Feature Article

The Metabolism of Prostate Malignancy: Insight Into the Pathogenesis of Prostate Cancer and New Approaches for Its Diagnosis and Treatment

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ABSTRACT

What is the role of altered metabolism in the pathogenesis of prostate cancer (PCa), and how can the relationship of altered metabolism to malignancy be applied to the diagnosis and treatment of PCa?

These are critical issues relating to the incidence and management of PCa. Compelling evidence exists that prostate malignancy involves and requires an alteration in zinc accumulation and citrate-related metabolism of prostate secretory epithelial cells of the peripheral zone. These metabolic alterations are the most consistent and persistent changes that uniquely differentiate malignant prostate tissue from normal and benign prostatic hyperplasia (BPH) tissue. The malignant process involves an early metabolic transformation of energy-inefficient, zinc-accumulating, citrate-producing, sane epithelial cells to malignant cells that have lost the ability to accumulate zinc; and, correspondingly, have become energy-efficient, citrate-oxidizing cells. The interrelationship between zinc and citrate metabolism has now been established. This relationship has served as the basis for a new and highly effective noninvasive magnetic resonance spectroscopy (MRS) procedure for the diagnosis of prostate cancer. The metabolic transformation required for the onset and progression of malignancy provides new approaches to the treatment of PCa, especially in the progressive and metastatic stages that are currently virtually untreatable.

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INTRODUCTION

Prostate cancer (PCa) is the second-leading cause of cancer deaths in males. However, the pathogenesis of this malignancy is poorly understood. The diagnosis of PCa is dependent largely on digital rectal examination and prostrate-specific antigen (PSA), with confirmation by biopsy examination. Treatment of PCa during the hormone-refractory stages and following metastasis is difficult, and most often it is unsuccessful. New approaches to the diagnosis, treatment, and possible prevention of PCa are urgently needed. However, the lack of understanding of the development and progression of prostate malignancy contributes greatly to the lack of progress in these areas.

The altered metabolism (the details of which are presented in this review) provides the most consistent and persistent characteristic for distinguishing malignant prostate cells from nonmalignant tissue. Unfortunately, this relationship is not widely acknowledged nor appreciated by the clinical and research community. As will be presented here, the altered metabolism in malignancy provides new insights into the pathogenesis and progression of malignancy that can provide novel approaches to the diagnosis, treatment, and prevention of PCa. For additional, extensive reviews and details of zinc and citrate-related metabolism of normal and malignant prostate, the reader is referred to our recent publications.[1-3]

TALKING POINTS Physicians Pharmacy Formulary Cancer Nurses

Altered metabolism is the most consistent and persistent characteristic distinguishing malignant prostate cells from nonmalignant tissues.

The reestablishment of high intracellular zinc levels in malignant prostate cells could arrest the progression of malignancy as well as prevent its development.

Understanding the metabolic alterations present in malignant prostate cells can provide new insights into the pathogenesis and progression of prostate cancer, which can result in novel approaches to the diagnosis and treatment of this malignancy.

Detecting metabolic alterations may lead to early diagnosis of prostate cancer, critical to the successful treatment of patients and management of the disease.

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CITRATE AND ZINC RELATIONSHIPS IN PROSTATE

The normal human prostate gland accumulates the highest levels of citrate and zinc of any soft tissue in the body (Table 1). This capability is due to the unique metabolic relationships of the glandular epithelium of the peripheral zone. Other regions of the normal prostate (eg, central zone) are not significantly involved. In this regard, it is also extremely important to emphasize that malignancy arises and develops mainly in the peripheral zone.

TABLE 1. REPRESENTATIVE CITRATE AND ZINC LEVELS IN PROSTATE

	Citrate	Zinc
Normal (mixed tissue)	8,000	209
Normal (central zone)	4,000	121
Normal (peripheral zone)	13,000	295
BPH	8,000–15,000	589
PCa (mixed tissue)	1,000–2,000	55
PCa (malignant tissue)	500	—
Other soft tissues	150–450	30
Blood plasma	90–110	1
Prostatic fluid	40,000–150,000	590

Note: Citrate values are nmols/gram wet weight. Zinc values are µg/gram wet weight.

