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Mitochondrial function, zinc, and intermediary metabolism relationships in normal prostate and prostate cancer

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Abstract

Human prostate secretory epithelial cells have the uniquely specialized function of accumulating and secreting extremely high levels of citrate. This is achieved by their ability to accumulate high cellular levels of zinc that inhibit citrate oxidation. This process of net citrate production requires unique metabolic/bioenergetic mitochondrial relationships. In prostate cancer, the malignant cells undergo a metabolic transformation from zinc-accumulating citrate-producing sane cells to citrate-oxidizing malignant cells that lost the ability to accumulate zinc. This review describes the metabolic/bioenergetic, zinc and mitochondrial relationships involved in normal and malignant prostate. Hopefully, this report will generate much needed interest and research in this neglected, but critically important, area of investigation.

Keywords

Prostate citrate metabolism; Terminal oxidation; Krebs cycle; Mitochondrial aconitase; Zinc transport; Apoptosis

1. Introduction

Future progress in combating prostate cancer (PCa) will be highly dependent upon an understanding of the pathogenesis of malignant prostate cells. The etiology and mechanisms involved in the development and progression of prostate malignancy remain a mystery. Irrefutable and overwhelming clinical and experimental evidence now exists that altered cellular and mitochondrial zinc accumulation and associated changes in citrate-related metabolism are essential to the development and progression of prostate malignancy. The compelling evidence leads to the concept that the malignant process in prostate cancer involves a required metabolic transformation of zinc-accumulating citrate-producing normal glandular secretory epithelial cells to citrate-oxidizing malignant cells that have lost the ability to accumulate zinc. The mitochondria play an essential role in this metabolic transformation. An understanding of these relationships provides important insight into the pathogenesis of prostate cancer. In this paper, the clinical and experimental evidence relating to zinc and citrate-related metabolism in normal and malignant prostate with special

focus on the mitochondrial involvement will be reviewed. The zinc/metabolic transformation has important implications on cellular bioenergetics, cell growth and proliferation, lipogenesis, and other metabolic-associated malignant activities. The uniqueness of the metabolic relationships of prostate malignancy, which are in contrast to the general concepts of tumor metabolism, will be highlighted. 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. Therefore, we hope to stimulate more awareness and interest in this neglected, albeit most important, area of prostate cancer. For extended background information, we direct the reader to our earlier reviews (Costello and Franklin, 1998, 2000, 2001, 2002; Costello et al., 1999, 2002, 2004a). Also, we have opted to make this review an instructive and provocative presentation that brings into focus the role and importance of metabolic alterations that are essential to the evolution and survival of highly specialized cell function.

2. Citrate metabolism in normal prostate epithelial cells

We must first define the cell type that is the focus of this presentation. The human prostate gland is a complex organ comprised of differing ontological, morphological and functional components defined as the peripheral zone, central zone, transition zone, and periurethral region. The peripheral zone comprises about 70% of the prostate and is responsible for the major function of production and secretion of enormously high levels of citrate (Table 1). This unique function resides within the highly specialized glandular secretory epithelial cells, which are the dominant cells of the peripheral zone. The peripheral zone is also the major site for the development of malignancy. It is the normal peripheral zone glandular epithelium and the malignant cells (adenocarcinomatous glands) that are the focus of this presentation; and, unless otherwise designated, are being described in the following relationships.

It is important to focus on some fundamental relationships that generally apply to normal mammalian cell intermediary metabolism, but are not applicable to prostate epithelial cells. 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 (@80%) of cellular energy (ATP production). In addition, the Krebs cycle and the re-cycling 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. This is evident from the lethal effects of fluoroacetate; which inhibits m-aconitase, i.e. the first step in the oxidation of citrate via the Krebs cycle. Since citrate oxidation is essential to the sustenance of most mammalian cells, the citrate-producing prostate cells must adopt alternate metabolic pathways and curtail other non-essential 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 (Huggins, 1946; Harkonen, 1981; Harkonen et al., 1982; Muntzing et al., 1975) 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.

Many of the common metabolic relationships that generally exist in most mammalian cells do not exist in the secretory epithelial cells of the peripheral zone. These are highly specialized cells that have evolved and adapted for the unique function of citrate production and secretion. 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, in prostate secretory cells most of the citrate becomes an endproduct of intermediary metabolism.

