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ZINC AND ZINC TRANSPORTERS IN NORMAL PROSTATE FUNCTION AND THE PATHOGENESIS OF PROSTATE CANCER

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1. ABSTRACT

Zinc is an essential metal for all cells. It plays a role in a wide variety of physiological and biochemical processes. In the prostate epithelial cell the accumulation of high cellular zinc is a specialized function that is necessary for these cells to carry out the major physiological functions of production and secretion of citrate. The production of citrate and its secretion into prostatic fluid is a differentiated function of the prostate epithelial cells that is apparently important for reproduction. The loss of citrate and zinc accumulation is the most consistent and persistent characteristic of prostate malignancy. This characteristic of prostate cancer indicates that the lost ability of the malignant cells to accumulate zinc and citrate is an important factor in the development and progression of malignancy. The lost ability of the epithelial cells to accumulate zinc and thus to also accumulate citrate is the result of decreased expression of specific zinc uptake transporters. The purpose of this presentation is to review the current understanding of zinc and zinc homeostasis in the prostate and the role of zinc and zinc transporters in the normal function of the prostate and the pathogenesis of prostate cancer.

2. INTRODUCTION

The physiology and biochemistry of zinc and its importance in normal cellular and bodily function has been the subject of numerous reviews (1-6). The regulation and maintenance of a "normal" concentration and distribution of cellular zinc is essential to the function, metabolism, growth, proliferation and survival of cells. In this paper, we will describe the role of zinc in the function of the normal prostate (particularly the human prostate) and its implication in prostate cancer (PCa). In regard to zinc, the prostate gland is uniquely different from essentially all other tissues/organs in the body. A major function of the human prostate gland, i.e. the production and secretion of extremely high levels of citrate, requires the accumulation of a high concentration of zinc by the glandular secretory epithelial cells. Therefore, these specialized cells developed unique zinc accumulating capabilities and unique metabolic relationships that permit their function and survival. This is especially relevant since most mammalian cells contain mechanisms that protect them from the accumulation of high cellular zinc and from the potential toxic effects of high cellular zinc levels. An especially significant clinical aspect of zinc is its role in the development and progression

Table 1. Representative Citrate and Zinc Levels in The Prostate

Tissue/Fluid	Citrate	Zinc
	nmoles/gm wet weight	nmoles/gm wet weight
Normal peripheral Zone	13000	3000
Normal central Zone	4000	1000
Peripheral Zone Adenocarcinoma	400-1000	500-900
Glandular BPH	14000	4000
Other Soft Tissues	150-450	200
Prostatic Fluid	40000-150000	9000
Blood Plasma	90-110	15

Table 2. Distribution of Intracellular Zinc in Human Prostate

Cellular Compartment	Zinc microg/gm dry wt
Total	540
Nuclear	200
Mitochondria	152
Microsome	92
Supernatant	64

Values from (11)

of prostate malignancy. We refer the reader to our previous reviews for an extensive description of the important contributions and reports of early investigators regarding zinc relationships in normal prostate function and in prostate cancer (7-10). This report will build upon the previous reviews and integrate the more recent developments in zinc-prostate relationships.

3. ZINC LEVELS IN NORMAL HUMAN PROSTATE

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 high zinc accumulation and 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. The central zone comprises about 25% of the prostate and is the region where benign prostatic hyperplasia (BPH) exists. In contrast to the peripheral zone, central zone glandular epithelium does not function as zinc-accumulating citrate-producing glands. The foci of this review are the normal peripheral zone glandular epithelium and the malignant cells (adenocarcinomatous glands); and, unless otherwise designated, are being described in the following relationships. It is evident from table 1 that zinc levels in normal peripheral zone are 3-10 fold greater than found in other soft tissues. Associated with its accumulation of zinc, the peripheral zone citrate level is 30-50 fold greater than found in other soft tissues. Even more revealing is the comparative concentration of zinc and citrate in prostatic fluid versus blood plasma. The prostatic fluid zinc and citrate concentrations are ~75-fold and ~1000-fold higher than found in plasma. Therefore we characterize the peripheral zone secretory epithelial cells as "zinc-accumulating citrate-producing" cells.

In addition to the total concentration of cellular zinc, an important factor is the distribution and chemical forms of cellular zinc. Vallee and Falchuk (4) conclude, "In biological systems, very little, if any, zinc is free in solution". They estimate that about 30-40% of the total cellular zinc is in the nucleus, ~50% is in the cytoplasm and its organelles, and the remainder is in the cell membrane and/or wall. Table 2 presents the distribution of zinc as determined by Dhar *et al* (11).