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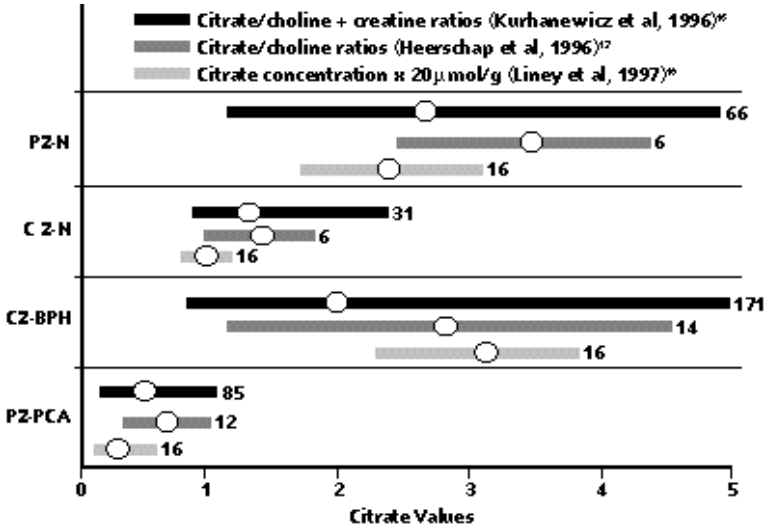
In contrast to normal prostate, the citrate and zinc levels of malignant prostate are dramatically decreased to the low levels that characterize most other soft tissues (Table 1). Recent advances in magnetic resonance spectroscopic imaging (MRSI) of the in situ prostate gland have confirmed and established this citrate and zinc relationship in normal, benign prostatic hyperplasia (BPH), and PCa subjects (Figure 1). Moreover, the decline in citrate and zinc levels occurs early in malignancy.[1-5] In BPH, the citrate and zinc levels remain as high as or higher than the normal levels. Of extreme significance is the fact that, in PCa, prostate tissue citrate or zinc levels rarely, if ever, exhibit the high levels of normal or BPH tissue. Consequently, the decrease in citrate and zinc levels is a consistent and persistent characteristic that distinguishes PCa from normal prostate and BPH (Figures 1 and 2).

THE METABOLIC RELATIONSHIP BETWEEN CITRATE AND ZINC

Net citrate production (ie, the production, accumulation, and secretion of extraordinarily high levels of citrate) requires a unique metabolic relationship of the prostatic secretory epithelial cells (ie, citrate-producing cells) that does not exist in typical citrate-oxidizing mammalian cells. Citrate is essentially an end product of intermediary metabolism of these epithelial cells, whereas citrate is a key intermediate for oxidation and energy production via the Krebs cycle and/or for lipogenesis in essentially all other cells. A pathway for net

FIGURE 1. IN SITU MAGNETIC RESONANCE SPECTROSCOPY DETERMINATION OF CITRATE LEVELS IN NORMAL, BPH, AND PROSTATE CANCER



This is a composite of independent studies from three different laboratories. The bars represent the range of the individual values and the open circles are the mean values. The numbers at the end of the bars represent the number of subjects.

BPH=benign prostatic hyperplasia; PZ-N=normal peripheral zone; CZ-N=normal central zone; CZ-BPH=BPH region of central zone; PZ-PCA=malignant region of peripheral zone.

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citrate production has been established (Figure 3). An important factor is the limiting mitochondrial (m-) aconitase activity that minimizes the oxidation of citrate at the initial step for citrate oxidation via the Krebs cycle. This necessitates a source of oxalacetate (OAA) for continued synthesis of citrate. To achieve this, aspartate is utilized for OAA production via the mitochondrial aspartate aminotransferase (mAAT) reaction. Glucose, via pyruvate oxidation, provides the acetyl Coenzyme A (CoA) to complete the synthsesis of citrate.

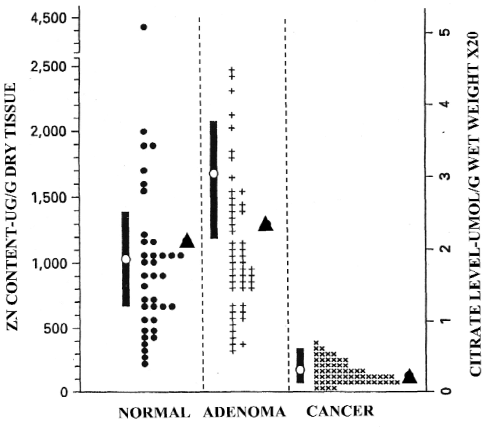
A key issue that we recently resolved was the mechanism by which m-aconitase activity was limiting in prostate cells; whereas m-aconitase is not a limiting enzyme reaction in the intermediary metabolism of other mammalian cells. Either the level of m-aconitase enzyme was uniquely low in the prostate cells or the enzyme activity was low due to mitochondrial environment conditions. The consistent correlation between zinc and citrate levels in prostate had indicated that zinc might be involved in the metabolic production of citrate by prostate secretory epithelial cells. It is now established that the citrate-producing prostate cells, which are also zinc-accumulating cells, contain high levels of mitochondrial zinc that inhibit the m-aconitase activity leading to the accumulation of citrate.[6]

