Published in final edited form as:

Oncology. 2000 November ; 59(4): 269–282.

The Intermediary Metabolism of the Prostate: A Key to Understanding the Pathogenesis and Progression of Prostate Malignancy

L.C. Costello and **R.B. Franklin**

Cellular and Molecular Biology Section, OCBS/Dental School, University of Maryland, Baltimore, Md., USA

Abstract

This review emphasizes the importance and role of altered intermediary metabolism of prostate cells in the pathogenesis of prostate adenocarcinoma (PCa) and the progression of malignancy. The focus of the presentation is a summary of the overwhelming evidence which implicates the metabolic transformation of citrate-producing sane cells to citrate-oxidizing malignant cells in the process of malignancy. The evidence now demonstrates that altered zinc accumulation is an important factor in this transformation. These metabolic relationships are uniquely different from the metabolic alterations associated with tumorigenesis of other mammalian cells. The metabolic transformation of zinc-accumulating citrate-producing normal prostate epithelial cells to citrate-oxidizing malignant cells has important implications on cellular bioenergetics, cell growth and apoptosis, lipogenesis, angiogenesis. Based on the metabolic considerations new concepts concerning the pathogenesis, diagnosis and treatment of prostate malignancy are presented. Unfortunately the metabolism of the prostate has been a seriously neglected and largely ignored area of prostate research. The importance of expanded research into the intermediary metabolism of normal and neoplastic prostate is essential to future significant advances in understanding and dealing with PCa.

Dr. L. Costello, PhD, OCBS/Dental School, University of Maryland, 666 West Baltimore Street, Baltimore, MD 21201 (USA), Tel./Fax +1 410 706 7618, lcc@dental.umaryland.edu.

Addendum

While reviewing the literature for this report, I (L.C.C.) revisited the reprint of the Harvey Lectures presentation of Dr. Huggins [5] in 1946. As an aside point, the reprint that I possess and treasure was personally inscripted and presented to me by Dr. Huggins over a decade ago. I was reminded that Dr. Huggins did present data that demonstrated that prostate tissue citrate levels were markedly decreased in prostate cancer. Although not emphasized by Dr. Huggins, to our knowledge this is the first report of the association of low citrate levels with prostate cancer. In our recent reviews and presentations we have credited the 1959 report of Cooper and Imfeld [24] as the first reported of this relationship. Cooper and Imfeld [24] did extend the earlier data of Huggins by the inclusion of normal prostate tissue analysis, and they directed specific attention to the significance of the low citrate values in prostate cancer. The pursuant report of Cooper and Farid [23] in 1964 provided the most comprehensive study at that time which substantiated the citrate relationships in normal prostate, BPH, and prostate cancer. Thus Huggins and his colleagues along with Cooper and colleagues share in the initial revelation of citrate relationships in prostate cancer. I regret the earlier omissions of the report of Dr. Huggins in some of our presentations. I should apologize in advance for any other early initial reports that I might have missed, and I would appreciate being advised of any corrections to this presentation.

Keywords

Prostate cancer; Prostate epithelial cells; Citrate metabolism; Zinc; Mitochondria; Krebs cycle; Glycolysis; Lipogenesis; Apoptosis; Angiogenesis; Magnetic resonance spectroscopy

Introduction

Future progress in combatting prostate cancer (PCa) will be highly dependent upon an understanding of the pathogenesis of malignant prostate cells. Presently, the etiology and mechanisms involved in the development and progression of prostate malignancy remain a mystery. The absence of such an understanding prevents the rational development of new approaches to the diagnosis, prevention, and treatment of PCa. Important recent advancements have been in the areas of public awareness and early detection so that effective treatment can be imposed prior to the onset of metastasis. The treatment of PCa is for the most part based on modifications and improvements of established treatments which are effective only when the malignancy is confined to the intraglandular origin. Moreover chemotherapy based on androgen ablation is effective only until the androgen-refractory malignant phase develops. Prevention of PCa and inhibition of the progression of malignancy are areas where little or no advances have been made. The advances in these areas are dependent upon the understanding of the pathogenesis of prostate malignancy, about which very little progress has been made over the past 40 years.

Although not widely recognized or accepted within the clinical and research community despite the available compelling evidence, altered citrate and zinc levels are the most consistent distinguishing hallmark characteristics identified to date which differentiate PCa from normal and benign prostatic hyperplasia (BPH) prostate. The overwhelming evidence supports the concept that prostate malignancy involves and requires a metabolic transformation of zinc-accumulating, citrate-producing nonmalignant epithelial cells to citrate-oxidizing malignant cells which do not accumulate zinc. Consequently, as we will describe, altered intermediary metabolism plays a major role in the pathogenesis and progression of PCa. Except for the pathway of net citrate production (fig. 1), pathways of prostate metabolism (unlike liver, kidney, muscle, etc.) have not been identified. Yet the metabolism of the prostate involves unique relationships which are not generally applicable to any tissues. Conversely, metabolic relationships established for other tissues are not applicable to the prostate. The importance of establishing intermediary metabolism relationships in normal and malignant prostate has been largely ignored over the last 40 years, which has stymied productive and important research. This report summarizes and highlights the important relationships which implicate zinc and altered citrate-related intermediary metabolism in the pathogenesis and progression of prostate malignancy. We will take this opportunity to update, based on new information, our concept of the metabolic involvement in the development of malignant prostate cells. Hopefully this presentation will convince other investigators and clinicians of the importance of intermediary energy metabolism in understanding the mechanisms of prostate disease, and the opportunity for new directions in the prevention, treatment and diagnosis of PCa based on these metabolic relationships.

We refer the reader to our recent articles [1–4], which provide extensive and detailed reviews of the literature concerning zinc and citrate metabolism of prostate. It is not our intention to repeat the presentation of the extensive literature which has been described in our recent publications, and which cite and acknowledge the important contributions of others.

Citrate Metabolism in Normal Human Prostate Cells

In addressing this issue, it is important to focus on some fundamental relationships that generally apply to normal mammalian cell intermediary metabolism. Citrate synthesis and oxidation via the Krebs cycle are central to the pathways of intermediary metabolism of aerobic cells. The complete oxidation of glucose and fats is achieved through the synthesis of citrate and its oxidation via the Krebs cycle. It is through the synthesis and oxidation of citrate that cells generate, by coupled phosphorylation, their major supply (approximately 80%) of cellular energy (ATP production). In addition, the Krebs cycle and the recycling of its intermediates provide major pathways for the biosynthesis and degradation reactions of amino acid metabolism. Synthesized citrate provides the source of acetyl-CoA required for lipogenesis. Thus citrate synthesis and oxidation via an operational Krebs cycle are critical for the normal metabolism, functional capabilities, growth and reproduction, and survival of aerobic mammalian cells. As is evident from the lethal effects of fluoroacetate which inhibits mitochondrial (m-) aconitase reaction (the first step in the oxidation of citrate via the Krebs cycle), aerobic mammalian cells generally are seriously compromised by an aborted Krebs cycle. Mammalian cells, in specific instances, do possess adaptive capabilities which permit the incorporation of alternative metabolic pathways to provide energetic and synthetic requirements of the cell. However, the significance of citrate-related intermediary metabolism and an operational Krebs cycle in mammalian cells is most apparent.

The major functions of the human prostate gland include the accumulation and secretion of extraordinarily high levels of citrate and zinc (table 1). It is now evident that this function is associated specifically with the glandular epithelium of the peripheral zone. Thus secretory epithelial cells of the peripheral zone are the highly specialized zinc-accumulating, citrate-producing cells of the human prostate. In contrast, the glandular cells of the central zone do not accumulate zinc and are citrate-oxidizing cells typical of most mammalian cells. No other cells in the body exhibit these functional and metabolic capabilities which uniquely characterize the peripheral zone secretory epithelial cells.

Clinicians and researchers involved in prostate must begin to recognize this distinction. The use of any terminology that infers the existence of a common singular secretory epithelial cell type associated with the human prostate is inaccurate and leads to misrepresentations and misconceptions. This especially applies to the description and identification of primary and immortalized prostate epithelial cells in cultures as being representative of ‘normal prostate epithelial cells’. A cell that does not express the functional and metabolic characteristics associated with zinc accumulation and citrate production cannot be identified as being representative of normal secretory epithelial cells of the peripheral zone; and the description of such cells as ‘normal prostate epithelial cells’, is meaningless. Therefore to be completely accurate and unambiguous, unless otherwise defined, the following described

relationships are applicable only to citrate-producing prostate epithelial cells, which in the human prostate are representative of the peripheral zone secretory epithelial cells.

In these prostate secretory epithelial cells, the accumulation and secretion of citrate (which we refer to as 'net citrate production') occurs at the expense of citrate oxidation. Instead of being an essential oxidizable intermediate of cellular metabolism, most of the citrate becomes an endproduct of intermediary metabolism. This occurs due to a uniquely limiting m-aconitase activity which minimizes the oxidation of citrate. This is the first reaction involved in citrate oxidation via the Krebs cycle. Therefore, in these cells, the operation of the Krebs cycle is aborted prior to the oxidation of the accumulated citrate.