For this discussion we will define three pools of zinc that comprise the total intracellular zinc: 1) tightly bound zinc (mainly metalloenzymes, metalloproteins and nucleoproteins) that is an immobile unreactive pool; 2) loosely bound zinc (such as amino acid and citrate bound) that constitutes a mobile reactive pool; and 3) free Zn++ ion that is a reactive pool. The total cellular zinc content of mammalian cells is estimated to approximate 0.2-1.0 mM (3, 5, 12). The cytosolic concentration of free Zn++ ions is estimated to be in the nM-fM range; i.e. a negligible pool of zinc (12,13). Essentially all of the cytosolic zinc exists bound to either immobile macromolecules (~90%) or to mobile low molecular weight ligands (~10%). In the prostate, the cytosolic zinc approximates 0.6-3.0 mM. The mobile transportable ZnLigand pool approximates 20-100 uM Zn in most mammalian cells and about 60-300 uM in prostate cells. Figure 1 represents the distribution and pools of zinc in prostate cells.

These relationships raise the question, "In the absence of the existence of a free Zn++ ion pool, how is mobile reactive zinc incorporated into the cell and trafficked through the cytosol and into the mitochondria. This issue requires the process for zinc uptake across the plasma membrane and into the cytosol, the delivery of the cytosolic zinc to the mitochondria, and the import of zinc into the mitochondrial space (Figure 2).

4. ZINC IMPORT INTO PROSTATE CELLS

The total zinc concentration in plasma is about 15 µM (4). About 85-90% of the plasma zinc is protein bound (4,13,14). The plasma free Zn++ ion concentration is negligible (0.1-1 nM) (4,15,16). The remaining 10-15 % includes zinc that is complexed to low molecular ligands (ZnLigands) such as amino acids and citrate, and approximates 2 uM zinc. However, it is the interstitial fluid, not the blood plasma, that is the direct source of zinc for its uptake by cells. Therefore the total zinc concentration of ISF approximates the low molecular weight ZnLigand pool of plasma; i.e., ~2 uM Zn.

4.1. Zinc uptake transporters

Transporters responsible for zinc uptake were identified in yeast, plants, and nematodes and grouped into

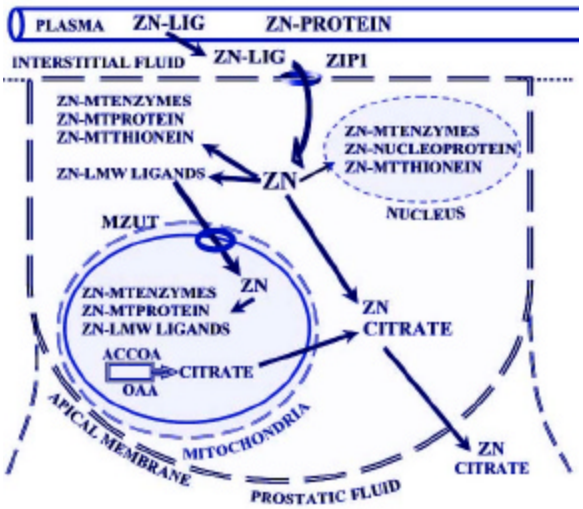

Figure 1. The Distribution of Zinc in Prostate Epithelial Cells. Free Zn++ ions are virtually absent in the represented compartments. Abbreviations are: MT=Metallo-: LMW=low molecular weight; MZUT=mitochondrial zinc uptake transporter