THE METABOLIC ALTERATIONS IN PROSTATE MALIGNANCY

We can now define normal peripheral zone and BPH secretory epithelial cells as zinc-accumulating, citrate-producing cells. In contrast, malignant prostate cells have lost the ability to accumulate zinc and citrate; and, in the absence of zinc inhibition of m-aconitase, can be represented as citrate-oxidizing cells with an operational Krebs cycle typical of other mammalian cells (Figure 3). In addition, strong evidence exists[1-3] that the metabolic transformation from zinc-accumulating, citrate-producing sane cells to citrate-oxidizing malignant cells that have lost the ability to accumulate zinc occurs early and prior to histopathological changes (Figure 4). This represents a premalignant stage. Because malignant prostate tissue virtually never exhibits high citrate levels, even in early, highly differentiated malignancy, it is evident that overt malignancy is not evident prior to the metabolic transformation. Obviously, the metabolic transformation is an essential step in the capability of the neoplastic cell to manifest its malignant activities. This metabolic relationship leads to the concept of the pathogenesis and progression of prostate malignancy represented in Figure 4.

It now becomes evident that the neoplastic (ie, genetically transformed) malignant cell has lost the ability to accumulate zinc, which leads to the metabolic transformation that is essential to malignancy. Currently, little information exists regarding the mechanism(s) involved in the ability of prostate cells to accumulate uniquely high zinc levels. Therefore, the zinc-accumulating processes that might be altered in the neoplastic malignant cell cannot yet be identified. Some recent studies have identified a zinc uptake transporter that is associated with zinc accumulation in prostate cells.[7] Whether or not altered expression of this transporter and/or other processes are responsible for the

FIGURE 2. COMPARISON OF ZINC AND CITRATE LEVELS IN PROSTATE



The citrate values are from Liney et al[18] as presented in Figure 1. The solid bars represent the range of citrate values, and the open circles are the mean values (n=16). Citrate was determined by in situ magnetic resonance spectroscopy. The zinc values are from Zaichick et al[10] and were determined by zinc analysis of biopsy tissue. Each dot, +, and x represents a value for each biopsy sample from a different subject. The triangles represent the mean zinc value for each population.

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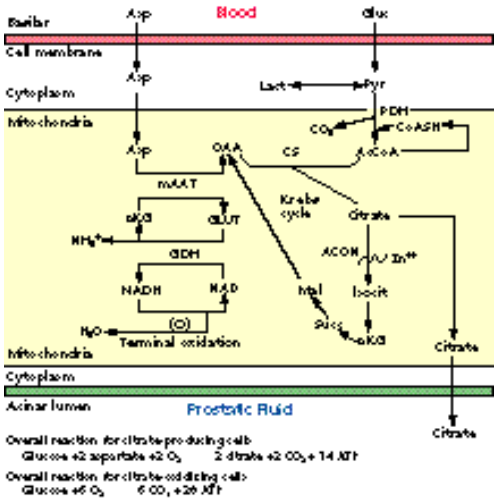
FIGURE 3. THE METABOLIC PATHWAY OF NET CITRATE PRODUCTION BY PROSTATE SECRETORY EPITHELIAL CELLS



Asp=aspartate; Gluc=glucose; Pyr=pyruvate; Lact=lactate; AcCoA=acetyl Coenzyme A; OAA=oxalacetate; Isocit=isocitrate; aKG=alpha ketoglutarate; Succ=succinate; Mal=malate; PDH=pyruvate dehydrogenase; mAAT=mitochondrial aspartate aminotransferase; CS=citrate synthase; ACON=aconitase; GDH=glutamate dehydrogenase.

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lack of zinc accumulation remains to be determined. For this discussion, we can expect that the zinc-accumulating apparatus of the sane cell is not functioning or is overridden in the malignant cell.

