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Cytotoxic/tumor suppressor role of zinc for the treatment of cancer: an enigma and an opportunity

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Abstract

A major issue relating to many cancers is the absence of effective chemotherapeutic agents; so that most often untreatable morbidity and death are prevalent once the cancer has been detected and has advanced. The search for efficacious anticancer agents is imperative. One potential agent is zinc, which is decreased in the development of some cancers in order to avoid its cytotoxic/tumor suppressor effects on the malignant cells. This provides the basis and opportunity to employ a treatment regimen that restores elevated zinc levels in the malignant cells and elicits the cytotoxic/tumor suppressor effects of zinc. The enigma is that this approach and expectation has not reached fruition. The question is “why?”. This article provides a discussion of relevant zinc issues that need to be considered and resolved. Important areas of research are identified as being essential for the successful application of zinc cytotoxicity/tumor suppression actions for the treatment of specific cancers.

Keywords

cancer chemotherapy; Clioquinol; prostate cancer; zinc; zinc; ionophore; ZIP transporters

Evolving and compelling evidence exists that proves that zinc is implicated as an important cytotoxic/tumor suppressor agent in several cancers. For example, that cellular zinc levels are markedly decreased in prostate cancer is well established [1–3]. This relationship exists because the concentration of zinc that exists in the normal prostate epithelial cells is cytotoxic in the malignant cells. Evolving evidence indicates that this zinc relationship also exists in other cancers, such as hepatocellular carcinoma [4–11], pancreatic adenocarcinoma [12], ovarian cancer [13] and other cancers described below. This relationship provides the basis and the opportunity for the potential development of zinc-associated chemotherapeutic agents for specific cancers for which no effective treatment currently exists. The enigma is the unfortunate fact that this potential role and therapeutic application of zinc treatment has not reached fruition.

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The important question is “Why?”. For some, there is recalcitrance regarding the fundamental role, effects and implications of this zinc relationship; despite the overwhelming clinical and experimental evidence of the decrease in zinc in these cancers. Such a subjective and scientifically unfounded view has serious consequences on the support and funding decisions for clinical and experimental studies required to develop the zinc treatment approach in cancer. For others, there exists a poor understanding or misunderstanding of the fundamental physiology, biochemistry, pathology and molecular biology of zinc relationships in mammalian systems. This leads to inappropriate and ill-informed translational conclusions and issues. Notwithstanding these subjective problems, we must also consider that adoption of the clinical and experimental cytotoxic/tumor suppressor implications of zinc for a therapeutic application could be a flawed concept for reasons that are not yet understood. Conversely, one must also consider that adoption of the clinical and experimental cytotoxic implications of zinc for a therapeutic application is a valid concept; but has not been successful due to zinc relationships that are not yet understood and revealed. Among all of the combinations of reasons and contributing factors for lack of success, the most important is the absence of sufficient information and understanding that are essential for a zinc treatment approach for cancer. This presents the enigma that will be discussed.

It is important for the reader to have some fundamental understanding of zinc relationships in mammalian systems; which we cannot fully provide in this report. Many excellent reviews of the physiology, biochemistry and molecular biology of zinc and zinc transporters in mammalian cells are available and such a list is presented, in part, in our recent zinc review [14]. This article will predominantly focus on the zinc relationships that exist in prostate cancer. The reason for this focus is because the prostate cancer–zinc relationships are the most established and most studied and we believe that the fundamental implications and relationships in prostate cancer will be applicable to other cancers that exhibit decreased zinc levels in malignancy. This will be followed by the zinc relationships that exist in other cancers.

Decrease in cellular zinc levels in malignant cells *in situ* in prostate cancer

Since the first report in 1954 by Mawson and Fisher of a decrease in zinc levels in prostate cancer [15], the ensuing 57 years of dozens of reported clinical studies have confirmed this decrease in zinc. With amazing consistency, these collective studies report a decrease in zinc levels of approximately 60–80% when compared to normal or benign prostate levels [1–3]. These studies involve measurements of zinc levels from extracts of human prostate tissue, *in situ* cellular and tissue zinc levels by zinc staining probes, and by direct x-ray fluorescence detection of zinc levels. By contrast, no contradictory substantiated reports exist. One rarely, if ever, finds malignant loci that exhibit the high zinc levels that characterize the normal glandular epithelial cells. The decrease in zinc is an early event in the development of malignancy [16–18], which is evident in premalignant cells/lesions (such as prostate intraepithelial neoplasia). Johnson *et al.* state:

“We demonstrate that Zn is depleted from the neoplastic as well as pre-neoplastic prostatic glandular epithelial cells.” [17].

and Cortesi *et al.* conclude with:

“The zinc depletion occurs not only in the cancerous tissue segments but also, though less pronouncedly, in the non-cancer components surrounding the lesion. This observation is consistent with the conclusions of Costello *et al.* that the zinc depletion is an early step in the cancer proliferation process and that zinc depletion precedes the transformation of cells from normal to cancerous type.” [18].

The decrease in zinc, along with the corresponding decrease in citrate levels that further substantiates the decrease in zinc, is the most consistent and persistent existing hallmark characteristic that differentiates prostate cancer from normal and benign prostate.

