Late detection of PCa in advanced or metastases stages and variations in resources for access to therapy are thought to contribute to higher mortality rates. A recent report by the American Cancer Society estimated new cases of PCa in the United States for 2023 as 1,958,310 and it has been predicted that 609,820 men will die from PCa [4]. In India, it is the second most common cancer in men as per the Indian Council of Medical Research (ICMR) and various state cancer

registries. The cancer rate in India is 9-10/100,000 population which is higher than in other parts of Asia and Africa but lower than Europe and the USA [5]. PCa is the second leading site of cancer among men in large Indian cities like Delhi, Kolkata, Pune, and Thiruvananthapuram, and third in cities like Bangalore and Mumbai [6].

To date, limited studies have shown the potential of the metabolic approach in blood and urine samples of PCa with metastases [7-8]. Furthermore, patients with early PCa present with insignificant tumors that may not be aggressive to produce pathological variable problems. Despite various studies using pathology and molecular basis of metastases PCa, not much information is available on the underlying biochemical and invasion, proliferation, and tumorigenesis processes. This review briefly discusses metabolic changes in cancer, focusing on metastasized prostate cancer.

2. Prostate cancer metastases: The metastatic cascades:

The majority of cases of metastatic cancer are still deadly and incurable. Figure 1 illustrates the multi-step, intricate process of metastasis. The process by which cancer cells travel from a primary tumor to other, remote locations in the body to form secondary tumors is known as metastasis [9]. Even though this is an ineffective technique, the results are distressing because metastatic disease is the cause of over 90% of patient deaths connected to cancer. A sequence of events that enable cancer cells to escape from the originating location, survive in the blood vessels or lymphatic system, and proliferate and thrive at distant places culminating in the production of metastases [10].

The primary reason that PCa patients die is metastasis. Prostatectomy is the initial line of treatment for individuals with advanced-stage PCa. Gonadotropin hormone-releasing hormone (GnRH) equivalents are then used in first-line hormonal therapy to block androgen receptor (AR) driven signaling pathways. Castration-resistant PCa (CR-PCa) is an incurable recurrent disease state that results from suppression of AR activity after a brief period of regression. At this point, the patient receives treatment on second-line castration regimens to further suppress AR function (enzalutamide) and/or adrenal androgen biosynthesis (abiraterone) [9, 11].

The majority of CR-PCa patients get metastases, and most patients eventually become resistant to these treatments despite their best efforts. A portion of patients with advanced CR-PCa may eventually develop an AR-independent phenotype, histologically exhibiting significant neuroendocrine (NE) features, as a possible mechanism of adaptive resistance to AR-targeted treatment. The most aggressive and lethal subtype of prostate cancer is called neuroendocrine prostate cancer, or NE-PCa [12]. It has strong metastatic propensities. Understanding the many types of PCa cells and the sequence of molecular changes that underlie this and other deadly forms of PCa will help in the search for and creation of innovative, effective treatments for both primary tumors and metastases [13]. The cascades of metastatic prostate cancer, also known as metastasis, is a multi-step, intricate process in Figure 2.