THE BIOENERGETICS OF NET CITRATE PRODUCTION AND PROSTATE MALIGNANCY

It has become evident since the early classical studies of Warburg and associates[8] that the malignant process involves and requires alterations in the intermediary metabolism of sane cells that give rise to tumor cells. The metabolic requirements of the tumor cells are different from those of the sane cells. Although not universal, tumorigenesis generally involves the metabolic alterations that transform aerobic, energy-efficient, sane cells to tumor cells characterized by an energy-inefficient high-aerobic glycolysis. This general relationship is not applicable to prostate cells. The sane prostate secretory epithelial cell, which is a citrate-producing cell, exhibits a high aerobic glycolysis and is energy-inefficent (Figure 3). Net citrate production results in incomplete oxidation of glucose, which results in the decrease of about 60% of the potential adenosine triphosphate [ATP] production. The metabolic transformation from citrate production to citrate oxidation provides a marked increase in energy production by the malignant prostate cells. Conceivably, an increased energy production is a requirement for the conduct of malignant activities of these cells.

CLINICAL SIGNIFICANCE

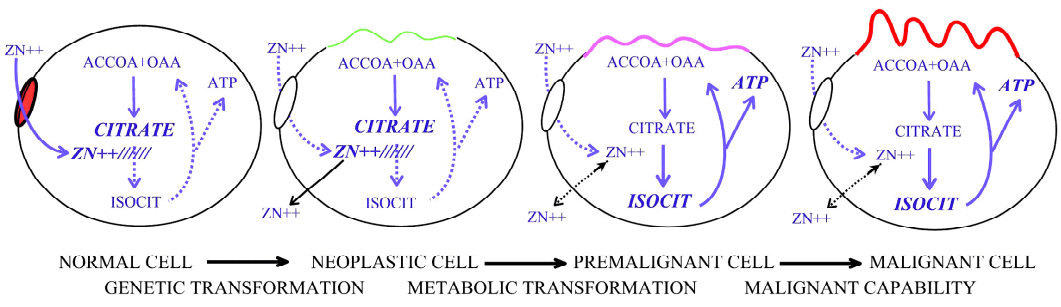
Hopefully, the preceding discussion has provided compelling and convincing evidence that alterations in zinc and citrate-related metabolism are intimately involved in the development and progression of prostate malignancy. In addition to providing an understanding of the pathogenesis of PCa, these metabolic relationships are of important clinical relevance. The two most apparent areas of relevance are 1) in the diagnosis of PCa, and 2) in the treatment and, perhaps, prevention of PCa.

1. Diagnosis

An accurate and early diagnosis of PCa is critical to the successful treatment of the patient and management of the disease. Although a valuable diagnostic marker for PCa detection, the widespread contemporary use of PSA is fraught with variables that affect its value, and it 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 additional biopsies. This is especially the case in early PCa.

Of immense value would be a noninvasive procedure for the direct detection of malignancy, particularly in the early stages. 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 extremely valuable. Because dramatic citrate changes occur very early in malignancy, citrate level could provide a valuable biochemical marker for the early detection of PCa. With the use of an endorectal coil, 1-H MRS (magnetic resonance spectroscopy) has been successfully applied to the in situ detection of citrate levels in prostate (Figure 5).[3,9] Consistent and corroborating results from several laboratories (Figure 1) have now demonstrated that malignant areas of the prostate can be readily identified on the basis of a decreased citrate content. It is extremely important to note the absence of any overlap of the citrate values of peripheral zone in the absence of malignancy vs the values when malignancy is present. In combination with magnetic resonance imaging (MRI), citrate analysis

FIGURE 4. A CONCEPT OF THE GENETIC AND METABOLIC RELATIONSHIPS IN THE DEVELOPMENT OF MALIGNANT PROSTATE CELLS IN HUMAN PROSTATE CANCER



The normal secretory epithelial cell is a zinc-accumulating citrate-producing cell. The cell contains a zinc-accumulating apparatus, and the accumulation of zinc inhibits citrate oxidation by the Krebs cycle. Coupled ATP production is minimal. The normal cell undergoes a genetic transformation to a neoplastic cell type that has lost the ability to accumulate zinc. As the intracellular zinc level decreases, the inhibitory effect of zinc on citrate oxidation is eliminated; the Krebs cycle is functional and coupled ATP production is increased in this premalignant cell. The cell is now metabolically and energetically capable of proceeding with its malignant activities, and the malignant cell can be identified by histopathological examination.

ACCOA=acetyl Coenzyme A; OAA=oxalacetate; ISOCIT=isocitrate; ATP=adenosine triphosphate.