One of the important and obvious consequences of this net citrate production relates to the bioenergetics of the citrate-producing cells (fig. 1). The oxidation of citrate via the Krebs cycle provides about 12mol ATP/mol citrate, and 24 mol ATP/mol glucose oxidized. This represents about 65% of the ATP derived from the complete oxidation of glucose. Thus it becomes evident that the function of citrate accumulation and secretion is bioenergetically extremely costly. This alone provides compelling evidence that this function of citrate production is a major and highly specialized function of these secretory epithelial cells. Since citrate oxidation is essential to the sustenance of most mammalian cells, the citrate-producing prostate cells likely adopt alternate metabolic pathways and curtail other unrequired activities in order to meet the energy requirements for their survival and propagation and to perform their specialized functions. For example, citrate-producing prostate cells reportedly exhibit a high aerobic glycolysis [5, 6], which would provide a costly metabolic pathway for energy production in the absence of citrate oxidation. Unfortunately knowledge of these alternative and additional metabolic pathways in prostate is lacking.

Clearly, the human prostate (specifically the peripheral zone) has evolved as a highly specialized organ for the primary function of secreting enormously high levels of citrate. The concentration of citrate in normal prostatic fluid generally ranges from 40 to 150 m*M*, and, as the sole source, gives rise to seminal fluid citrate level of about 10–30 m*M* [7]. The high citrate content of seminal fluid occurs in many animals although the source of citrate is derived from various accessory reproductive glands depending upon the species. This leads to the intriguing question, 'What is the function of the high citrate content of semen?' Unfortunately this remains an unanswered question. Suggested functions include a role of citrate as a buffer to maintain the pH of semen; as a chelator for zinc and other cations which are also highly concentrated and involved in liquefaction of semen; as an energy source for the viability of sperm and/or the capacitation process of fertilization [7–11]. Its role might be complex in relation to other components uniquely associated with prostatic fluid. For example, the high concentration of polyamines possibly provides a cationic component for the excess of citrate anion [7]. The bioenergetic consequences and the unique specialization of citrate-producing prostate dictate that there must be some significant reproductive role of citrate which could not be achieved by some less energetically costly process. Alternatively, this might be a vestigial relationship that is no longer required for human reproductive capability.

Glucose Metabolism and Citrate

Huggins [5] and Barron and Huggins [12] in 1944 reported that human prostatic adenoma tissue exhibited a low respiratory rate, a high anaerobic glycolysis, and a high aerobic glycolysis. Muntzing et al. [13] in 1975 reported a high dependency of human prostate tissue on aerobic glycolysis. Little additional information concerning glucose metabolism of human prostate exists. Studies with rat ventral prostate (a citrate-producing gland) also reveal the existence of a high aerobic glycolysis [6, 14–16]. All these studies were conducted with complex tissue preparations containing mixtures of epithelium and various stromal components. However, isolated ventral prostate cells also exhibit a high aerobic glycolysis [17; also our unpublished information]. Thus the available information leads to the likely conclusion that citrate-producing prostate secretory epithelium, including human prostate, exhibits a high aerobic glycolysis. However, direct studies with human prostate cells are needed to confirm this expectation.

There exists a serious, yet intriguing, apparent paradox in the combination of a high rate of glycolysis along with high citrate accumulation. A key step in controlling the rate of glycolysis in mammalian cells is the phosphofructokinase (PFK) reaction [for review of PFK see ref. 18–21]. The enzyme is negatively modulated by citrate and by ATP. When the citrate levels rise in mammalian cells, a feedback control occurs that decreases glucose utilization via glycolysis. This provides a logical interdependent mechanism to synchronize glycolysis and Krebs cycle activity. Altered ATP levels achieve the same result. The intracellular concentration of citrate in most mammalian cells ranges from approximately 0.1 to 0.5 m*M*. In contrast the citrate concentration is estimated to be in the range of 1.0–3.0 m*M* in normal human prostate cells. Although the intracellular distribution and compartmentalization of citrate are difficult to establish, it is reasonable to expect that the cytosolic citrate concentration should approximate the total intracellular citrate concentration. For citrate to be a physiological regulator of glycolysis in mammalian cells, its repressive effect on PFK must occur at cellular concentrations up to 0.5 m*M*. Consistent with this Li et al. [22] reported that mammalian PFK was inhibited 50 and 90% by 0.25 and 0.5 m*M* citrate, respectively. One should then expect that at the extremely high citrate levels that exist in normal human prostate cells, PFK should by virtually completely inhibited, which should prevent glucose utilization via glycolysis in contradiction to the high rate of glycolysis that characterizes citrate-producing prostate cells. This implies that some unique relationships associated with PFK and the regulation of glycolysis must exist in these prostate cells. Information concerning the levels of ATP in these cells is needed. PFK is also regulated by positive effectors, about which no information currently exists for human prostate cells. Clearly, research concerning these important metabolic issues is urgently needed.

Citrate Relationship in PCa

The tissue concentration of citrate in normal human prostate ranges from 8,000 to 15,000 nmol/g tissue, whereas all other soft tissues contain about 150– 450 nmol/g (table 1). The citrate level of BPH tissue approximates the high levels associated with normal prostate so that BPH epithelial cells, like normal peripheral zone epithelial cells, are also citrate-

producing cells. In contrast to normal or BPH prostate, in PCa the tissue citrate levels (generally 1,000–2,000 nmol/g) are dramatically and consistently decreased. When estimated on the basis of the malignant tissue component, the value approximates the low citrate level observed in other nonprostate tissue. Thus malignant prostate cells, unlike normal peripheral zone or BPH cells, are citrate-oxidizing cells. As will be discussed below, malignant prostate tissue contains low levels of zinc, which is in contrast to the uniquely high zinc accumulation associated with normal peripheral zone. These relationships are especially relevant since prostate malignancy is associated predominantly with the peripheral zone.

The evidence in support of this citrate relationship in prostate cancer is overwhelming. Several early reports [5, 23–26] consistently demonstrated that resected prostate tissue samples (prostatectomy, biopsy, TURP) obtained from PCa subjects exhibited a significantly lower citrate content than normal or BPH tissue samples. The compelling confirmation of these early reports resulted from the recent in situ determination of prostate citrate levels by magnetic resonance spectroscopy (MRS) [see ref. 4, 27 for extensive reviews of MRS]. Figure 2 is a summary of the independent MRS studies from three different facilities. In every case, malignant loci in peripheral zone exhibit extremely low citrate levels as compared to the characteristic high citrate levels in normal peripheral zone. Just as impressive is the fact that there exists no report of prostate malignancy in which the citrate levels of malignant tissue remain elevated at the levels of normal and BPH prostate. No longer should there be any question that a decreased citrate level is a distinguishing hallmark characteristic which clearly differentiates PCa from the normal and BPH conditions, and that altered citrate metabolism is intimately associated with prostate malignancy.

In his now classical presentation in 1946, Dr. Huggins [5] reported that the citrate content of prostate cancer tissue was 0.012–0.137 g/100 g tissue as compared to BPH tissue values of 0.218–1.533. Although not emphasized in that presentation, the values demonstrate the marked decrease in prostate citrate content as compared to BPH (see Addendum). In 1959 Cooper and Imfeld [24] reported that citrate levels were significantly decreased in prostate cancer tissue as compared to either normal prostate or BPH. In that report, and later corroborated [23], they suggested that, ‘It is probable that biochemical alteration (i.e., altered citrate metabolism) of prostatic tissue may well occur long before malignant changes are histologically obvious to the pathologist.’ They further suggested that, ‘… prostatic citrate determinations may be of value in the detection of latent, unsuspected prostatic carcinoma where routine histological sections have failed to demonstrate malignancy.’ It now seems apparent that these early prophetic conclusions are accurate and applicable to the pathogenesis of PCa. A low citrate level is readily detectable even when the histologically identifiable malignancy represents a small proportion of the glandular component of the tissue section, i.e., when the tissue is dominated by nonmalignant (normal and BPH) tissue which expectedly would contain high citrate levels. Similarly, in situ MRS detection of low citrate is evident when the malignant cells occupy only a portion of the targeted voxel. Such conditions lead to the conclusion that a significant population of cells in proximity to the malignant loci, and which are not histopathologically identifiable as malignant cells, no longer possess the ability to accumulate high citrate levels. In other words a significant

population of citrate-oxidizing cells must exist which do not present histopathologically identifiable malignant characteristics.

These observations lead us to propose that the pathogenesis of prostate malignancy involves an early metabolic transformation of sane citrate-producing cells to malignant citrate-oxidizing cells. This event precedes the onset of the appearance of histopathologically identifiable malignant changes so that it occurs during a premalignant stage which follows the initial genetic neoplastic step as represented in figure 3. Once the neoplastic cell exhibits the metabolic transformation to a citrate-oxidizing cell (the premalignant stage), the cell is capable of performing the activities and exhibiting the characteristics of a malignant cell. The progression of malignancy will then be manifested. In the absence of the metabolic transformation to citrate oxidation, the neoplastic cell will remain in an arrested state incapable of proceeding through malignancy. Moreover, this concept proposes that sane prostate epithelial cells that have not been genetically transformed to a neoplastic malignant cell type will not exhibit malignant capabilities even if they are metabolically transformed to citrate-oxidizing cells. The concept raises important questions which need to be addressed. Why is a metabolic transformation of neoplastic malignant cells from citrate-producing cells to citrate-oxidizing cells a requirement for prostate malignancy? How is this alteration in citrate metabolism achieved? How can the metabolic alteration be prevented and will inhibition of citrate oxidation arrest the progression of malignancy?