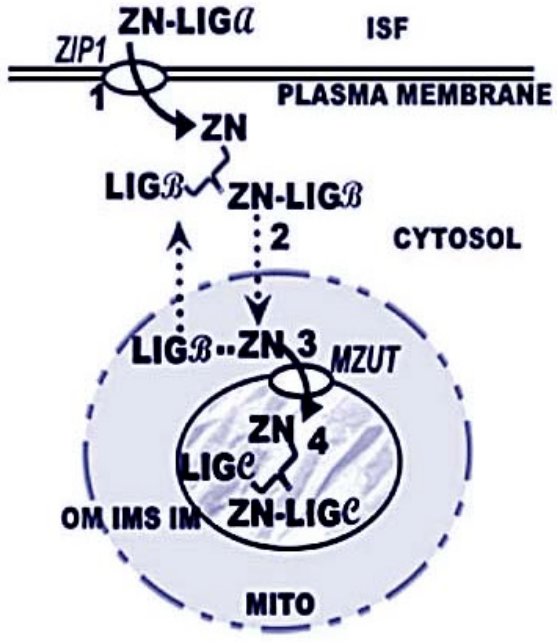

Figure 2. Zinc Trafficking and Transport Into Mitochondria. 1. Zinc exists in interstitial fluid in a mobile ligand form. Zinc is donated from the ligand to Zip1 and transported across the plasma membrane into the cell where it is bound to a cytosolic ligand. 2. The mobile cytosolic zinc ligand permeates the mitochondrial outer membrane and enters the intermembrane space. 3. At the inner membrane, zinc is donated from the ligand to MZUT and transported into the matrix where it associates with mitochondrial ligands. MZUT= mitochondrial zinc uptake transporter.

the closely related subfamilies of the ZIP super family (17,18,19). The ZIP (Zrt/Irt like Proteins) family is named for the first two members, Zrt in *Saccharmyces* (18) and Irt in *Arabidopsis thaliana* (20) to be identified. Currently there are at least 90 members of the Zip family divided into four subfamilies (21,22). Subfamily I consists of mainly fungal and plant genes; subfamily II is made up of nematode, insect and mammalian sequences. Two additional subfamilies LIV-1 and gutA have been identified by PSI-BLAST analysis (22). In the human genome 14 ZIP family proteins encoded by SLC (solute-linked carrier) related genes have been identified. However, only three of these have been functionally characterized. Included in subfamily II are two putative zinc uptake transporters in humans identified as hZIP1 (SLC39A1) and hZIP2 (SLC39A2). Costello *et al* (23) first demonstrated that prostate cells contain a rapid zinc-uptake transport process that is operational at low concentrations of zinc representative of plasma zinc levels; and was increased by testosterone and by prolactin treatment of the cells. We identified the constitutive expression of hZIP1 in prostate cells; and the increase in hZIP1 expression by treatment with testosterone and prolactin. Pursuant studies (24) established hZIP1 as the transporter that functions in the uptake and accumulation of zinc in prostate cells. Gaither and Eide (25,26) confirmed that hZIP1 was an important zinc uptake transporter in mammalian cells. Milon *et al* (27) also confirmed the expression of hZIP1 in PC-3 cells. hZIP2, was identified based on its homology to other members of the zip family. The endogenous expression of this transporter in mammalian cells is at very low levels and only in a few tissues, thus its role in zinc homeostasis in mammalian cells remains to be established (22). In contrast to hZIP1, we have not detected (either by Western blot or by RT- PCR) ZIP2 in rat or human prostate cells. However, Rishi *et al* (28) reported the presence of ZIP1 and ZIP2 expression in tissue sections of human prostate. Therefore, at this time, we must conclude that ZIP1 is a major zinc uptake transporter in prostate cells, and the role of ZIP2 requires further investigation.

How is the transport from interstitial fluid across the plasma membrane into the cytosol achieved by the zinc uptake transporter? The ZIP1 Km value ranges from 3 to 7 uM zinc, but the interstitial fluid concentration of free Zn++ is ~ 0.7 nM; i.e. 10,000-fold lower than the available zinc level. Gaither and Eide (26) proposed that hZIP1 transport involved the transport of free zinc ions as a result of the high capacity of the transporter despite the extremely low availability of Zn++ ions. It seems to us that the evolution of an effective transporter would involve properties that would be consistent with the environmental conditions of its intended operation. In this case we would expect that the Km of the transporter would approximate the physiological concentration of the transport substance. Based on this expectation, we determined the effectiveness of the transporter for ZnLigands as compared to free Zn++ ions (24). The transport of zinc is dependent upon the total concentration of zinc and independent of the presence of any free Zn++ ions. With ZnEDTA no transport of zinc occurs. Therefore, ZnLigands with relatively low zinc-binding affinities (log Kf ~10 or lower) serve as zinc donors for ZIP1 transport equally as effectively as free Zn++ ions. The interstitial fluid concentration of such ZnLigands (citrate, aspartate, cysteine, histidine and others)