3. m-Aconitase and net citrate production

The key reaction responsible for this accumulation of citrate is a uniquely limiting mitochondrial (m-) aconitase activity that minimizes the oxidation of citrate in the prostate cells (Fig. 1). Typically, in mammalian cells m-aconitase is generally described as catalyzing an equilibrium reaction, is present in excessive amounts, and is not a rate-limiting reaction. m-Aconitase catalyzes the equilibrium reaction:

which results in a citrate/isocitrate ratio w10/1. Because m-aconitase activity is generally high and not rate-limiting, this cellular steady-state ratio is maintained regardless of the cellular citrate concentration.

In pioneering studies, Huggins (1946) emphasized that prostate preparations exhibited a low citrate oxidation capability and a high citrate synthesis rate. Early studies (Barron and Huggins, 1944; Williams-Ashman, 1954) reported the existence of aconitase activity in prostate homogenate preparations, which led to the conclusion that aconitase was not involved in limiting citrate oxidation. Since those studies did not employ isolated mitochondrial preparations, both cytosolic and mitochondrial aconitase likely existed. Pursuant studies by Costello et al. (1976) demonstrated that prostate mitochondria exhibited a low m-aconitase activity that limited citrate oxidation. Further evidence for a limiting m-aconitase activity in prostate cells is obtained from the uniquely high citrate/isocitrate ratio ~30–40/1 (Costello et al., 1978; Franklin et al., 1977). Recent studies demonstrated that normal citrate-producing prostate cells contain typical levels of m-aconitase enzyme (Costello et al., 1996; Liu et al., 1996) Therefore m-aconitase activity must be limited by (1) unique properties of prostate m-aconitase which limit the conversion of citrate to isocitrate, and/or (2) unique intramitochondrial conditions which inhibit the m-aconitase reaction.

4. Zinc and m-aconitase in prostate cells

Several parallelisms exist between citrate production and zinc levels in prostate (Costello and Franklin, 1998, 2000, 2001). Along with citrate the prostate gland also has the highest level of zinc of all the tissues in the body (Table 1). Moreover, changes in the level of citrate are generally accompanied by corresponding changes in the level of zinc in prostate. For example PCa is characterized by a decrease in both citrate and zinc levels. BPH is characterized by an increase in citrate and zinc levels. Such relationships indicated the possibility that zinc might be involved in prostate citrate production. This possibility was strengthened by the report that zinc could inhibit prostate m-aconitase activity and citrate oxidation (Costello and Franklin, 1981). Liu et al. (1997) recently established that normal prostate cells contain a higher level of mitochondrial zinc than that found in other cells. Zinc, at levels that represent prostate mitochondria, has now been shown to be an inhibitor of m-aconitase (Costello et al., 1997). Although the mechanism of the interaction of zinc with m-aconitase has not been established, the kinetic properties are indicative of a competitive inhibition model. Of added significance is the reaction site of zinc inhibition of the aconitase equilibrium as follows:

The inhibition occurs at the conversion of citrate to cis-aconitate, which is the entry step for citrate oxidation. This minimizes the flux of synthesized citrate toward isocitrate and subsequent oxidation.

Zinc also causes a further shift in the apparent equilibrium of the aconitase reaction toward citrate specifically in prostate mitochondria (Costello et al., 2000a). Unlike its inhibitory action, this effect is not evident in liver mitochondria or with purified m-aconitase. Preliminary evidence indicates that a prostate mitochondrial protein interaction with zinc is a component of this effect; but more study is necessary to elucidate the mechanism. However, the combination of these two unique effects of zinc on m-aconitase provides a process that conserves and maximizes the accumulation of citrate, which is essential to the function of these highly specialized citrate-producing cells.

5. The bioenergetics of net citrate production

One of the important and obvious consequences of this net citrate production relates to the bioenergetics of the citrate-producing cells (Fig. 1). The complete oxidation of glucose results in 38ATP/glucose. The incomplete oxidation leading to citrate production results in 14ATP/glucose. Thus the process of net citrate production results in a sacrifice of about 65% of the ATP that could be derived from the complete oxidation of glucose. Some of this loss might be recovered by the oxidation of glutamate, and this needs to be investigated. Nevertheless, net citrate production is energetically extremely costly, so we characterize the cells as 'energetically-inefficient' cells.