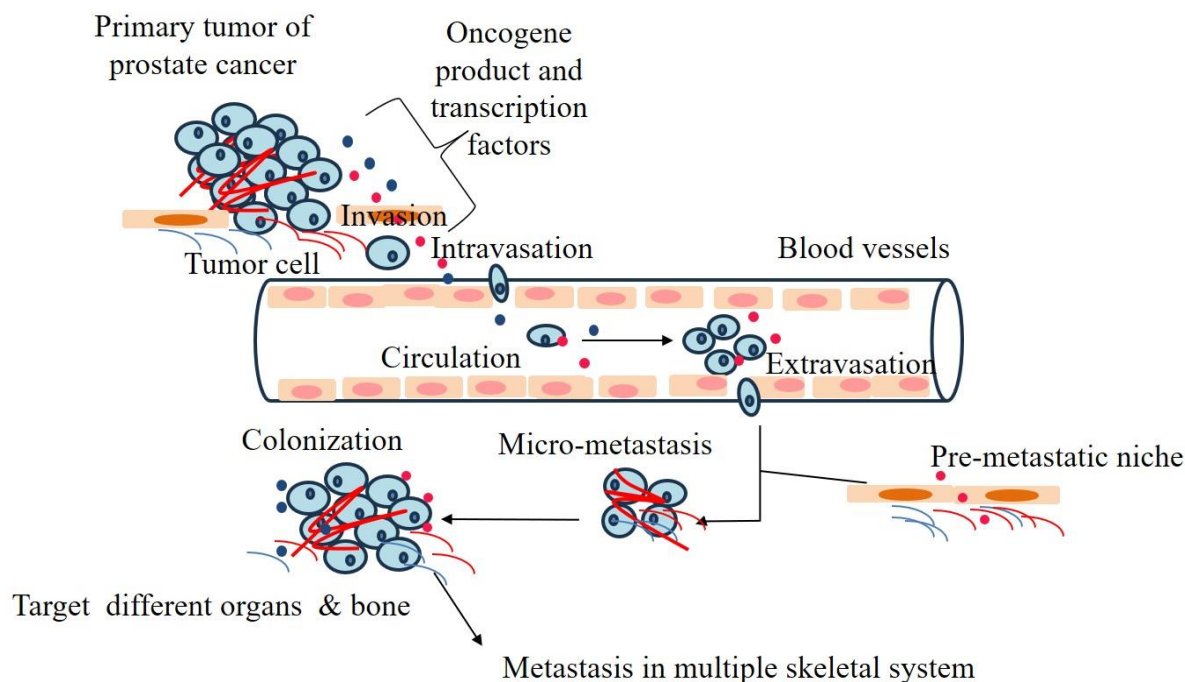


Figure 1: Metastatic prostate cancer cascades- metastasis is a complex and multi-step process.

3. Metabolomic approaches:

Metabolomics is a novel field of ‘omics’ that provides downstream analysis of proteomics and transcriptomics. It is a promising tool to discover new disease biomarker/s and provide novel insights into disease biochemical mechanisms and metabolic pathways. The goal of metabolomics is to comprehensively identify and quantify all endogenous and exogenous small molecule metabolites in a biological system [13]. “Metabolome” was first used by Oliver in 1985 to describe the collection of small molecules in an organism. Numerous biologically varied compounds, including lipids, organic acids, carbohydrates, amino acids, nucleotides, energy molecules, and others, are found in the metabolome [14]. Metabolites can be measured in a variety of different samples including tissues, biofluids (blood, urine, feces, seminal fluid, saliva, bile, cerebrospinal fluid), and cell culture [15]. Three main methods are available for measuring metabolites: nuclear magnetic resonance spectroscopy (NMR), liquid chromatography (LC-MS), and gas chromatography-mass spectrometry (GC-MS). Metabolomics is a rapidly growing approach that can provide integrated insight into complex disorders including prostate disease. Furthermore, as metabolomics is complementary to genomics, transcriptomics and proteomics, full integration of metabolites will ultimately lead to personalized molecular diagnosis and treatment of diseases [15-16].