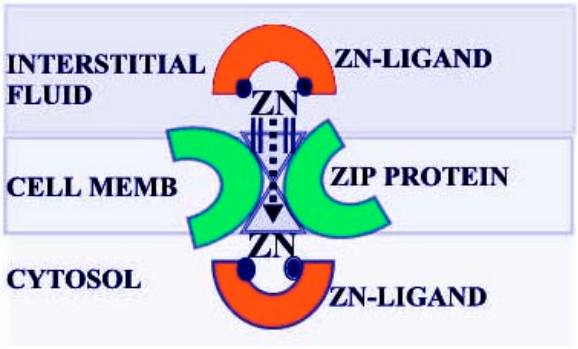

Figure 3. Concept of ZIP Transport of Zinc. This concept depicts the transport of zinc in the absence of free Zn++ ions in the interstitial fluid. Zinc is passed from extracellular ligand to Zip transporter to intracellular ligand.

The export of zinc could also be important in the maintenance of zinc homeostasis and net zinc accumulation in prostate epithelial cells. However no information currently exists concerning the functional role of zinc exporters in prostate cells. ZnT4 has been identified in prostate cells (30). ZnT4 is associated with the sequestering of cytosolic zinc into organelles, and is not involved as a plasma membrane zinc exporter. ZnT1 expression, which functions as a plasma membrane-associated zinc exporter in some cells has also been identified in prostate (31). However, its role in zinc homeostasis in prostate cells needs to be established. More studies regarding the functional role of zinc transporters in prostate are required.

4.2. Zinc Import Into Mitochondria

The next issue is "What happens when zinc enters the cell; i.e. in the cytosolic compartment?" As zinc is transported to the cytosolic side of the plasma membrane it is immediately bound to cytosolic ligands so that a cytosolic free Zn++ ion pool is essentially non-existent. The cytosolic zinc exists bound to either immobile macromolecules (~90%) or to mobile low molecular weight ligands (~10%). 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 intermembrane space. This potential pool of zinc donors for interaction within the mitochondria would approximate a low uM range. The intermembranous pool of ZnLigands is available for interaction with mitochondrial components that reside in and have exposure in the intermembrane space, such as 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. These low molecular weight ZnLigands (such as ZnMetallothionein, ZnCitrate, ZnAspartate, ZnHistidine) 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++ ion as the reactive form of zinc. This possibility was proposed for the effects of zinc metallothionein (ZnMT) on terminal oxidation of liver mitochondria (32,33). 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 (29). 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 (34). It is now apparent that cytosolic low molecular weight ZnLigands (such as ZnCitrate, ZnAspartate, ZnCysteine) that have zinc binding affinities equal to or less than log Kf~10 are equally as effective as free Zn++ ions in inhibiting the electron transport activity and respiration of prostate and liver mitochondria. ZnLigands with log Kf >~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 uM concentration that exists in the cytosol of the prostate cells.

The ZnLigands that enter the intermembranous space also comprise the pool of zinc donors for import into the mitochondrial matrix. This raises a critical issue about which little information exists, "How is zinc transported into mitochondria; especially into prostate mitochondria that contain high levels of zinc?" Brierly and Knight (35) reported that heart mitochondria accumulated zinc by energy-dependent and by passive processes depending upon the conditions employed; but free Zn++ ion concentrations in the range of 100–500uM were employed in those studies. It has been suggested that free Zn++ ions enter the mitochondria via the calcium uniporter (36). 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 that 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 (logKf~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 (37). This zinc uptake process involves a kinetically-identified zinc uptake transporter (that we designate as mZUT) that is associated with the inner mitochondrial membrane (38). The kinetic properties (Table 3) indicate that mZUT is operational under the expected cellular conditions. The mechanism involves the direct intermolecular exchange of zinc from the donor ZnLigand to the recipient putative uptake transporter protein which has an apparent logKf of ~10-11. Free Zn++ ions are not required for the transport process. Therefore the transport process is similar to that described above for ZIP1. Presently, the putative transporter, mZUT, has not been identified. No specific mitochondrial zinc uptake transporter has been identified in any mammalian cells. Some mitochondrial cation transporters do exist; but none

Table 3. Kinetic Parameters of Zinc Uptake Transporter in Rat Ventral Prostate Mitochondria

Zn Form	Km	Vmax
	Mean ± s.e.m	**Mean ± s.e.m**
Zinc chloride	59.93 ± 9.98	0.63 ± 0.04
Zinc aspartate	54.74 ± 8.53	0.67 ± 0.04
Zinc citrate	31.14 ± 3.23	0.42 ± 0.01

Km = MicroM; V_{max} = nmols Zn/min/mg mito. Protein combined would approximate the Km value of the transporter. Therefore, we propose that the donor ZnLigands undergo a direct intermolecular transfer of zinc to the transporter protein as represented in figure 3. This is consistent with demonstrated peptide to peptide exchange of zinc without any intermediate free Zn++ ion availability (29).