These prostate cells must adapt and limit their activities to the reduced availability of mitochondrial production of ATP; and/or modify their energy metabolism to increase the availability of ATP. Coincidental with the inhibition of citrate oxidation, the citrate-producing prostate cells exhibit a high aerobic glycolysis (Barron and Huggins, 1944; Huggins, 1946; Muntzing et al., 1978); a condition that does not typically occur in normal mammalian cells. This accelerated utilization of glucose leads to two major end-products; lactate and citrate. The former would result in 8ATP/glucose and the latter (pyruvate→acetylCoA) would provide an additional 6ATP/glucose. Another result of the accelerated aerobic glycolysis is the increased production of acetylCoA that is required for increased synthesis of citrate.

Despite the inefficiencies of this energy metabolism, the level of ATP in prostate seems to rank with other tissues (Harkonen, 1981). For example, normal rat ventral prostate reportedly contains 2.9 mmol/g tissue which compares with liver=2.5, kidney=1.4, skeletal muscle=2.3, and submaxillary gland=1.0; and the energy charge of ventral prostate is 0.9 which is within the range of the other tissues (0.6–0.9). Thus, the highly specialized citrate-producing prostate cells have adapted unique metabolic/bioenergetic pathways that compensate for and are suitable to the evolution of their specialized function, survival, and propagation.

6. Zinc and terminal oxidation in prostate cells

Prostate had been characterized as a tissue that possesses a low respiration (Huggins, 1946; Muntzing et al., 1975). This suggested that zinc inhibition of citrate oxidation could result in low substrate availability for oxidation via terminal respiration. Alternatively, the possibility existed that the activity of components of terminal oxidation might be a limiting factor that results in low oxygen consumption. Studies have established that the activities of components of complexes 1–4 and respiration are markedly lower in prostate mitochondria than found in other cells (Costello et al., 1976, 2004a). The mechanism of low terminal oxidation in prostate mitochondria needs to be addressed. These mitochondria accumulate and contain uniquely high zinc levels that might inhibit terminal oxidation. Several reports have identified zinc as an inhibitor of terminal oxidation in mammalian cell mitochondria (Hunter and Ford, 1955; Skulachev et al., 1967; Nicholls and Malviya, 1968; Kleiner and von Jagow, 1972; Lorusso et al., 1991). We recently reported that physiological levels and forms of zinc inhibit the respiration and terminal oxidation of prostate mitochondria (Costello et al., 2004b). The inhibition occurs at Complex III, and possibly at Complex I and/or II; but no inhibition at Complex IV. The inhibition occurs also with isolated liver mitochondria when supplemented with zinc. Additionally, the constitutive levels of activity of complexes I–IV of prostate mitochondria are markedly lower (50–80% lower) than liver mitochondria. This suggests that the abundance of terminal oxidation components is inherently and uniquely low in prostate cells. Therefore the combination of low levels of electron transport components and the inhibitory effect of high zinc accumulation on terminal oxidation are major factors associated with the low respiration of these prostate cells.

7. Zinc trafficking into mitochondria

In studies that address zinc relationships and associations with mitochondria, one must recognize the existing in situ cellular environmental conditions that relate to zinc. It is evident that experimental studies of zinc-mitochondrial interactions traditionally involved the employment of free Zn^{++} ions and zinc concentrations that are unphysiological and often non-existent in living systems. Vallee and Falchuk (1993) conclude, 'In biological systems, very little, if any, zinc is free in solution'. The total cellular zinc content of mammalian cells is estimated to approximate 0.2 mM (Outten and O'Halloran, 2001); of which about 50% is cytoplasmic (cytosol and organelles). The cytosolic concentration of free Zn^{++} ions is estimated to be in the nM–fM range; i.e. a negligible pool of zinc (Williams and Frausto da Silva, 2000; Outten and O'Halloran, 2001). Essentially all the cytosolic zinc exists bound to either immobile macromolecules (~90%) or to mobile low molecular weight ligands (~10%). The former would not provide an available source of zinc for delivery into mitochondria. Therefore, the cytosolic pool of low molecular zinc ligands (ZnLigands) is the source of zinc for incorporation into and interaction with the mitochondria. This means that free Zn^{++} ions do not represent either the cytosolic source of zinc incorporation into the mitochondria or zinc effects on mitochondrial function.