These relationships also argue against the possibility that the decline in citrate levels in PCa is due principally to a decrease in luminal space and secretory content of malignant glands. The extraordinarily high citrate level of prostatic fluid (table 1) that exists in the acinar lumen contributes to the high tissue citrate level of normal prostate. Obviously a decrease in the prostatic fluid content of the acini would decrease the tissue citrate level. However, as already discussed, the citrate level is markedly decreased in the early stages of malignancy, and when there is little, if any, histological appearance of acinar changes. The precipitous decrease in citrate values of tissue sections bears little, of any, relationship to the structural changes and lumenal volume of the malignant component of the tissue sample. The composite of virtually all reports, which involve all stages of PCa and involve citrate analysis of resected and in situ (MRS) prostate, demonstrate a significant decrease in citrate. There is no evidence that highly differentiated PCa that contains normal acinar organization and secretory content exhibit the high citrate levels that are representative of normal prostate tissue (fig. 2). Consequently the principal reason for the characteristic decrease in citrate levels in PCa is the metabolic transformation and is not due to a decrease in prostatic fluid volume, although the latter could be a contributing factor particularly in later undifferentiated stages of malignancy.

Tangential to the issue of PCa, MRS detection of prostate citrate distribution in situ reveals an important relationship in BPH. As we earlier proposed [28], it is now evident that the normal central zone contains low citrate levels and is not significantly involved in the production and secretion of high citrate levels (fig. 2). In BPH (particularly glandular BPH) the citrate levels of the central zone are markedly increased to levels that exceed the normal peripheral zone. These observations reveal that BPH involves the proliferation and invasion of citrate-producing glandular epithelium into the central zone. Such information provides

new insight into the pathogenesis of BPH. The origin of these citrate-producing glandular epithelial cells needs to be established.

Altered Metabolism and Bioenergetics of Tumor Cells

That malignant cells generally exhibit major changes in intermediary metabolism from that which characterized their precursor sane cells has been well documented since the early classical studies of Warburg et al. [29]. Obviously, altered metabolism is not a cause of malignancy. The potential for malignancy resides within the genetic alterations of the neoplastic cell. However, it seems clear that altered metabolism is essential for the neoplastic cell to exhibit its malignant activities, i.e., to progress through the malignant process. An exciting renewed interest in the metabolism of tumor cells is beginning to focus on the relationship of altered metabolism to the process of malignancy.

While there exists no metabolic alterations that universally apply to all tumor cells, altered metabolic characteristics that are generally represented in most tumor cells do exist [for reviews see ref. 30–32]. For this presentation we will highlight some characteristics that will relate to the discussion of prostate malignancy. Generally, the normal cells, from which tumor cells are derived, exhibit glycolytic and Krebs cycle activities which result in the complete oxidation of glucose, and therefore are energy-efficient cells. In contrast, most tumor cells exhibit a faulty aerobic oxidation with impaired Krebs cycle activity. These tumor cells exhibit a high rate of aerobic glycolysis leading to the accumulation of lactate with the accompanying generation of 2 ATP/glucose utilized (possibly an additional 6 ATP through mitochondrial oxidation of NADH), which compares with 38 ATP generated from the complete oxidation of glucose (fig. 1). Thus, in general, tumorigenesis involves a metabolic transformation of energy-efficient sane cells to energy-inefficient tumor cells.

While this metabolic alteration applies widely to tumor cells, it is not applicable to prostate malignancy. The sane precursor prostate epithelial cell is already an energy-inefficient cell which exhibits high aerobic glycolysis and low aerobic oxidation with an impaired Krebs cycle. The malignant prostate epithelial cell exhibits increased aerobic oxidation due to restoration of a fully operational Krebs cycle and their ability to oxidize citrate. Thus in prostate, the metabolic transformation is from an energy-inefficient, anaerobic-like metabolism of the sane cell to an energy-efficient, aerobic metabolism of the malignant cell. An important implication of this metabolic transformation is the bioenergetic consequence. The neoplastic malignant prostate cell discards the secretory epithelial cell function of net citrate production in favor of citrate oxidation for increased generation of ATP from complete oxidation of glucose. This increased energy production (fig. 1) is apparently essential for the neoplastic prostate cell to perform its malignant processes, which we describe as the ‘bioenergetic basis of prostate malignancy’ [1–4, 33]. To reemphasize this relationship, we reiterate that malignant prostate cells never retain the energy-inefficient metabolic process of net citrate production.

Altered Metabolism and Angiogenesis

The in situ perfusion of any tissue by its associated blood supply is determined by the metabolic requirements of the cells comprising the tissue. Angiogenesis is the process by

which new blood vessels are recruited to meet the metabolic demands of the cells. Cellular metabolic parameters regulate angiogenesis to provide the appropriate perfusion to meet the demands of the cells in relation to their growth and functional activities. Therefore, angiogenesis cannot be divorced from altered cellular metabolism. In tumor growth and development, two important metabolic parameters that regulate angiogenesis are hypoxia and hypoglycemia. Both conditions reportedly induce the expresssion of angiogenic factors, particularly vascular endothelial growth factor (VEGF). We refer the reader to Dang and Semenza [34] for an excellent overview of these relationships. In most cancers tumor development occurs in normal tissue which is aerobic, and in which the associated circulation would allow for high oxygen availability to support the normally aerobic cells. The low-respiring tumor cells initially exist in an adequate oxygen environment. As the mass of tumor cells increases, the requirement for oxygen will increase although the oxygen utilization per tumor cell is comparatively less than the normal cell. (For clarification, tumor cells are not true anaerobic cells despite the fact that they exhibit a high aerobic glycolysis. They require oxygen for their employment of alternative oxidative pathways such as glutamine/glutamate oxidation.) Eventually, tumor growth will result in hypoxia, at which time induction of expression of hypoxia-inducible factor (HIF) will stimulate VEGF and subsequent angiogenesis. During the hypoxic phase, further tumor cell progression will be arrested (a dormant phase) until additional circulation through angiogenesis becomes available. This description of the metabolic-angiogenesis relationship is a superficial view of a complex process, but it provides an introduction to the following discussion relating to prostate malignancy.

As already discussed, human prostate tissue is characterized as being a low-respiring tissue that exhibits high aerobic glycolysis. Although more direct studies with peripheral zone glandular epithelium and with isolated epithelial cells are required to confirm this characterization, it is consistent with the metabolic relationship of net citrate production. The metabolic transformation from functional citrate-producing secretory epithelial cells to neoplastic citrate-oxidizing malignant cells would be accompanied by an increase in oxygen consumption (i.e. increased respiration). Consistent with this are our observations [unpublished information] that the treatment of citrate-producing LNCaP cells to increase citrate oxidation produces a 125% increase in oxygen consumption. Therefore the normal peripheral zone tissue, which constitutes about 70% of the prostate tissue mass, would be characterized by a low oxygen consumption and should have a circulatory supply consistent with this low demand for oxygen.

The initial development of malignant prostate cells which have a higher oxygen consumption than the citrate-producing normal epithelial cells would expectedly rapidly deplete the oxygen tension at the malignant site. This should result in rapid development of hypoxic regions that would arrest further growth and progression of the malignant cells (a dormant phase of malignancy). Consistent with this expectation, Movsas et al. [35] reported the presence of hypoxia in malignant regions of the prostate. In order for further growth and progression of the aerobic malignant cells to occur, increased circulation will be essential. This is consistent with reports that malignant prostate cells exposed to hypoxia initiate the HIF induction of angiogenesis [36, 37]. In essence, in PCa the prostate tissue becomes more aerobic with an increasing utilization of oxygen. The increased metabolic requirement of the

aerobic malignant cells plus the increase in cell mass increases the demand for oxygen in an environment that normally exists for low respiring normal cells. Under these conditions, hypoxic states will exist in the early stage of malignancy and will be more rapidly induced by a smaller increase in proliferation of the aerobic malignant cells. Proliferation will be arrested while HIF angiogenesis cycles occur to meet the oxygen demand of proliferating malignant cells. We believe that this concept possibly provides an explanation for the characteristic slow malignant growth in PCa.

Citrate Metabolism and Lipogenesis in Prostate Malignancy

In previous reports and in the preceding discussion, we focused on the relationship of citrate as an oxidizable substrate and its bioenergetic consequences. However, to represent that the decrease in citrate levels in prostate malignancy is solely due to the oxidation of citrate could be misleading. Citrate serves two important functions in cell metabolism: as an oxidizable intermediate in the operation and function of the Krebs cycle (as we have been discussing), and as an essential precursor for lipogenesis. The latter function is especially important in tumor growth. Membrane biogenesis is essential to the growth and reproduction of cells. Lipogenesis is essential to the process of membrane biogenesis. An important general characteristic of tumor cells is an accelerated lipogenesis consistent with their propensity for growth. Indeed, tumor cells exhibit increased citrate production and low citrate oxidation [31]. However, this is not manifested as a dramatic increase in accumulated tumor citrate levels because the citrate is utilized for the accelerated lipogenesis [30, 31]. In this process, citrate is exported from the mitochondria to cytosol where it is cleaved to oxalacetate and acetyl-CoA by ATP-citrate lyase. Acetyl-CoA is the building block for fatty acid synthesis. This metabolic process is essential to prostate malignancy as it is to all cancers. Therefore, the decrease in citrate levels in PCa would be due to (1) the increase in citrate oxidation due to increased m-aconitase activity of the malignant cells, and (2) the increase in citrate utilized for the accelerated lipogenesis of the malignant cells. These are likely to be linked events in that the energy for lipogenesis is derived from the oxidation of citrate.