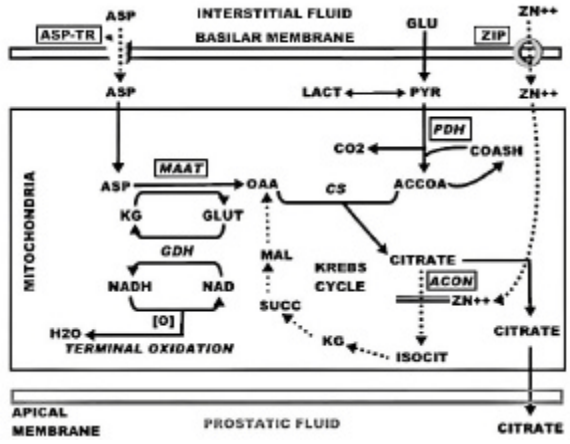

Figure 4. The Pathway of Net Citrate Production in Prostate Epithelial Cells. A major effect of intramitochondrial zinc is the inhibition of m-aconitase activity and thus citrate oxidation. Inhibition of citrate oxidation makes citrate available for secretion to prostatic fluid.

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 an essential issue to resolve.

5. EFFECTS OF ZINC ACCUMULATION ON PROSTATE CELLS

The unique accumulation of high zinc levels must impose effects in prostate cells that other mammalian cells attempt to avoid by their prevention of high zinc accumulation. The questions become, "Why do the specialized prostate cells accumulate high zinc levels?; What are the consequences of high zinc accumulation?" These issues are addressed in our recent reviews (8,9,39) and will be summarized as follows.

5.1. Zinc and Net Citrate Production

The major prostate function of high citrate production and secretion ("net citrate production") is achieved by and is the reason for the unique accumulation of zinc by the specialized prostate epithelial cells. The key reaction responsible for this accumulation of citrate is a uniquely limiting mitochondrial (m-) aconitase activity that minimizes the oxidation of citrate in prostate cells (figure 4). This limited m-aconitase activity results from the inhibitory effects of zinc on the enzyme (40,41). This prevents the oxidation of citrate via the Krebs cycle so that it accumulates for secretion as a major component of prostatic fluid. Therefore, citrate is an end-product of intermediary metabolism in prostate cells. In virtually all other mammalian cells, citrate is a critical intermediate that is oxidized via the Krebs cycle for essential ATP production and/or utilized as the source of acetyl coenzyme A in lipogenesis and cholesterogenesis. In fact, the inhibition of m-aconitase (as occurs in fluoroacetate/fluorocitrate poisoning) is lethal to most mammalian cells. Consequently, the zinc-citrate connection is unique to these prostate cells.

5.2. Zinc and Terminal Oxidation

Prostate had been characterized as a tissue that possesses a low respiration (42,43). This suggested the possibility 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 (34,44). Several reports have identified zinc as an inhibitor of terminal oxidation in mammalian cell mitochondria (45-49). We recently reported that physiological levels and forms of zinc inhibit the respiration and terminal oxidation of prostate mitochondria (34). The inhibition occurs at Complex lll, and possibly at Complex l and/or ll; but no inhibition at Complex lV. The inhibition occurs also with isolated liver mitochondria when supplemented with zinc. Additionally, the constituitive levels of activity of complexes l-lV of prostate mitochondria are markedly lower (50-80% lower) than liver mitochondria. 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.

5.3. Zinc Effects On Mitochondrial Apoptogenesis

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 (50,51,52). This effect results from a direct action of zinc on the mitochondrial release of cytochrome c followed by caspase activation and cascading apoptotic events. 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 (figure 5, steps 3-5). 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 (53) similarly reported that zinc induces apoptosis in neurons that results from a direct zinc-induced release of cytochrome c from the neuronal mitochondria. In addition, zinc also increases the cellular level of Bax, presumably by enhancing Bax gene expression; and facilitates the translocation of Bax to