Cytotoxic/tumor suppressor effects of zinc

Numerous studies on the effects of cytotoxic/tumor suppressor effects of zinc on prostate cells have been reported (reviewed in [1–3,19]). These effects can be categorized as follows: altered bio-energetic/metabolic effects; inhibition of growth/proliferation and inhibition of invasion/migration. The metabolic effects arise in part from the effects of zinc on inhibition of citrate oxidation and on terminal oxidation, which are essential for the synthetic and energetic requirements for the malignant process. The growth/proliferation effects are manifested by zinc promotion of apoptosis and inhibition of cell cycle activity. Zinc inhibition of invasion and migration prevents the malignant advancement to metastasis. The combination of these effects has been established by *in vitro* effects of zinc treatment of malignant cell lines, and by *in vivo* animal tumorigenesis effects of zinc treatment.

Which came first: the decrease in zinc or the development of malignant cells?

We are often confronted with the suggestion that the decrease in zinc is a result of malignancy rather than a contributing factor in the development of malignancy. This is a variation of the issue of ‘which came first, the chicken or the egg?’. Others have misinterpreted our concept by implicating the decrease in zinc as a cause of cancer. The response to such concerns and issues require further clarification of the concept of the role of zinc.

The fact that the decrease in zinc and concurrent decrease in the ZIP1 (SLC39A1) transporter in prostate cancer (described below) occur as early events that exist in premalignant cells/lesions indicates that these changes precede malignancy, and do not result from malignancy. The dilemma to this question is best resolved by an understanding of the multistep progressive events that constitute the carcinogenesis process. The issues, as raised above, often evolve from a view that malignant cells directly arise from the oncogenetic transformation of the precursor normal cells and such a view equates the neoplastic cell with the malignant cell. We believe that such a view is misguided; and we propose a modified concept of the carcinogenesis process that incorporates a multistep oncogenetic transformation of normal cells to neoplastic cells, and genetic/metabolic transformation of the neoplastic cells to premalignant cells and ultimately to malignant cells. This concept with its supporting basis appears in Costello and Franklin [20] and is represented in Figure 1.

When viewed with this perspective, the implications of zinc become clearer. It becomes apparent that a decrease in zinc will not cause malignancy in the absence of the initial oncogenetic transformation of the normal cell to a neoplastic cell with malignant potential. However, the decrease in zinc and the downregulation of *ZIP1* are required events in the progressive development of the neoplastic cell leading to the malignant cell with manifestation of malignancy. In the absence of this genetic/metabolic transformation, the neoplastic cell will not develop to full malignancy. Thus, the issue of ‘which came first’ and the role of zinc are fully explicable and plausible. This sets the stage for the consideration of restoration of zinc in malignant cells as an approach for treatment of cancer.

Zinc transporter status is an essential factor in the concept of the zinc implication in prostate cancer

The preceding clinical and experimental relationships provide the rational basis for a concept that includes the following conclusions and expectations:

- Under *in vivo* conditions, the cellular zinc level that exists in the normal peripheral zone (the major origin of malignancy) of glandular epithelial cells is potentially cytotoxic in the malignant cells;
- To avoid the cytotoxic effects of zinc, the development of malignant cells involves mechanisms that decrease the cellular zinc to levels that are not cytotoxic to the malignant cells;
- The restoration of elevated levels of zinc in malignant or premalignant cells will restore the cytotoxic/tumor suppressor effects of zinc, which will prevent and/or abort malignancy.

The enigma arises from the 'simplistic' view and expectation that providing a typical zinc supplement regimen to increase the circulating level of zinc delivered to the malignant site should abort and/or arrest the development and advancement of prostate cancer. This is a view that we had held until recent new observations of factors associated with the decrease in zinc in malignancy were identified. We now know that such a simplistic approach will not be effective. Therefore, the concept leads to issues that must be considered and elucidated in defining the necessary conditions that will be required to achieve an efficacious approach for the zinc treatment of prostate cancer.

In 2003, Feng *et al.* showed that zinc treatment to elevate the plasma zinc levels of PC-3 tumorigenic xenograft animals results in increased tumor cell zinc levels and suppression of tumor growth [21]. The enigma was subsequently discovered by our studies of the role of zinc transporters in the uptake and accumulation of zinc in prostate cells. In 1999 ZIP1 was identified as an important zinc uptake transporter in malignant prostate cells [22], including PC-3 cells, with further confirmation in 2003 [23]. This led to the expectation and 'successful' results that were obtained in the Feng *et al.* study [21]. However, in 2005 we found that the ZIP1 expression that exists *in situ* in the normal prostate peripheral zone of the glandular epithelium is downregulated in malignant cells *in situ* in prostate cancer [16]. This has been recently confirmed by Johnson *et al.* [17]. Thus, the ZIP1 constitutive expression status that exists in PC-3 cells, and other malignant cell lines, is not representative of the *in vivo* status in prostate cancer. Therefore, the zinc treatment that resulted in the accumulation of zinc and its suppression of wild-type PC-3 tumor growth did not reflect the possible efficacy of a treatment regimen for prostate cancer that is based on the elevation of plasma zinc level.