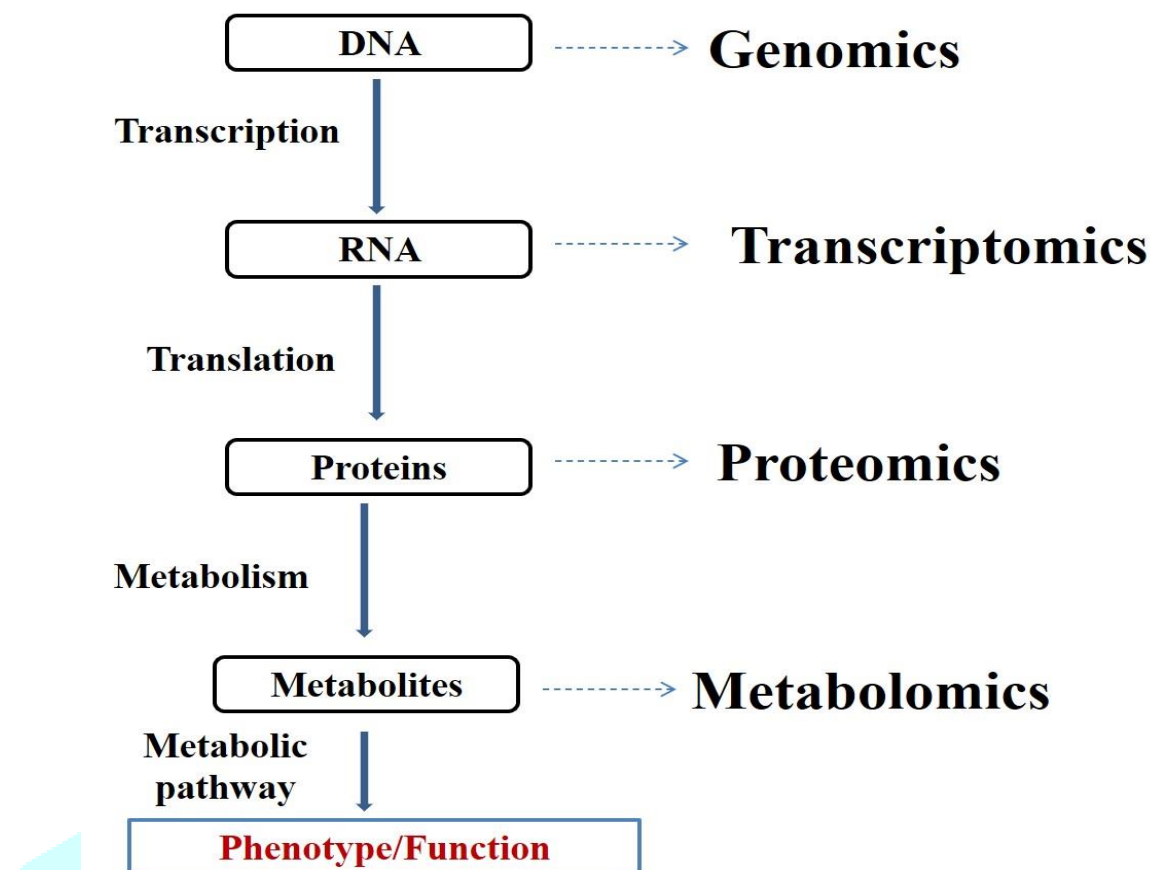


Figure 2: Overview of 'OMICS' cascade.

4. Metabolomic associated with prostate cancer metastases:

Numerous studies have been conducted to determine the genetic and proteomic features of PCa and metabolic disorders associated with PCa have been used to benefit from magnetic resonance spectroscopy. However, Sreekumar and colleagues recently used liquid and gas chromatography-time of flight mass spectrometry (GC/TOFMS) to profile the metabolome in tissue, urine, and plasma from PCa patients and discovered alterations associated with the progression of the illness [18]. It has been particularly shown that sarcosine, an N-methyl derivative of glycine, may be a major marker for PCa cell invasion, migration, and aggressiveness [18].

Metabolites in bone metastases were assessed in a highly thorough metabolomic profiling investigation to find indicators of prostate cancer aggressiveness. Using GC/time of flight MS (GC/TOFMS), biopsies of bone metastases from patients who had been hormone naïve and those who were castration-resistant were compared to the matched normal-appearing bone from the same patients, respectively [7]. Thirty-four identified metastatic bone specimens were shown to be significantly discriminated against by seventy-one metabolites. A different group of bone metastases samples from patients who were resistant to castration and matched normal bone from the patients was employed to validate these metabolites. The primary method for separating the metastatic samples was amino acid metabolism [7].