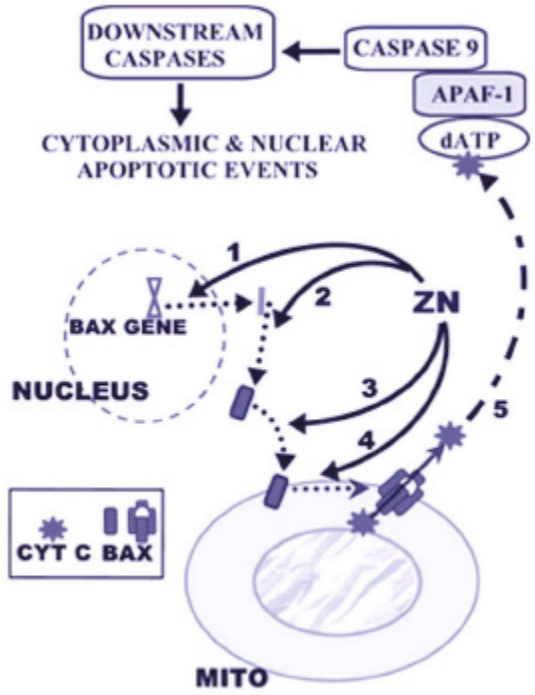

Figure 5. Zinc Induction of Mitochondrial Apoptogenesis in Prostate Cells. Zinc increases cellular Bax levels by increasing gene transcription (1) and/or post-transcriptional biosynthesis (2). Zinc facilitates the translocation of Bax to the mitochondria (3). Zinc facilitates Bax pore formation (4). Cytochrome c effluxes to cytosol and activates the caspase cascade (5) that leads to apoptosis.

the mitochondrial membrane (figure 5, steps 1-3; unpublished information).

It is important to note that most other reports demonstrate that zinc inhibits apoptosis in mammalian cells ((54) 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 (53,55-57). 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.

5.4. The Effect of Zinc on the Bioenergetics of the Prostate Cell

One of the important and obvious consequences of net citrate production relates to the bioenergetics of the citrate-producing cells. The complete oxidation of glucose, as typically occurs in mammalian cells, results in 38 ATP/glucose. The incomplete oxidation leading to citrate production, as occurs in normal prostate epithelial cells, results in 14 ATP/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. However, the inhibitory effect of zinc on terminal oxidation would further decrease the potential ATP production from coupled phosphorylation. To accommodate this loss of ATP, the prostate cells exhibit an accelerated aerobic glycolysis. Therefore, bioenergetically, the zinc-accumulating, citrate-producing prostate cells are energy-inefficient cells. This is the price that these specialized cells must pay for their function of net citrate production.

6. HORMONAL REGULATION OF ZINC IN PROSTATE

Zinc accumulation in some mammalian cells has been reported to be under hormonal regulation, e.g., glucocorticoid (58,59). However the regulation is achieved mainly through regulation of metallothioneins and the levels of accumulated zinc are significantly lower than the uniquely high levels associated with prostate cells. In prostate cells, both testosterone and prolactin have been implicated in the regulation of zinc, but reports concerning the specific effects of these hormones have been inconsistent and often conflicting. These inconsistencies arise from variability in the experimental conditions employed. Most reports have involved *in vivo* studies employing long-term castration and/or hypophysectomy. Such conditions result in complex systemic effects and in degenerative effects on the prostate gland. Moreover, a variety of different prostate sources (e.g., rat prostate lobes, dog prostate, human prostate) have been employed which present different responses and different relationships. Most studies have demonstrated that testosterone and prolactin increase the level and accumulation of zinc in the rat dorsolateral prostate; but the reports concerning the ventral prostate were conflicting and inconclusive (60-62). Liu *et al* (61) established with *in vivo* and *in vitro* studies the short-term and direct effects of testosterone and prolactin on the zinc levels of epithelial cells from the rat prostate lobes. The results demonstrate that both testosterone and prolactin independently increase the zinc levels in lateral prostate, decrease the zinc levels in ventral prostate, and have no effect on dorsal prostate, kidney or liver cells. Most significantly, the citrate responses to both hormones coincide perfectly with the zinc responses. Thus the hormonal effects are cell-specific, being related to cell lineage and to the functional relationship of the cells. Special attention must be directed at the major differences between rat dorsal and lateral prostate lobes regarding the levels of zinc, the responses to hormones, and the function of citrate production. Studies regarding such relationships should not be conducted with the combined dorsolateral complex, as has been commonly done. An important issue yet to be established is the hormone-zinc relationship in normal and neoplastic human prostate. Because the human prostate is a complex structure with differing embryological and functional components, it is likely that different epithelial cell types exist. One might expect that the hormonal-zinc-citrate relationships of the rat lateral prostate probable exist in the human peripheral zone since these are ontologically homologous glands. In support of