This relationship raised the critical issues as to how zinc is trafficked from cytosol into prostate mitochondria; and into mitochondria of all cells. The outer mitochondrial membrane pore structure permits the passage of molecules up to ~10 kDa. ZnLigands of this size and smaller would constitute the cytosolic pool that delivers zinc into the mitochondrial inter-membrane space. This potential pool of zinc donors for interaction within the mitochondria would approximate a low micromolar range. The intermembranous pool of ZnLigands is available for interaction with mitochondrial components that reside in and have exposure in the intermembrane space; and this is applicable to the electron transport components. The intermembranous pool of ZnLigands is also the source of zinc for transport across the highly impermeable inner membrane and into the mitochondrial matrix.

Consequently, the low molecular weight ZnLigands constitute the source of 'mobile reactive zinc' for interaction with mitochondria. There are two ways in which the ZnLigand can contribute reactive zinc. Once delivered to the intermembranous space, the ZnLigand could be exposed to conditions (such as oxidative environment; pH change) that facilitate the release of zinc from the ligand; thereby providing a localized pool of free Zn^{++} as the reactive form of zinc. This possibility was proposed for the effects of zinc metallothionein (ZnMT) on terminal oxidation of liver mitochondria (Maret, 1994; Ye et al., 2001). Alternatively or additionally, the ZnLigand can donate its zinc to a recipient molecule by a direct transfer that does not involve free Zn^{++} ions as an intermediary (Halhout et al., 2001). This intermolecular exchange is determined by the relative zinc binding affinities of the interacting ligands. These relationships were applied to the inhibitory effects of zinc on terminal oxidation and respiration of prostate and liver mitochondria (Costello et al., 2004b). It is now apparent that cytosolic low molecular weight ZnLigands (such as ZnCitrate, ZnAspartate, Zn Cysteine) that have binding affinities equal to or less than $\log K_f \sim 10$ are equally as effective as free Zn^{++} ions in inhibiting the electron transport activity and respiration (as previously described in the above sections) of prostate and liver

mitochondria. ZnLigands with $\log K_f > \sim 11$ have no effect and do not serve as zinc donors for the mitochondrial interactions. The effective concentration of these mobile reactive ZnLigands approximates the expected low micromolar concentration that exists in the cytosol of the prostate cells.

Another critical issue about which little information exists is, 'How is zinc transported into mitochondria; especially into prostate mitochondria that contain high levels of zinc?' Brierly and Knight (1967) reported that heart mitochondria accumulated zinc by energy-dependent and by passive processes depending upon the conditions employed; but free Zn^{++} ions in the range of 100–500 μM was employed in those studies. It has been suggested that free Zn^{++} ions enter the mitochondria via the calcium uniporter (Saris and Niva, 1994). However those studies were also conducted with unphysiological concentrations of free Zn^{++} ions. Also no direct measurements of mitochondrial uptake of zinc were provided. We have demonstrated the mobile reactive ZnLigands described above were as effective as free Zn^{++} ions as donors for zinc uptake across the inner mitochondrial membrane into the mitochondrial matrix. In addition, ZnMT ($\log K_f \sim 10$) is an effective donor for zinc uptake by mitochondria, and this might be an important zinc chaperone in cells that have high expression of MT and low levels of other mobile reactive ZnLigands such as liver cells (Costello et al., 2004b). This zinc uptake process involves a kinetically-identified zinc uptake transporter that is associated with the inner mitochondrial membrane (Guan et al., 2003). The mechanism involves the direct intermolecular exchange (as described above) of zinc from the donor ZnLigand to the recipient putative uptake transporter protein which has an apparent $\log K_f \sim 10$ –11. Presently, the putative transporter has not been identified. Presently, no specific mitochondrial zinc uptake transporter has been identified in any mammalian cells. Some mitochondrial cation transporters do exist; but none is specific for zinc, and none exhibit the kinetic properties that we have identified for the zinc uptake transport process. The identification of this transporter is a current essential issue to resolve.