Presently very little information exists regarding lipogenesis in normal and malignant prostate. Swinnen et al. [38] and Swinnen and Verhoeven [39] reported that ATP-citrate lyase along with other lipogenic enzymes in LNCaP cells is regulated by testosterone. Studies with monkeys revealed that the ATP-citrate lyase of the normal prostate was regulated by testosterone and by prolactin [40]. Most significant is the observation that human malignant prostate tissue contained lower citrate and elevated triacylglyceride levels when compared to adjacent nonmalignant tissue [41]. The authors concluded that ATP-citrate lyase activity and/or concentration was increased in prostate tumors. It is also significant that in situ MRS spectra of the human prostate reveal that the decrease in citrate in malignant loci is accompanied by an increase in choline compounds which are generally associated with membrane structures [4, 27, 42]. These limited studies support our proposition that the altered citrate metabolism of malignant prostate cells is essential to the process of malignancy through the bioenergetic and lipogenic consequences which we have described. It is also clear that much-needed research is required to establish these relationships.

The Role of Zinc in the Pathogenesis of Prostate Malignancy

Several compelling correlations between citrate and zinc in prostate exist [see ref. 3 for detailed review]. Normal and BPH prostate cells accumulate the highest zinc levels in the body along with the highest citrate levels (table 1). In PCa the zinc levels are dramatically decreased as is the case with citrate. This relationship is strikingly demonstrated in figure 4 in which we have superimposed the zinc studies of Zaichick et al. [43] with the citrate studies of Liney et al. [44] (see also fig. 2). For entirely different populations, there exists no case in which the cancer subjects exhibited either a prostate citrate or zinc level representative of the high levels of noncancer subjects. Moreover the zinc values were obtained from mixed prostate tissue samples, whereas the citrate values were determined for peripheral zone in situ. If the zinc levels were determined for peripheral zone, the noncancer values would be higher and the malignant zinc values would be lower than represented in this study. Despite this, the relationship is still clearly and strikingly apparent.

These and other correlations indicated that zinc might be involved in citrate metabolism of normal and malignant prostate epithelial cells. We have now demonstrated that the high intramitochondrial zinc levels inhibit m-aconitase activity and citrate oxidation in prostate epithelial cells [45–47]. Thus the zinc-citrate relationships are not simply correlations of corresponding changes in zinc and citrate. More importantly this is a cause-and-effect relationship. By this mechanism, zinc is a unique potent regulator of the operation of the Krebs cycle and the related intermediary energy metabolism of prostate epithelial cells.

Because of this metabolic effect of zinc, we theorized that the accumulation of high zinc levels might have an inhibitory effect on the growth (i.e., increase in cell number) of prostate epithelial cells. This proved to be the case in that the addition of physiological levels of zinc to medium inhibits the growth of zinc-accumulating prostate cells while having no effect on cells which do not accumulate high zinc levels [48]. The inhibition of growth results from the inhibition of the cell cycle (i.e., inhibited cell proliferation) and from the stimulation of apoptosis. Our recent studies [49] have revealed that the accumulation of zinc induces the release of cytochrome c from mitochondria into cytosol which then initiates the activation of caspase 9 and the subsequent caspase cascade leading to cell death. Consequently another metabolic effect of zinc is the induction of mitochondrial apoptogenesis. This effect (e.g. loss of mitochondrial cytochrome) of zinc will undoubtedly have profound consequences on terminal oxidation and related mitochondrial-metabolic functions yet to be identified. In the absence of high zinc levels, the malignant cells will not be subjected to the growth-inhibitory (stimulated apoptosis and inhibited cell cycle) effects of zinc. In this regard it is important to note that, in PCa, an increase in the expression of bcl-2 occurs in the malignant cells which prevents apoptosis [50–52]. Bcl-2 inhibits mitochondrial apoptogenesis by preventing the opening of the permeability transition pore. This raises the likelihood that both the loss of accumulated zinc and the expression of bcl-2 ar related events to prevent apoptosis of the malignant cells, thereby allowing their proliferation and progression.

That normal peripheral zone secretory epithelial cells must contain special mechanisms that permit the intracellular accumulation of uniquely high zinc levels is clearly evident,

particularly in the form of mobile reactive zinc. This is in contrast to most mammalian cells which possess mechanisms that protect against the accumulation of reactive zinc, such as zinc exporters and zinc binding (e.g. metallothionein) activities. Most importantly, it is incontrovertible that, in PCa, the malignant cells have lost the unique capability to accumulate high levels of zinc. It is this lost ability that triggers the altered metabolic and growth effects that appear to be essential to the malignant process. This brings into focus the urgent need to identify the zinc-accumulating apparatus that is not functioning in the malignant cell and to establish why the apparatus is not functioning. This will be an important key to revealing the pathogenesis of PCa.

Unfortunately very little information exists concerning the mechanisms associated with the accumulation of high zinc levels in the normal cells or with the inability of malignant prostate cells to accumulate zinc. Our studies have demonstrated that LNCaP and PC-3 cells are capable of accumulating high intracellular zinc levels [53]. Associated with this capability is the expression and existence of a zinc uptake transporter (Zip-type transporter). The genotypic and phenotypic representation in vitro of these malignant cell lines to the ‘true’ malignant cells (i.e., the natural malignant prostate cells in situ in PCa) from which they were derived is questionable. The ‘true’ malignant cells have lost the ability to accumulate zinc, whereas, under specific in vitro conditions, these immortalized malignant cells exhibit the ability to accumulate high zinc levels and to express a zinc uptake transport apparatus. The gene complement of these cell lines is likely representative of the gene complement of the in situ ‘true’ malignant cells in prostate cancer. It would follow that ‘true’ malignant prostate cells and normal prostate epithelial cells (from which the neoplastic cell is derived) contain the Zip-type transporter. However, in situ, either the ‘true’ malignant cell expression of the transporter is repressed, or other in situ conditions inhibit or override the function of zinc accumulation. Obviously this concept is speculative, but it points to the importance of elucidating the mechanisms and operation of zinc accumulation in normal and malignant prostate cells as a basis for the understanding of the pathogenesis and progression of PCa.

The early observation of Habib et al. [54] that the decrease in zinc levels occurs early in malignancy as does the decrease in citrate levels become extremely important. This provides the mechanism associated with the metabolic transformation from a citrate-producing cell to a citrate-oxidizing malignant cell (fig. 3). Apparently the neoplastic cell loses the ability to accumulate the high zinc levels required to inhibit m-aconitase activity. As the zinc levels decline, m-aconitase activity is increased, citrate oxidation increases, and ATP production increases. At this point the neoplastic cell is metabolically capable of performing its malignant activities.

Clinical Significance

We have attempted to provide a convincing presentation in support of the concept that alterations in zinc and citrate-related metabolism are intimately involved in the development and progression of prostate malignancy. In addition to providing an understanding of the pathogenesis of PCa, how can these relationships be clinically applied? The two most

apparent areas are (1) in the diagnosis of PCa, and (2) in the treatment and, perhaps, prevention of PCa.

Diagnosis

An accurate and early diagnosis of PCa is critical to the successful treatment and management of the patient. While providing a valuable diagnostic marker for PCa detection, the widespread contemporary use of prostate-specific antigen is fraught with variables that affect its values. As such, prostate-specific antigen testing is quite often inconclusive. Digital rectal examination and biopsy analysis are required for additional confirmation of possible malignancy. The latter often does not reveal malignant loci and requires repeated multiple biopsies. This is especially the case in early PCa.

A noninvasive procedure for the direct detection of malignancy, particularly in the early stages, will be of immense value. In addition, a procedure that could provide the location and volume of the malignancy and a permanent mapping of the prostate for follow-up examination would be of immense value. As earlier suggested [23, 24, 26, 28], citrate changes could prove to be a valuable biochemical marker for the detection of PCa. 1-H MRS has been successfully applied to the in situ detection of citrate levels in prostate with the use of an endorectal coil [for reviews see ref. 4, 27]. Consistent and corroborating results from several laboratories [42, 44, 55] now demonstrate that malignant areas of the prostate can be readily identified on the basis of a decreased citrate content (fig. 2). Most strikingly there is virtually no overlap of the citrate values of peripheral zone in the absence of malignancy versus the values when malignancy is present. When combined with magnetic resonance imaging (MRI), citrate analysis (i.e., choline + creatine/citrate ratio) exhibits a positive predictive value of 90% for detection of malignancy and excludes the presence of cancer with an 83% negative predictive value [27]. Most importantly, this imaging procedure directly locates the presence of malignant loci and provides a map of the prostate. Estimates of the volume of malignancy can be obtained. Thus it is possible to track the development, progression, and/or regression of the malignancy over time following watchful waiting or treatment regimens. Targeting and localizing radiation therapy directly at the malignant loci is more accurate and reliable in combination with this MRI/MRS procedure, and should reduce the unwanted damage to surrounding healthy tissue [56]. Although still in the technological development stage, MRS detection of altered citrate metabolism provides the most promising diagnostic procedure for PCa.