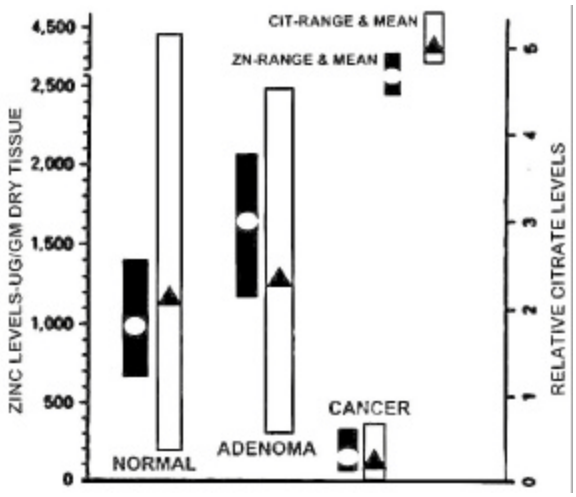

Figure 6. Comparison of the zinc and citrate changes in prostate cancer. The citrate data are taken from Liney *et al.* (66). The values were determined by *in situ* MRS measurements. The normal and cancer citrate values are for the peripheral zone; and the adenoma values are for the central zone. The zinc data are from Zaichick *et al.* (67). The values were determined by analysis of biopsy.

this, Costello *et al* (23) established that both prolactin and testosterone increased the accumulation of zinc and the expression of ZIP1 in LNCaP and PC-3 cells.

7. 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 (Table 1, figure 6) (8,10). In fact, the decrease in citrate is now being employed for *in situ* magnetic resonance spectroscopy imaging, identification and mapping of malignant foci with an accuracy of nearly 100% (63-65). 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 citrate-oxidizing malignant cells that lose the ability to accumulate zinc (figure 7). Two transformations are essential: a genetic transformation of the sane cell to the 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. The metabolic transformation involves the lost ability of the neoplastic cell to accumulate zinc. This eliminates the inhibitory effect of zinc on m-aconitase and 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. It is interesting to note that this metabolic/bioenergetic transformation is opposite to the metabolic transformation described for most tumor cells. The latter involves the transformation of the typical normoxic glucose oxidizing, energy-efficient cells to highly aerobic glycolytic energy-inefficient cells. Therefore, the generalizations of tumor metabolism cannot be applied to prostate malignancy.

The critical issue is "What is the cause of the inability of the neoplastic cell to accumulate zinc. Because ZIP1 has been identified as an important transporter for the accumulation of zinc in prostate cells, it is a logical candidate for this transformation. In recent studies (unpublished information), we have identified the down-regulation of ZIP1 expression and a loss of ZIP1 transporter protein in the adenocarcinomatous glands of prostate tissue sections from prostate cancer subjects. Correspondingly, the malignant glands also exhibit a depletion of cellular zinc. The importance of ZIP1 is also corroborated by Rishi *et al* (28) who reported that ZIP1 expression is down-regulated in African-American males in contrast to Caucasian males. This correlates with the increased incidence of prostate cancer in the Black male population. Thus, ZIP1 might be a tumor suppressor gene in prostate cancer.

An increase in the export of zinc could also decrease zinc accumulation in malignant glandular epithelium. However no information currently exists concerning the functional role of zinc exporters in prostate cells. Beck *et al* (30) reported that ZnT4 was decreased in peripheral zone malignant tissue when compared to normal peripheral zone tissue samples. ZnT4 is associated with the sequestering of cytosolic zinc into organelles, and not involved as a plasma membrane zinc exporter. Moreover, a decrease in ZnT4 would not be associated with a decrease in cellular zinc level, even as a secretory process. ZnT1 expression was unchanged in malignant versus normal peripheral zone. ZnT1 functions as a plasma membrane-associated zinc exporter in some cells and possibly in prostate cells. Hasumi *et al* (31) reported that ZnT1 expression was significantly lower in malignant prostate tissue samples when compared to BPH samples, which led them to conclude that ZnT1 was not likely to be associated with the decreased zinc accumulation in "true" malignant cells. Consequently, a possible role of altered expression of zinc exporters in the genetic/metabolic transformation of the malignant glands in prostate cancer is not evident, but more research is required regarding this issue.