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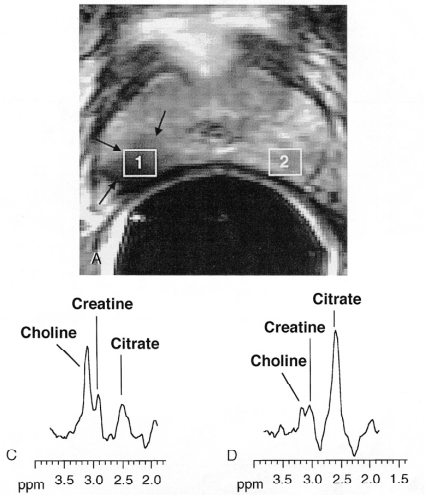
FIGURE 5. A MAGNETIC RESONANCE SPECTROSCOPY IMAGE OF THE IN SITU HUMAN PROSTATE



The rectangle insert 1 in the right lobe of the peripheral zone is at the site of a malignant locus. The rectangle insert 2 in the left lobe of the peripheral zone is a normal region. The corresponding 1-H magnetic resonance spectroscopy spectra demonstrate the high citrate signal in the normal peripheral zone as compared with the low signal at the malignant site. The change in citrate becomes more dramatic when referenced to the creatine + choline peaks.

Reprinted with permission from Kurhanewicz.[9]

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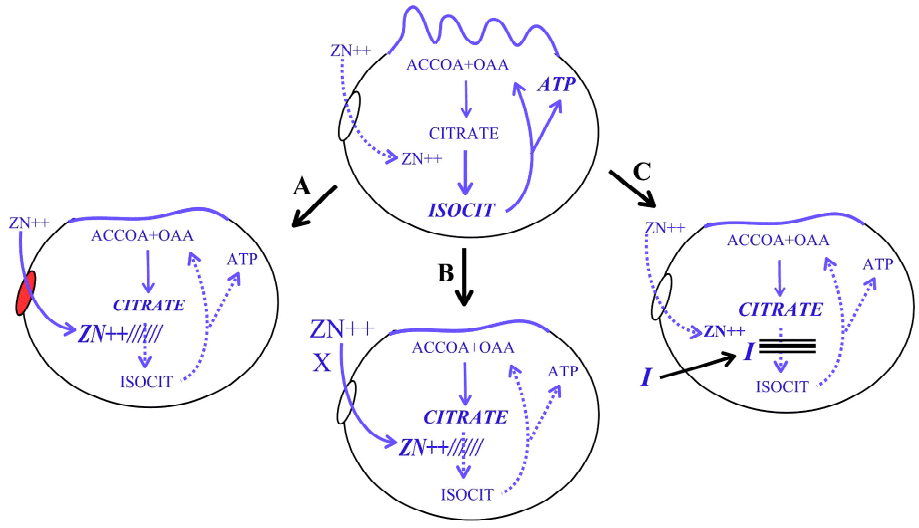
exhibits a positive predictive value of 90% for detection of malignancy and excludes the presence of cancer with an 83% negative predictive value.[9] This imaging procedure directly locates the presence of malignant loci and provides a map of the prostate. Estimates of the volume of malignancy can be obtained. Therefore, 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. Although still in the technological development stage and undergoing clinical testing, the noninvasive in situ MRS detection of altered citrate metabolism provides the most promising diagnostic procedure for PCa.

The possible use of changes in zinc levels for the diagnosis of PCa warrants serious consideration. 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 (Table1; Figure 2). Habib et al[5] reported that the decrease in prostate zinc levels occurs early in malignancy and suggested that changes in zinc could be employed in the early detection of PCa. Zaichick et al[10] reported 98% sensitivity and specificity for the differential diagnosis of PCa based on zinc analysis of prostate biopsy samples. Inconclusive histopathological examination of biopsy samples could be subjected to zinc analysis, which could reveal early

FIGURE 6. NEW CONCEPTS FOR THE TREATMENT OF PROSTATE MALIGNANCY BASED ON THE METABOLIC RELATIONSHIPS OF THE PATHOGENESIS OF MALIGNANT CELLS



The transformation of the citrate-oxidizing malignant cell to a citrate-producing cell that is metabolically incapable of conducting malignant activities. (A) Restoration of the zinc-accumulating apparatus that became dysfunctional in the malignant cell. (B) Increase of the availability of zinc through dietary supplementation and the use of an agent that will increase the uptake of zinc by the cell. (C) Administration of an inhibitor of citrate oxidation that will be selectively taken up by the prostate cell.

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malignancy and premalignant stages not detected by microscopic examination. 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.