In addition to citrate, the possible use of changes in zinc levels for the diagnosis of PCa warrants serious consideration. As already discussed, a marked decrease in prostate tissue zinc levels, as is the case with citrate, is always associated with PCa, as contrasted with the high zinc levels observed in normal prostate and BPH. Habib et al. [54] reported that the decrease in prostate zinc levels occurred early in malignancy and suggested that changes in zinc could be employed in the early detection of PCa. In light of new technological advancements, the measurement of zinc levels in relation to PCa should be explored. Zaichick et al. [43] reported 98% sensitivity and specificity for the differential diagnosis of PCa based on zinc analysis of prostate biopsy samples. It is possible that the changes in zinc

levels could be coupled to the MRS determination of citrate changes to provide a more effective diagnostic procedure for detecting PCa and the progression of malignancy.

Recent studies by Zaichick et al. [57] demonstrated a dramatic decrease in the zinc content of expressed prostatic fluid of cancer subjects vs. normal, prostatitis, and BPH subjects. They reported a 93% accuracy in diagnostic PCa based on the decreased zinc level of prostatic fluid. Although not conclusive, Anderson and Fair [58] reported a decrease in zinc levels of seminal fluid from cancer subjects. However, other studies [59] did not reveal a significant decrease in prostatic fluid zinc levels in PCa. Moreover, zinc levels reportedly are decreased in prostatitis [59, 60]; however, Zaichick et al. [57] did not observe such a decrease. If the report of Zaichick et al. [57] can be corroborated and the discrepancies among various reports resolved, the use of zinc levels in expressed prostatic fluid samples could provide a simple test for an initial diagnosis of PCa.

Treatment

The zinc-citrate metabolism relationship opens opportunities for the development of new approaches to the treatment and perhaps prevention of Pca. It is now evident that the ability of malignant prostate to accumulate high zinc levels is a major factor in the metabolic transformation associated with the malignant process (fig. 3). Therefore the reestablishment of high intracellular zinc levels in malignant cells could arrest the progression of malignancy as well as prevent the development of malignancy. To achieve this, a treatment regimen which restores the accumulation of zinc in prostate cells needs to be established. This requires the identification of an appropriate agent to stimulate zinc uptake by prostate epithelial cells. The fact that prolactin and testosterone can increase zinc accumulation in prostate cells [45, 53] demonstrates that the development of such an agent for human use is feasible. Once the mechanism of zinc accumulation in prostate is established, approaches designed to reestablish the activity of the zinc-accumulating apparatus in malignant cells can be developed. Because other mammalian cells lack the ability to accumulate high zinc levels and employ processes to minimize the accumulation of reactive zinc, a regimen to increase zinc accumulation should be highly specific for prostate cells with minimal adverse effects on other tissues.

The approach described above takes advantage of the role of zinc as a ‘natural and specific’ inhibitor of m-aconitase activity and citrate oxidation of prostate cells. However, alternative approaches can be directed at other mechanisms to inhibit citrate oxidation of malignant cells. The possibility of developing specific inhibitors of prostate citrate oxidation should be pursued. For example, fluoroacetate is an effective inhibitor of mammalian cell m-aconitase activity and citrate oxidation. If such an inhibitor could be modified so as to be targeted at prostate cells, it could provide an effective treatment without adverse systemic effects.

Another approach could take advantage of the observations that the level of m-aconitase is under gene regulation, and, specifically in prostate cells (including LNCaP and PC-3), the m-aconitase gene is hormonally (prolactin and testosterone) regulated [61]. Possibly, prevention of hormonally stimulated gene expression in the malignant prostate cell could result in decreased m-aconitase activity and inhibited citrate oxidation, thereby arresting malignancy even when the inhibitory effect of zinc is not present. These are examples of

novel rational approaches to the treatment of PCa based on the metabolic implications involved in the pathogenesis of PCa.

Summary

1. Alterations in the intermediary metabolism of all neoplastic cells are required and essential for the successful manifestations of their malignant capabilities. The metabolic transformation of malignant prostate cells is uniquely different from that of other tumor cells.
2. Normal peripheral zone glandular epithelium is characterized by the accumulation of extraordinarily high levels of citrate and zinc. PCa derived principally from peripheral zone is characterized by low levels of citrate and zinc.
3. The pathogenesis of PCa involves the metabolic transformation of sane zinc-accumulating, citrate-producing cells to citrate-oxidizing cells that have lost the ability to accumulate zinc. In the absence of this metabolic transformation, the neoplastic cell is incapable of manifesting its potential malignant capability.
4. In normal prostate glandular epithelial cells, the role of zinc is to inhibit m-aconitase which limits the oxidation of citrate and allows its accumulation. In malignant cells, the lost ability to accumulate high zinc levels eliminates its inhibitory effect on m-aconitase, which permits the oxidation of citrate via a functional Krebs cycle, and eliminates the growth-inhibitory effects of zinc. Thus, altered zinc accumulation is an important factor in the pathogenesis of PCa.
5. The metabolic transformation from citrate-producing sane cell to citrate-oxidizing malignant cells has widespread implications in the intermediary energy metabolism of the cells. These implications include altered cellular energy production, altered glucose and lipid metabolism, altered oxygen requirements and angiogenic requirements, altered cell proliferation capabilities.
6. An understanding of the metabolic implications in the pathogenesis and progression of prostate malignancy will lead to new approaches to the diagnosis, treatment and prevention of prostate cancer.

Concluding Comments

The relationships described herein demonstrate the importance of metabolic consideration in establishing the pathogenesis and progression of PCa. The metabolic relationships discussed represent a limited segment of the area of intermediary energy metabolism. Other metabolic relationships need to be studied. Because of the unique metabolic functions of the prostate gland, the extrapolation of the ‘typical’ metabolic relationships described for most mammalian cells to prostate cells leads to misperceptions of the pathways and roles of intermediary energy metabolism in both normal and neoplastic prostate. This is an area of prostate research which has been woefully neglected since the pioneering studies of Huggins, Mann, Williams-Ashman, Farnsworth, Grayhack, and a few others over 30 years ago. Hopefully, we have demonstrated that vital information and future advances concerning the pathogenesis, diagnosis and treatment of prostate disease will depend on the elucidation

and understanding of the metabolic relationships. Finally, we would echo the comment of Dang and Semenza [34], 'Rekindling of interest in the classical biochemical pathways and their intersections with newly developed signal transduction methods [and, we would add, emerging relationships of gene regulation of metabolic pathways] will provide novel insights into the alterations in metabolic profile that have long been known to occur in cancers.' This is especially relevant to prostate cancer.

Acknowledgments

This report and the studies of L.C.C. and R.B.F. herein were supported by NIH research grants DK 42839, DK 28015, CA 71207, CA 79903.

References

1. Costello LC, Franklin RB. Citrate metabolism of normal and malignant prostate epithelial cells. Urology. 1997; 50:3–12. [PubMed: 9218011]
2. Costello, LC.; Franklin, RB. Intermediary energy metabolism of normal and malignant prostate epithelial cells. In: Naz, RK., editor. Prostate: Basic and Clinical Aspects. New York: CRC Press; 1997. p. 115-150.
3. Costello LC, Franklin RB. The novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. Prostate. 1998; 35:285–296. [PubMed: 9609552]
4. Costello LC, Franklin RB, Narayan P. Citrate in the diagnosis of prostate cancer. Prostate. 1999; 38:237–245. [PubMed: 10068348]
5. Huggins C. The prostate secretion. Harvey Lect. 1946; 52:148–193.
6. Harkonen PL. Androgenic control of glycolysis, the pentose cycle and pyruvate dehydroge-nase in the rat ventral prostate. J Steroid Bio-chem Mol Biol. 1981; 14:1075–1084.
7. Kavanagh JP. Sodium, potassium, calcium, magnesium, zinc, citrate and chloride content of human prostatic and seminal fluid. J Reprod. 1985; 75:35–41.
8. Mann, T.; Lutwak-Mann, C. Male Reproductive Function and Semen. Berlin: Springer; 1981.
9. Hicks JJ, Martinez-Manautou J, Pedron N, Rosado A. Metabolic changes in human spermatozoa related to capacitation. Fertil Steril. 1972; 23:172–179. [PubMed: 4333765]
10. Arver S. Zinc and zinc ligands in human seminal plasma. Acta Physiol Scand. 1982; 116:67–73. [PubMed: 7158392]
11. Tomlins AM, Foxall PJ, Lynch MJ, Parkinson J, Everett JR, Nicholson JK. High resolution 1H NMR spectroscopic studies on dynamic biochemical processes in incubated human seminal fluid samples. Biochim Biophys Acta. 1998; 1379:367–380. [PubMed: 9545599]
12. Barron ESG, Huggins C. The metabolism of isolated prostatic tissue. J Urol. 1944; 51:630–634.
13. Muntzing J, Varkarakis MJ, Saroff J, Murphy GP. Comparison and significance of respiration and glycolysis of prostatic tissue from various species. J Med Primatol. 1975; 4:245–251. [PubMed: 808627]
14. Farnsworth WE. Testosterone stimulation of citric acid synthesis in the rat prostate. Biochim Biophys Acta. 1966; 117:247–254. [PubMed: 5950245]
15. Harkonen P, Isotala A, Santti R. Studies on the mechanism of testosterone action on glucose metabolism in rat ventral prostate. J Steroid Biochem. 1975; 6:1405–1413. [PubMed: 1052841]
16. Santti RS, Villee CA. Hormonal control of hexokinase in male sex accessory glands. Endocrinology. 1971; 89:1162–1170. [PubMed: 5096992]
17. Costello LC, Akuffo V, Franklin RB. Net citrate production by isolated prostate epithelial cells. Enzyme. 1988; 39:125–133. [PubMed: 3378541]
18. Feichter A, Gmunder FK. Metabolic control of glucose degradation in yeast and tumor cells. Adv Biochem Eng Biotechnol. 1989; 39:1–28.
19. Cai G-Z, Callaci TP, Luther MA, Lee JC. Regulation of rabbit muscle phosphofructokinase by phosphorylation. Biophys Chem. 1997; 64:199–209. [PubMed: 9127945]