8. CONCLUSIONS AND PRESPECTIVES

The physiological function of the normal prostate gland is the accumulation of very high levels of citrate and zinc. These are apparently important components of semen and play an essential role in reproduction. The prostate luminal epithelial cell has evolved to carry out the function of zinc and citrate accumulation and secretion. Thus, these cells have adapted to accommodate intracellular zinc levels that would be toxic to other cells. Zinc and citrate accumulation are linked in that the cellular mechanism for citrate accumulation involves the inhibition of m-aconitase activity by the very high level of intramitochondrial zinc.

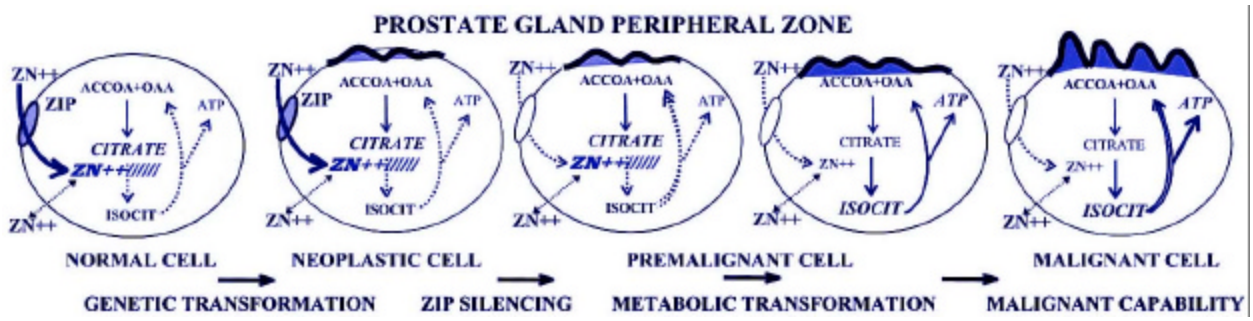

Figure 7. The Concept of Zinc in the pathogenesis of prostate malignancy. The normal glandular epithelial cell expresses ZIP1 that permits zinc accumulation, which inhibits citrate oxidation and terminal respiration. A genetic transformation results in a neoplastic cell with potential malignant capability. ZIP1 expression is down-regulated, which eliminates Zip1 transporter and accumulation of zinc in the premalignant cell. The level of cellular zinc decreases which removes the inhibitory effects on citrate oxidation and terminal oxidation. The malignant cell is metabolically and bioenergetically capable of manifesting its malignant potential. Also, the apoptogenic effect of zinc is removed, which allows growth and progression of the malignant cell.

Inhibition of m-aconitase results in a greatly reduced rate of citrate oxidation and thus allows for citrate secretion into prostatic fluid. The sacrifice of this major oxidizable substrate and the associated potential energy is further evidence of the importance of the specialized function of citrate secretion.

Zinc accumulation by the epithelial cells is achieved through the activity of zinc uptake transporter proteins that are members of the ZIP family of transporters. The ZIP proteins are encoded by SLC39 related genes. hZIP1, one member of the family has been reported to be the major zinc uptake transporter in prostate cells. Evidence of a role for hZIP1 in the mechanism of citrate accumulation is strengthened by the demonstration that prolactin and testosterone, two hormones that regulation citrate accumulation by the prostate gland, also regulate the expression of this transporter in prostate cells. In addition to the cellular uptake of zinc from interstitial fluid by the ZIP transporters, intracellular zinc is transported to the mitochondrial matrix by specific zinc transporters that have been kinetically identified and that we have designated mZUT . Once in mitochondria, zinc inhibits terminal oxidation by prostate cells. The role of this effect of zinc on prostate epithelial cell proliferation and epithelial cell function remains to be established.

Overwhelming clinical and experimental evidence establish that malignant prostate, in contrast to normal prostate, is characterized by a decrease in both zinc and citrate. We propose that this decrease in zinc and citrate level represents a metabolic transformation that is essential for the expression of the neoplastic transformation and the expression of prostate malignancy. Recent evidence suggests that down-regulation of hZIP1 expression and loss of the transporter function results in loss of zinc accumulation, which leads to decreased inhibition of m-aconitase and terminal oxidation. These effects of lost zinc accumulation contribute to the metabolic transformation of normal citrate producing prostate epithelial cells to citrate-oxidizing, energy-efficient prostate cancer cells.

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