The one metabolite that discriminated the most was cholesterol, which had considerably higher levels in the metastatic samples. It was also shown that bone metastases from prostate cancer had far more cholesterol than those from breast, lung, kidney, and oesophageal cancers. Additional metabolites that showed significant

increases in the metastatic samples included fatty acids, myo-inositol-1-phosphate, fumarate, citric acid, and glycerol-3-phosphate [7]. Several of these metabolites were also linked to progression and may be a sign of the high bioenergetic requirements of cellular proliferation in bone metastases. Further analysis comparing men's initial prostate cancers with and without bone metastases identified eight distinct metabolites with discriminant capacity. Among them, the collection of major discriminating metabolites in bone metastases comprised asparagine, threonine, fumaric acid, and linoleic acid [7].

It is still very difficult to identify PCa and its metastases in clinical research backgrounds. A different study discovered that patients with PCa had several distinctive changes in their NMR-based serum metabolism, such as elevated trends of alanine and decreased trends of 3-HB and acetone when compared to BPH subjects, and decreased levels of alanine and increased levels of 3-HB and acetone when compared to PCa with bone metastases patients. They also reported on the use of clinical factors such as age and body mass index in conjunction with metabolic panels of discriminant metabolites to distinguish prostate cancer patients with metastatic from no metastasized disease. These studies have been demonstrated to be effective in differentiating between BPH, PCa, and PCa in bone metastases patients. They have additionally been demonstrated to characterize metabolic changes that may be connected to the development of PCa and its metastases, such as those involving 3-HB, acetone, and alanine.

Therefore, an extensive study needs to be carried out to achieve a detailed understanding of the biochemistry of metastases cascades and if possible, determine the targeted metabolic biomarker/s that is specific to PCa. Analyzing the metabolic differences between different stages in patients might provide insight into the underlying histopathological and metabolite differences after disease conditions. However, no comprehensive studies have been performed on blood and urine samples of PCa patients using NMR and MS-based metabolomics for simultaneous measurement and quantification of metabolites.

In clinical practice, the identified biomarker panels may be used to help with PCa diagnosis and categorization. The summary is shown in some biopsied tissue and blood metabolomic studies in different analytical methods in Table 1.

Table 1: List a few of the abnormalities in the metabolomics profile of metastases of prostate cancer patients using different analytical approaches.

Samples	Subjects	Metabolites	References
Biopsy Tissues	Prostate cancer Patients with bone metastases vs. non-metastases	Higher levels of asparagine, threonine, fumaric acid and linoleic acid	7
Blood	Prostate cancer patients with bone metastases vs. non-metastases	Increased levels of glutamic acid, taurine, and phenylalanine. Decreased stearic acid	7
Blood	Bone metastases prostate cancer patients vs. with non-metastases	Higher levels of pyruvate, citrate, 3-HB, acetone, and phenylalanine Lower levels of alanine, creatine, isoleucine, LDL/VLDL, leucine, and valine levels	8
Blood	Bone metastases prostate cancer patients vs. BPH	Increased levels of formate, phenylalanine, and pyruvate Decreased levels of acetoacetate, creatine, glutamine, histidine, isoleucine, leucine, and valine	8

BPH: Benign prostatic hyperplasia; 3-HB: 3-hydroxybutyrate

5. Conclusion and future perspective:

One of the characteristics associated with cancer is metabolic reprogramming. Metabolomics offers diverse avenues for comprehending the onset, progression, and metastasis of prostate cancer. The metabolomics profile has the potential to yield mechanism-based preventive and therapeutic solutions for various kinds of malignancies, including prostate cancer in an advanced stage. It will probably happen that metabolic profiling will distinguish between aggressive and non-aggressive prostate cancer. It may also predict the result and how a therapy will be perceived. Understanding the dynamic alterations that take place in malignant cells thanks to metabolomics may help us understand the process of carcinogenesis better. This may lead to a significant breakthrough in determining if a benign lesion can spread and become malignant. Consequently, there will be less overdiagnosis and overtreatment of prostate cancer.

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