20. Feichter A, Gmunder FK. Metabolic control of glucose degradation in yeast and tumor cells. Adv Biochem Eng Biotechnol. 1989; 39:1–28.
21. Ramaiah A. Pasteur effect and phosphofructo-kinase. Curr Topics Cell Regul. 1974; 8:297–345.
22. Li Y, Rivera D, Ru W, Gunasekers D, Kemo RG. Identification of allosteric sites in rabbit phosphofructo-1-kinase. Biochemistry. 1999; 38:16407–16412. [PubMed: 10587466]
23. Cooper JE, Farid I. The role of citric acid in the physiology of the prostate: Lactic/citrate ratios in benign and malignant prostatic homogenates as an index of prostatic malignancy. J Urol. 1964; 92:533–536. [PubMed: 14226486]
24. Cooper JF, Imfeld H. The role of citric acid in the physiology of the prostate: A preliminary report. J Urol. 1959; 81:157–163. [PubMed: 13631793]
25. Marberger H, Marberger E, Mann T, Lutwak-Mann C. Citric acid in human prostatic secretion and metastasizing cancer of prostate gland. BMJ. 1962; i:835–836. [PubMed: 14469565]
26. Costello, LC.; Littleton, GK.; Franklin, RB. Regulation of citrate-related metabolism in normal and neoplastic prostate. In: Sharam, RK.; Criss, WE., editors. Endocrine Control in Neoplasia. New York: Raven Press; 1978. p. 303-314.
27. Kurhanewicz J, Vigneron DB, Males RG, Swanson MG, Yu KY, Hricak H. The prostate: Imaging and spectroscopy. Radiol Clin North Am. 2000; 38:115–138. [PubMed: 10664669]
28. Costello LC, Franklin RB. Concepts of citrate production and secretion by prostate. 2. Hormonal relationships in normal and neoplastic prostate. Prostate. 1991; 19:191–205.
29. Warburg O, Wind F, Negelein E. Über den Stoffwechsel von Tumoren im Körper. Klin Wochenschr. 1926; 5:829–832.
30. Parlo RA, Coleman PS. Enhanced rate of citrate export from cholesterol-rich hepatoma mitochondria. J Biol Chem. 1984; 259:9997–10003. [PubMed: 6469976]
31. Baggetto LG. Deviant energetic metabolism of glycolytic cancer cells. Biochimie. 1992; 74:959–974. [PubMed: 1477140]
32. Argiles JM, Lopez-Soriano FJ. Why do cancer cells have such a high glycolytic rate. Med Hypotheses. 1990; 32:151–155. [PubMed: 2142979]
33. Costello LC, Franklin RB. Bioenergetic theory of prostate malignancy. Prostate. 1994; 25:162–166. [PubMed: 7520580]
34. Dang CV, Semenza GL. Oncogenic alterations of metabolism. Trends Biochem Sci. 1999; 24:68–72. [PubMed: 10098401]
35. Movsas B, Chapman JD, Horwitz EM, Pinover WH, Greenberg RE, Hanlon AL, Iyer R, Hanks GE. Hypoxic regions exist in human prostate carcinoma. Urology. 1999; 53:11–18. [PubMed: 9886581]
36. Zhong H, Agani F, Baccala AA, Laughner E, Rioseco-Camacho N, Isaacs WB, Simons JW, Semenza GL. Increased expression of hypoxia inducible factor-1 alpha in rat and human prostate cancer. Cancer Res. 1998; 58:5280–5284. [PubMed: 9850048]
37. Zhong H, De Marzo AM, Laughner E, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Over-expression of hypoxia-inducible factor 1 alpha in common human cancers and their metastases. Cancer Res. 1999; 59:5830–5835. [PubMed: 10582706]
38. Swinnen J, Ulrix W, Heyns W, Verhoeven G. Coordinate regulation of lipogenic gene expression by androgens: Evidence for a cascade mechanism involving sterol regulatory element binding proteins. Proc Natl Acad Sci USA. 1997; 94:12975–12980. [PubMed: 9371785]
39. Swinnen J, Verhoeven G. Androgens and the control of lipid metabolism in human prostate cancer cells. J Steroid Biochem Mol Biol. 1998; 65:191–198. [PubMed: 9699873]
40. Arunakaran J, Balasubramanian K, Srinivasan N, Aruldhas MM, Govindarajulu P. Interaction of androgens and prolactin on prostatic enzymes of the pyruvate-malate cycle involved in lipogenesis in castrated mature monkey, *Macaca radiata*. Cytobios. 1992; 70:33–40. [PubMed: 1511627]
41. Halliday KR, Fenoglio-Preiser C, Sillerud LO. Differentiation of human tumors from non-malignant tissue by natural-abundance 13C NMR spectroscopy. Magn Reson Med. 1988; 7:384–411. [PubMed: 2459580]

42. Kurhanewicz J, Vigneron DB, Hricak H, Narayan P, Carroll P, Nelson SJ. Three dimensional hydrogen-1 MR spectroscopic imaging of the situ human prostate with high spatial resolution. Radiology. 1996; 198:795–805. [PubMed: 8628874]
43. Zaichick VY, Sviridova TV, Zaichick SV. Zinc in the human prostate gland: Normal hyperplasia, cancerous. Int Urol Nephrol. 1997; 29:565–574. [PubMed: 9413764]
44. Liney GP, Turnbull LW, Lowry M, Turnbull LS, Knowles AJ, Horsman A. In vivo quantification of citrate concentration and water T2 relaxation time of the pathologic prostate gland using 1H MRS and MRI. Magn Reson Imaging. 1997; 15:1177–1186. [PubMed: 9408138]
45. Liu Y, Costello LC, Franklin RB. Prolactin and testosterone regulation of mitochondrial zinc in prostate epithelial cells. Prostate. 1997; 30:26–32. [PubMed: 9018332]
46. Costello LC, Liu Y, Franklin RB, Kennedy MC. Zinc inhibition of mitochondrial aconitase and its importance in citrate metabolism of prostate epithelial cells. J Biol Chem. 1997; 272:28875–28881. [PubMed: 9360955]
47. Costello LC, Franklin RB, Liu Y, Kennedy MC. Zinc causes a shift toward citrate at equilibrium of the m-aconitase reaction of prostate mitochondria. Inorg Biochem. 2000; 78:161–165.
48. Liang J-Y, Liu Y, Zou J, Franklin RB, Costello LC, Feng P. Inhibitory effect of zinc on human prostatic cell growth. Prostate. 1999; 40:200–207. [PubMed: 10398282]
49. Feng P, Liang J-Y, Li T-L, Guan Z-X, Zou J, Franklin RB, Costello LC. Zinc induces mitochondrial apoptogenesis in prostate cells. Mol Urol. 2000; 4:31–35. [PubMed: 10851304]
50. Perlman H, Zhang X, Chen MW, Walsh K, Buttyan R. An elevated bax/bcl-2 ratio corresponds with the onset of prostate epithelial cell apoptosis. Cell Death Differ. 1999; 6:48–54. [PubMed: 10200547]
51. Furuya Y, Krajewski S, Epstein JI, Reed JC, Isaacs JT. Expression of bcl-2 and the progression of human and rodent prostatic cancers. Clin Cancer Res. 1996; 2:389–398. [PubMed: 9816182]
52. Colembel M, Symmans F, Gils S, O'Toole KM, Chopin D, Benson M, Olsson CA, Korsmeyer S, Buttyan R. Detection of the apoptosis-sup-pressing oncoprotein bcl-2 in hormone-refactory human prostate cancers. Am J Pathol. 1993; 143:390–400. [PubMed: 7688182]
53. Costello LC, Liu Y, Zou J, Franklin RB. Evidence for a zinc uptake transporter in human prostate cancer cells which is regulated by prolactin and testosterone. J Biol Chem. 1999; 274:17499–17504. [PubMed: 10364181]
54. Habib FK, Mason MK, Smith PH, Stitch SR. Cancer of the prostate: Early diagnosis by zinc and hormone analysis. Br J Cancer. 1979; 39:700–704. [PubMed: 87214]
55. Heerschap A, Jager GJ, van der Graaf M, Barentsz JO, De La Rossette JJMCH, Oosterhof GON, Ruijter ETG, Ruijs SHJ. In vivo proton MR spectroscopy reveals altered metabolite content in malignant prostate tissue. Anticancer Res. 1997; 17:1455–1460. [PubMed: 9179183]
56. Pickett B, Vigneault E, Kurhanewicz J, Verhey L, Roach M. Static field intensity modulation to treat a dominant intraprostatic lesion to 90 Gy compared to seven field 3-dimensional radiotherapy. Int J Radiat Oncol Biol Phys. 1999; 43:921–929. [PubMed: 10098448]
57. Zaichick VY, Sviridova TV, Zaichick SV. Zinc concentration in human prostatic fluid: Normal, chronic prostatitis, adenoma and cancer. Int Urol Nephrol. 1996; 28:687–694. [PubMed: 9061429]
58. Anderson RU, Fair WR. Physical and chemical determinations of prostatic secretion in benign hyperplasia, prostatitis and adenocarcinoma. Invest Urol. 1976; 14:137–140. [PubMed: 61187]
59. Kavanagh JP, Darby C, Costello CB. The response of seven prostatic fluid components to prostatic disease. Int J Androl. 1982; 5:487–496. [PubMed: 7174128]
60. Fair WR, Cordonnier JJ. The pH of prostatic fluid: A reappraisal and therapeutic implications. J Urol. 1978; 120:695–698. [PubMed: 32404]
61. Costello LC, Liu Y, Zou J, Franklin RB. Mitochondrial aconitase gene expression is regulated by testosterone and prolactin in prostate epithelial cells. Prostate. 2000; 42:196–202. [PubMed: 10639190]