8. Zinc effects on mitochondrial apoptosis

A consequence of the cellular accumulation of zinc is an inhibitory effect on proliferation of these prostate cells that results from its induction of apoptosis (Liang et al., 1999; Feng et al., 2000). This effect results from a direct action of zinc on the mitochondrial release of cytochrome *c* followed by caspase activation and cascading apoptotic events (Feng et al., 2000, 2002). Although the mechanism of this effect needs to be elucidated, our recent studies (unpublished) reveal that cytosolic zinc directly interacts with the mitochondria to facilitate Bax pore-forming activity (Fig. 2). It is notable that this zinc-mitochondrial interaction is cell specific for citrate-producing prostate cells. Mitochondria from some other prostate cells do not exhibit the zinc-induced Bax-cytochrome *c* release effect; and do not exhibit zinc-induced apoptosis. Thus, zinc induction of apoptosis in citrate-producing prostate cells results from a mitochondrial apoptogenic effect of zinc. Jiang et al. (2001) similarly reported that zinc induces apoptosis in neurons that results from a direct zinc-induced release of cytochrome *c* from the neuronal mitochondria.

It is important to note that most other reports demonstrate that zinc inhibits apoptosis in mammalian cells (Truong-Tran et al., 2000 for review). Many of those studies employed

extremely high unphysiologic levels of zinc that would never occur in situ even under severe pathological conditions. However some studies have demonstrated that zinc induces apoptosis in selective cells (Provinciali et al., 1995; Hamatake, 2000; Iitaka et al., 2001; Haase et al., 2001; Jiang et al., 2001). In the case of prostate cells, two requirements are essential for the manifestation of zinc-induced apoptosis: the cells must possess the capability of accumulating high cellular levels of mobile reactive zinc; and the mitochondria of the cells must be directly responsive to zinc induction of cytochrome *c* release.

9. Altered zinc and citrate-related metabolism in prostate cancer

There now exists overwhelming compelling clinical and experimental evidence that, in contrast to normal glandular epithelium, the malignant prostate cells in situ virtually always exhibit low zinc and low citrate levels (Fig. 3; for reviews see Costello and Franklin, 1998, 2000, 2001; Costello et al., 2002, 2004a). To our knowledge, there exist no reports of prostate adenocarcinomatous glands that retain the high zinc and citrate levels that characterize the normal peripheral zone glands. These relationships lead us to propose that the development and progression of prostate malignancy involves and requires the metabolic transformation of normal zinc-accumulating citrate-producing cells to citrateoxidizing malignant cells that lose the ability to accumulate zinc (Fig. 4). In this concept of prostate malignancy, two transformations are essential: a genetic transformation of a sane cell to a neoplastic cell that is endowed with the potential for malignancy; and a metabolic transformation that is essential for the neoplastic cell to manifest its malignant activities. This metabolic transformation results in the elimination of the inhibitory of zinc on m-aconitase; which permits citrate oxidation via a functional Krebs cycle. The inhibitory effect of zinc on terminal oxidation is also alleviated. Consequently, the malignant cell becomes more energy-efficient in regard to ATP production to meet the metabolic/bioenergetic requirements of malignancy. In addition, the apoptogenic effect of zinc is alleviated, thereby permitting proliferation of the malignant cell.

This metabolic transformation distinguishes prostate malignancy from the relationships that are generally described for tumor metabolism. 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 (1926). While there exists no metabolic alterations that universally apply to all tumor cells, some altered metabolic characteristics that are represented in most tumor cells do exist (for reviews see Pedersen, 1978; Parlo and Coleman, 1984; Baggetto, 1992; Argiles and Lopez-Soriano, 1990; Modica-Napolitano and Singh, 2004; Dang and Semenza, 1999) such as: High Aerobic Glycolysis and Low Respiration; Truncated Krebs Cycle; Citrate Export and Lipogenesis; Glutaminolysis. Consequently, tumorigenesis generally involves the transformation of typical aerobic metabolism citrate-oxidizing energy-efficient normal cells to citrate-producing energy-inefficient tumor cells. Strikingly, the metabolic pathway of the citrateproducing normal prostate epithelial cells (Fig. 1) is similar to the metabolism of other tumor cells; and the metabolism of the malignant prostate cells is similar to that of typical normal mammalian cells. Because of these relationships, one cannot presume that generalizations regarding the intermediary metabolism of mammalian cells and tumor cells are applicable to normal prostate epithelial or malignant cells.