Zaichick et al[11] demonstrated a dramatic decrease in the zinc content of the expressed prostatic fluid of cancer patients vs fluid from normal, prostatitis, and BPH patients. The investigators reported a 93% accuracy in diagnosing PCa based on the decreased zinc level of prostatic fluid. Other studies[12] did not reveal a significant decrease in prostatic fluid zinc levels in PCa. If the report of Zaichick et al 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.

2. Treatment and Prevention of PCa

The effective treatment of PCa is highly dependent upon its early diagnosis while the malignancy is confined to the intracapsular prostate gland. Effective treatment is lessened and becomes nonexistent as the malignancy spreads beyond the capsule and metastasizes to distant tissues. In addition, no established protocols exist for the prevention of PCa.

The inability of malignant prostate cells to accumulate high zinc levels is a major factor and essential step in the metabolic transformation associated with the malignant process (Figure 4). The re-establishment of high intracellular zinc levels in the malignant cells could arrest the progression of malignancy as well as prevent the development of malignancy. It is interesting to note that increased dietary zinc reportedly decreases the incidence of PCa.[13] Also, oral administration of zinc salts can increase the tissue accumulation of zinc.[14] Based on these relationships, a treatment regimen that restores the accumulation of zinc in prostate cells needs to be established (Figure 6). This requires the availability of zinc in the blood for uptake by the prostate cells and the identification of an appropriate agent to stimulate zinc uptake by prostate epithelial cells. Conceivably, a high dietary supplement of zinc (perhaps 300–1,000 mg daily) could result in an effective accumulation of zinc in the prostate. The fact that prolactin and testosterone can increase zinc accumulation in prostate cells[14] demonstrates that the development for human use of an agent that facilitates the accumulation of zinc in the prostate cells is feasible. Once the mechanism of zinc accumulation in prostate is established, approaches designed to re-establish 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. Alternative approaches can be directed at other mechanisms to inhibit citrate oxidation of malignant cells. The development of specific inhibitors of prostate citrate oxidation should be pursued (Figure 6). For example, fluoroacetate is an effective inhibitor of mammalian cell m-aconitase activity and citrate oxidation. If such an inhibitor could be modified to target prostate cells, it could provide an effective treatment without adverse systemic effects.

Another approach could take advantage of the observations that have been made that show that the level of m-aconitase is under gene regulation, and that, specifically in prostate cells, the m-aconitase gene is hormonally (prolactin and testosterone) regulated.[15] The 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. OS

REFERENCES

1. Costello LC, Franklin RB. The intermediary metabolism of the prostate: a key to understanding the pathogenesis and progression of prostate malignancy. *Oncology*. 2000;59:269-282.
2. 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.
3. Costello LC, Franklin RB, Narayan P. Citrate in the diagnosis of prostate cancer. *Prostate*. 1999;38:237-245.
4. 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.
5. 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.
6. 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.
7. 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.
8. Warburg O, Wind F, Negelein E. Uber den Stoffwechsel von Tumoren im Korper. *Klin Woch*. 1926;5:829-832.
9. Kurhanewicz J, Vigneron DB, Males RG, et al. The prostate: MR imaging and spectroscopy. *Radiol Clin North Am*. 2000;38:115-138.
10. Zaichick VY, Sviridova TV, Zaichick SV. Zinc in the human prostate gland: normal, hyperplastic, cancerous. *Intern Urol Nephr*. 1997;29:565-574.
11. Zaichick VY, Sviridova TV, Zaichick SV. Zinc concentration in human prostatic fluid: normal, chronic prostatitis, adenoma and cancer. *Intern Urol Nephr*. 1996;28:687-694.
12. Kavanagh JP. Sodium, potassium, calcium, magnesium, zinc, citrate and chloride content of human prostatic and seminal fluid. *J Reprod*. 1985;75:35-41.
13. Kristal AR, Stanford JL, Cohen JH, et al. Vitamin and mineral supplement use is associated with reduced risk of prostate cancer. *Cancer Epidemiol Biomark Prevent*. 1999;8:887-892.
14. 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.
15. 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.
16. Kurhanewicz J, Vigneron DB, Hricak, H, Narayan P, Carroll P, Nelson SJ. Three dimensional hydrogen-1 MR spectroscopic imaging of the in situ human prostate with high spatial resolution. *Radiology*.1996;198:795-805.
17. Heerschap A, Jager GJ, van der Graaf M, Barentsz JO, De La Rosette 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.
18. 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. *Mag Res Imag*. 1997;15:1177-1186.