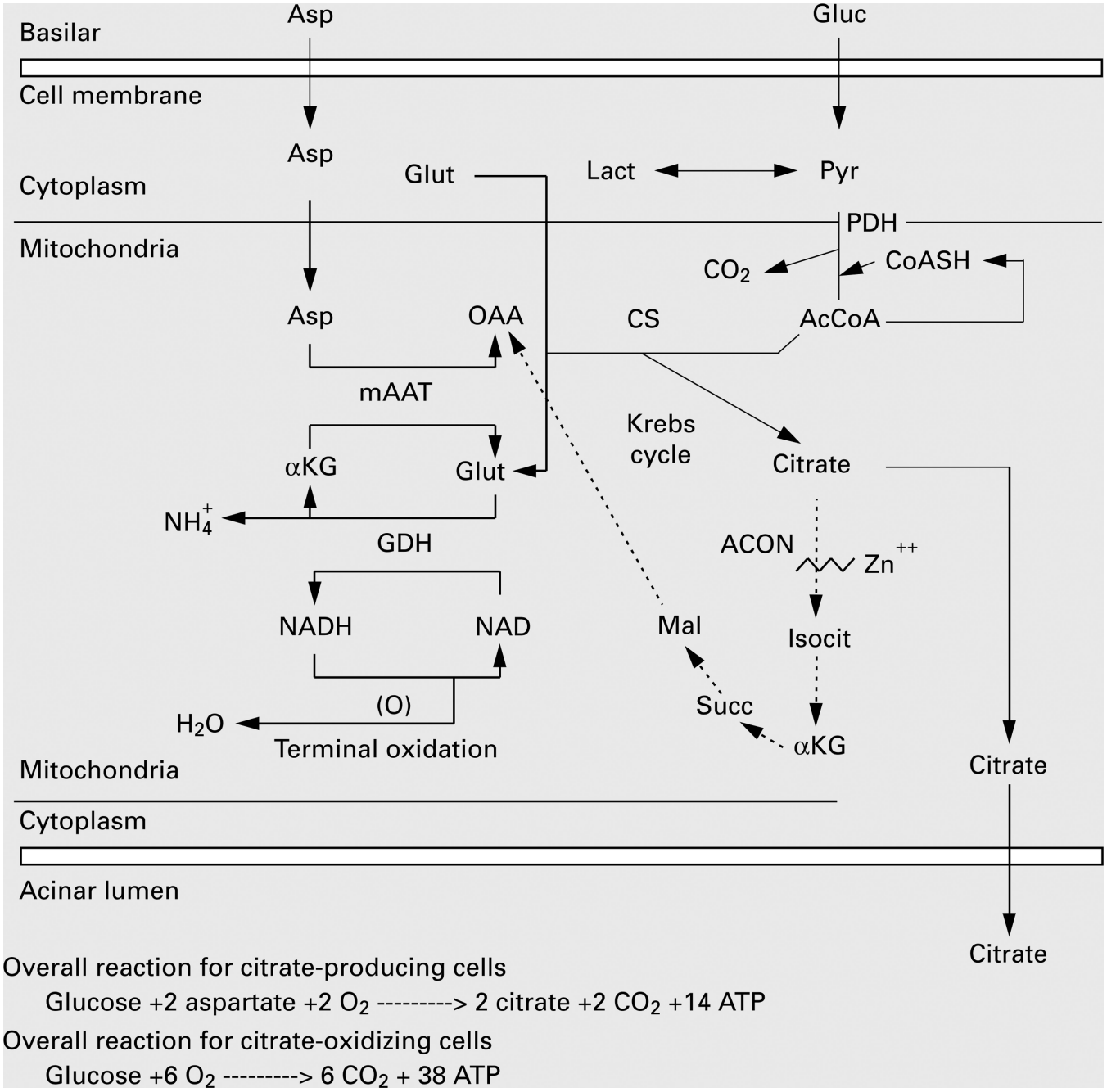

Fig. 1.

The pathway of net citrate production in citrate-producing prostate epithelial cells. Aspartate provides the intramitochondrial source of oxaloacetate and glucose provides the source of acetyl-CoA for citrate synthesis. The key regulatory enzymes for citrate synthesis are mitochondrial aspartate aminotransferase and pyruvate dehydrogenase. Citrate oxidation is limited due to the inhibition of m-aconitase activity by the in-tramitochondrial accumulation of high zinc levels. Citrate accumulates within the cell and is ultimately secreted as a major constituent of prostatic fluid. Asp = Aspartate; Gluc = glucose; Glut = glutamate; OAA = oxalacetate; CoASH = coenzyme A; AcCoA = acetyl coenzyme A; Isocit = isocitrate; αKG

= α-ketoglutarate; Succ = succinate; Mal = ma-late; Pyr = pyruvate; Lact = lactate; PDH = pyruvate dehydrogenase; CS = citrate synthase; ACON = aconitase; mAAT = mitochondrial aspartate aminotransferase; GDH = glutamic dehydrogenase.

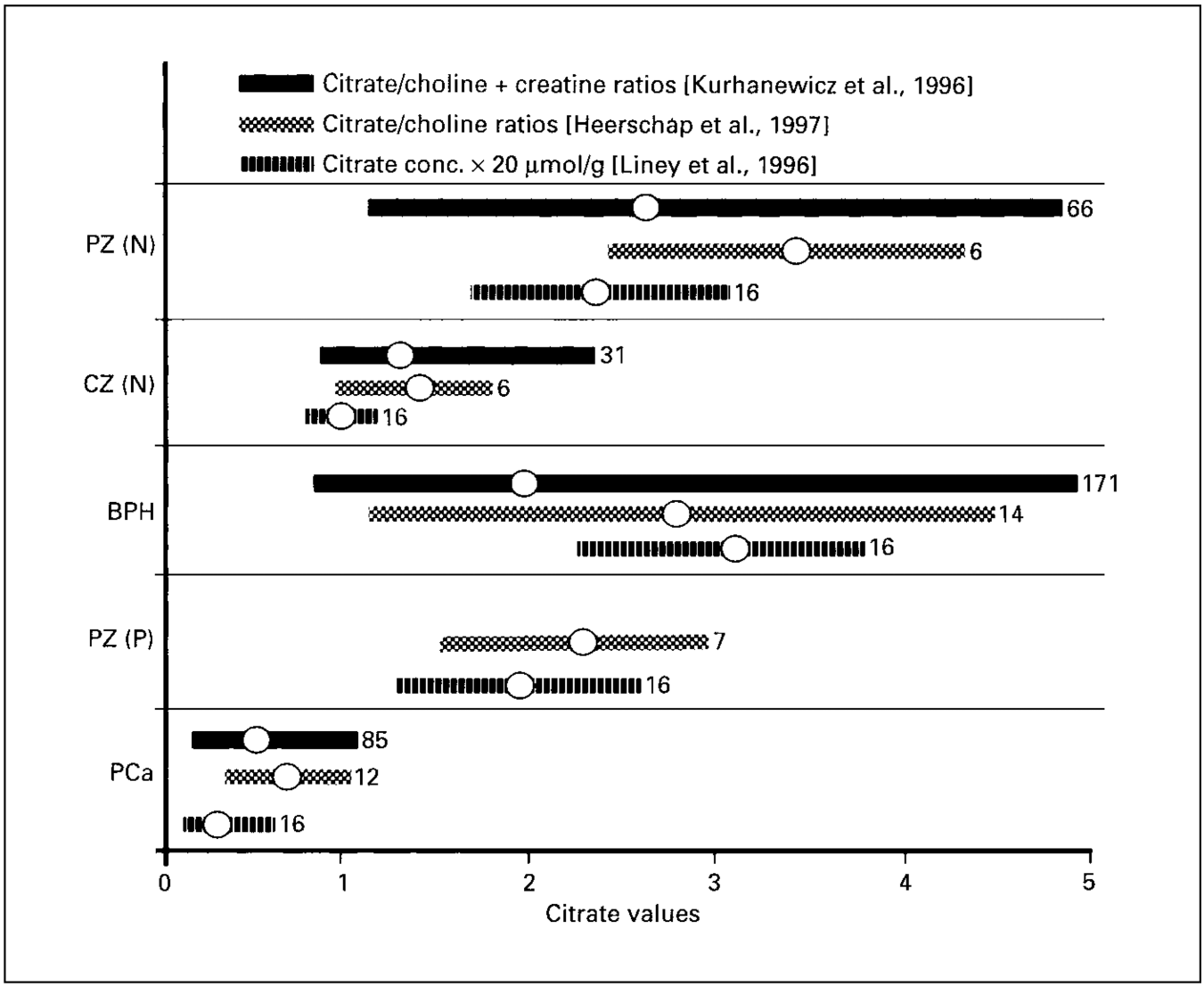

Fig. 2.