We have emphasized that the depletion of zinc in prostate malignancy removes the inhibition of m-aconitase, so that citrate oxidation will result. However, it is unlikely that the decrease in citrate in prostate malignancy is solely due to its oxidation. It is not surprising that tumor cell metabolism involves a truncated Krebs cycle with decreased citrate oxidation. The tumor cells actually engage in net citrate production; but the citrate does not accumulate as it does in normal prostate cells. Instead, the citrate is exported (via a shuttle such as the citrate:malate shuttle) from the mitochondria to cytosol where it is converted to acetyl CoA for lipogenesis. Increased lipogenesis is essential for tumor cell proliferation and growth; and citrate is the essential precursor for lipogenesis. Since this process is also essential for the growth and development of malignant prostate cells, a significant proportion of synthesized citrate must be utilized for lipogenesis in addition to being oxidized. This is an essential area for further investigation regarding the involvement of citrate-related intermediary metabolism and mitochondrial function in prostate malignancy.

10. Summary

1. Normal peripheral zone glandular epithelium is characterized by the accumulation of extraordinarily high levels of citrate and zinc. In prostate cancer, the adenocarcinomatous glands are characterized by low levels of citrate and zinc.
2. Overwhelming and compelling clinical and experimental evidence reveals that the development and progression of malignancy in prostate cancer involves the metabolic transformation of sane zinc-accumulating, citrate-producing cells to citrate-oxidizing cells that lost the ability to accumulate zinc. In the absence of this metabolic transformation, the neoplastic cell is incapable of manifesting its potential malignant capability.
3. The accumulation of high cytosolic and mitochondrial levels of zinc in normal prostate secretory epithelial cells inhibits m-aconitase, which limits the oxidation of citrate and permits net citrate production and inhibits terminal oxidation and respiration. In malignant cells, the lost ability to accumulate high zinc levels eliminates its inhibitory effect on m-aconitase and on terminal oxidation, which permits the oxidation of citrate via a functional Krebs cycle with increased production of ATP. In addition, the accumulation of high zinc levels induces mitochondrial apoptosis that inhibits cell proliferation. This mitochondrial apoptogenic effect is eliminated in malignant cells, which permits their proliferation.
4. In lieu of a sufficient free Zn^{++} ion pool, the transport of zinc into mitochondria is achieved by direct intermolecular exchange between mobile reactive donor ZnLigands and a mitochondrial zinc uptake transporter. Donor ZnLigands provide the source of zinc that inhibits terminal oxidation Complex components.
5. The metabolic transformation that is essential for the manifestation of the malignant activities of the neoplastic prostate cells is uniquely different from the metabolic alterations generally associated with most tumor cells. Prostate malignancy involves the transformation of energy-inefficient normal cells to

energy-efficient malignant cells; in contrast to the transformation of normal energy-efficient cells to energy-inefficient tumor cells.

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. Unfortunately, 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. We would echo the comment of Dang and Semenza (1999), “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.

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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. Citrate oxidation is limited due to the inhibition of m-aconitase activity by the intramitochondrial 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; aKG, alpha ketoglutarate; succ, succinate; mal, malate; pyr, pyruvate; lact, lactate; PDH, pyruvate dehydrogenase; CS, citrate synthase; ACON, aconitase; mAAT, mitochondrial aspartate aminotransferase; GDH, glutamic dehydrogenase.

Fig. 2.
Zinc induction of mitochondrial apoptogenesis in prostate cells.

Fig. 3.

Comparison of the zinc and citrate changes in prostate cancer. The citrate data are taken from Liney et al. (1997). The values were determined by in situ MRS measurements. The normal and cancer citrate values are for the peripheral zone; and the adenoma values are for the central zone. The zinc data are from Zaichick et al. (1997). The values were determined by analysis of biopsy samples.

Fig. 4.

A proposed concept of the role of zinc and 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; represented as ZIP zinc uptake transporter. 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 accompanying production of ATP which provides the metabolic requirements for the full expression of malignant activities and proliferation of the malignant cells. Consequently, the pre-malignant 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.

Table 1

Representative citrate and zinc levels in human prostate

	Citrate (nmol/g wwt)	Zinc (nmol/g wwt)
Normal (mixed tissue)	8000	3200
Normal (central zone)	4000	1860
Normal (peripheral zone)	13000	4538
BPH (glandular)	15000	9061
PCa (mixed tissue)	500–1000	808
Other soft tissues	150–450	461
Blood plasma	90–110	15
Prostatic fluid	40,000–150,000	9076