In situ 1-H MRS of prostate citrate in the diagnosis of PCa. Note that there is no overlap in the citrate content of malignant peripheral zone (PCa) vs. nonmalignant peripheral zone [PZ (N), PZ (P)], and no malignant tissue exhibited a high peripheral zone citrate level. PZ (N) = Peripheral zone, normal subjects; PZ (P) = peripheral zone, patients without PCa; CZ (N) = central zone, normal subjects; BPH = central zone, BPH patients; PCa = peripheral zone, malignant loci. The number at each bar represents the number of subjects. [Figure taken from ref. 4.]

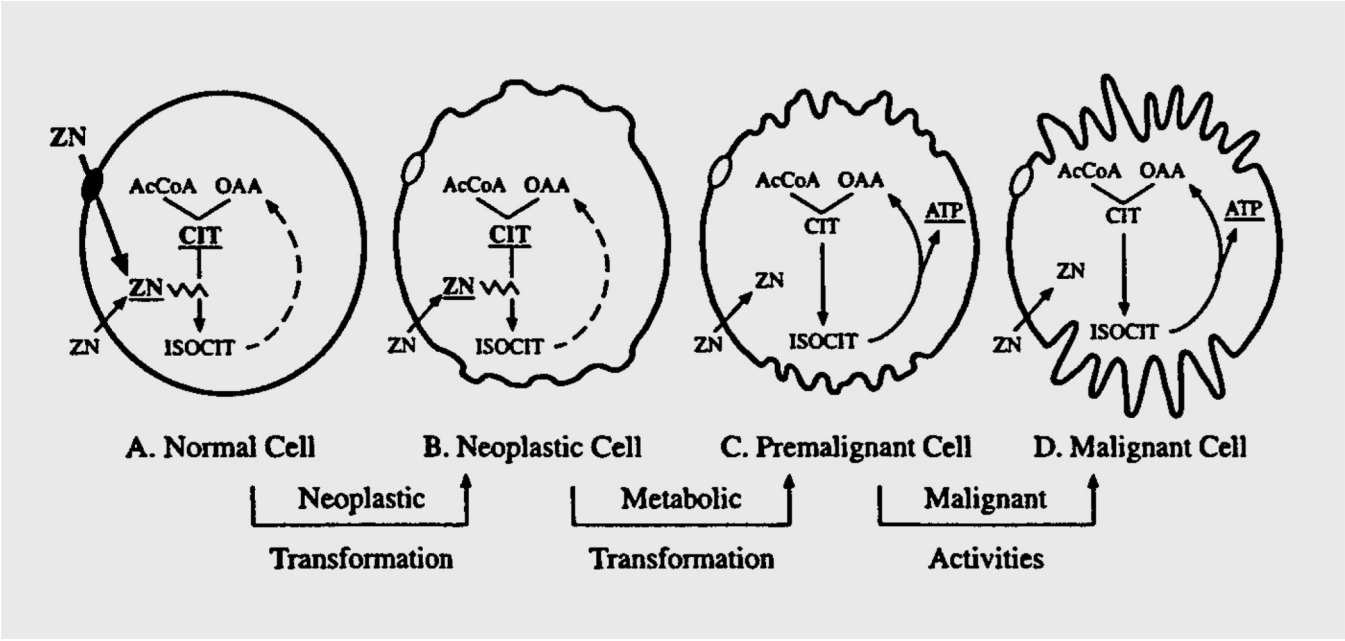

Fig. 3.

A proposed concept of the role of zinc citrate-related energy metabolism in the pathogenesis and treatment of prostate malignancy. Normal prostate peripheral zone epithelial cells contain a transport mechanism for the accumulation of high zinc levels. Due to zinc inhibition of citrate oxidation, these cells accumulate high levels of citrate and sacrifice the potential energy derived from citrate oxidation. The normal cell is genetically transformed to a neoplastic malignant cell type which initially is metabolically incapable of manifesting malignant activities. The neoplastic cell loses the ability to accumulate zinc and begins to exhibit the metabolic conversion to a pre-malignant citrate-oxidizing cell. Citrate oxidation proceeds with the accompagnying production of ATP which provides the metabolic requirements for the full expression of malignant activities and proliferation of the malignant cells. Consequently, the premalignant and malignant cells will be arrested by restoration of the accumulation of zinc, or by agents which will selectively inhibit m-aconitase and citrate oxidation, or by repression of the expression of m-aconitase.

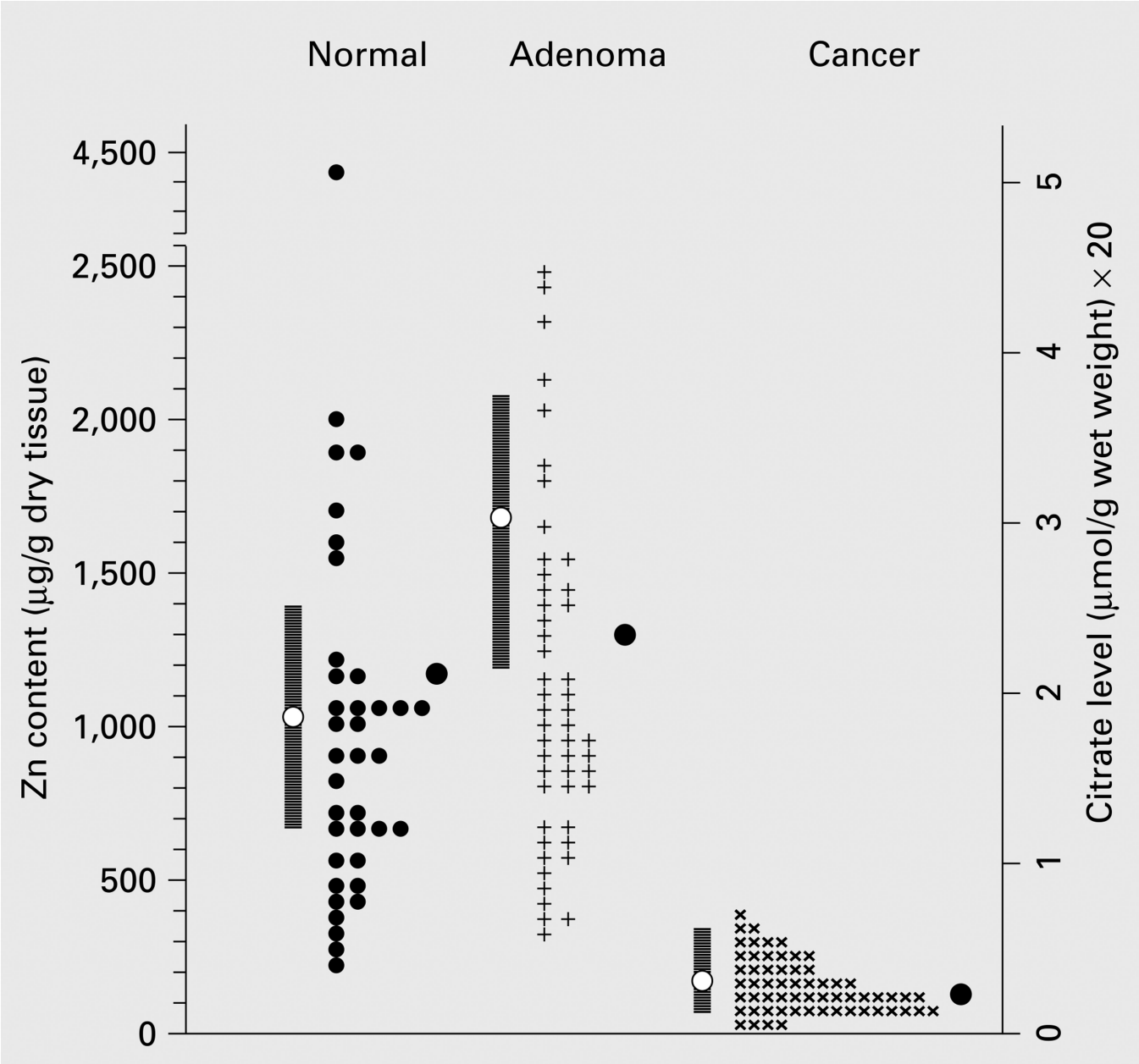

Fig. 4.

Comparison of the zinc and citrate changes in prostate cancer. The citrate data are taken from Liney et al. [44]. The values were determined by in situ MRS measurements. The bars represent the distribution of citrate values obtained from the subjects and the open circles ar the mean values. The normal and cancer citrate values are for the peripheral zone, and the adenoma values are for the central zone. The individual zinc values and the mean values (black circles) for the subjects are taken from Zaichick et al. [43]. The values were determined by analysis of biopsy samples.

Table 1

Representative citrate and zinc levels in prostate

	Citrate, nmol/g	Zinc, μg/g
Normal (mixed tissue)	8,000	209
Normal (central zone)	4,000	–
Normal (peripheral zone)	13,000	–
BPH	8,000–15,000	589
PCa (mixed tissue)	1,000–2,000	55
PCa (malignant tissue)	500	–
Other soft tissue	150–450	30
Blood plasma	90–110	1
Prostatic fluid	40,000–150,000	590