The appropriate model for prostate cancer to elucidate an efficacious zinc treatment approach must employ ZIP1-deficient tumor development and growth. This requires an approach that includes an agent or mechanism that will deliver zinc to the prostate gland and facilitate the malignant cellular uptake and increase in zinc in a mobile reactive form that exhibits the cytotoxic/tumor suppressor effects of zinc. Currently, there are no reported zinc treatment studies that employ such an appropriate model for prostate cancer. The experimental conditions and models for such studies have not considered and/or identified the transporter relationship and status in relation to the *in vivo* prostate cancer status. Therefore, the translational application to human prostate cancer of the results for such studies has become highly questionable.

The form of cellular zinc status is an essential factor

In the preceding section, we specifically referred to a 'mobile reactive' form of zinc. This is an important factor that must be understood and considered. For important background information we refer the reader to our recent paper and the references cited therein [14]. The important consideration is that there are two pools of zinc that constitute the total zinc that exists within cells. Most (>90%) of the zinc is tightly bound to zinc metalloproteins, which are an unexchangeable form that are not available for the manifestation of cellular effects of zinc. The remaining <10% is zinc that is loosely/moderately bound to ligands, such as amino acids, citrate and metallothionein. This is the exchangeable pool of zinc that is available for manifestation of the cellular effects of zinc. This pool is characterized as Zn Ligands with formation constants of log Kf~10 and lower. There is no relevant free Zn²⁺ ions pool (concentration in pM range) in mammalian cells. With these relationships in mind, it is important that the facilitation of zinc for uptake and reactivity in the cells must be an exchangeable reactive form of zinc. It is this pool, rather than the total cellular zinc, that must be established. The delivery of tightly bound unexchangeable zinc in the cell will likely be ineffective despite an increase in the total cellular zinc.

A zinc ionophore model for an approach to the development of efficacious zinc treatment of cancer

For prostate cancer, the key issue is to deliver zinc into ZIP1-deficient malignant cells and increase the cellular level of mobile reactive zinc, which will manifest the cytotoxic/tumor suppressor effects of zinc. Establishing the conditions to achieve this outcome requires:

- The selection of a zinc delivery agent or mechanism;
- An animal model to determine the efficacy of the agent or process.

Relative to a zinc delivery agent, a zinc ionophore provides one approach. One such ionophore is Clioquinol (CQ; 5-chloro-7-iodo-8-hydroxy-quinoline). CQ has a zinc binding affinity of log Kf~8, which would characterize it as a mobile reactive Zn Ligand that should exhibit the intracellular cytotoxic effects of zinc. Consistent with this expectation, CQ treatment of ZIP1-deficient cells in the presence of physiological levels of zinc results in 80% inhibition of proliferation [Costello LC, Franklin RB, Unpublished Data]. However, the important issue is whether or not such cytotoxic effects of Zn-CQ can be achieved under the complex *in vivo* conditions in a model that provides a representation of human cancer. The important initial criterion is that the model must involve human malignant cells. This can only be achieved with the mouse xenograft model involving the development and growth of tumors derived from inoculation of human malignant cell lines such as PC-3 cells. In addition, the tumors must exhibit the ZIP-deficient status of the malignant cells as exists *in situ* in human cancer. To achieve this, stable PC-3 cell lines with downregulated *ZIP1* can be employed.

There are reported studies that purport to show that CQ treatment inhibits tumor growth in xenograft animals [24]. However, those studies did not consider or employ malignant cell lines and developing tumors, which are representative of the ZIP-deficient malignant cell status that exist in human cancers. Consequently, the relevance and translational value of such studies are questionable. The important point is that any model and any agent must mimic the zinc-associated status that exists *in vivo* in the human cancer condition.

To be cytotoxic or not to be cytotoxic; that is the enigma!

Before further discussion, it is important to address a common view: increased exposure to zinc readily results in toxic effects. More correct is that zinc toxicity is relatively difficult to

induce in normal circumstances. This is well expressed and described in the excellent review of Vallee and Falchuk who state:

“Clearly, a metal that is known to be essential to the inheritance of the genetic endowment and the induction of development, growth, and differentiation could not easily be intended to be deleterious to the perpetuation and evolution of the species. Instead, one would expect zinc to be regulated carefully to ensure the preservation and continuity of life. In fact, zinc is the only pre-, post-, and transitional element that has proven to be essentially nontoxic.” [25].

The regulatory mechanisms involve systemic factors that maintain a normal level of extracellular zinc and cellular factors that maintain a normal condition of intracellular zinc.

A criticism of our concept is the presumption that the cytotoxic effect of zinc in malignant cells is questionable, and a paradox, since normal cells with high zinc levels do not exhibit zinc cytotoxicity. While this brings to the surface an important issue, it is not a paradox or inconsistency. Instead, it is a critical issue that impacts the zinc relationship; and it requires more attention and resolution than past and current research has addressed. Zinc is required for the normal growth, proliferation, metabolism and function of all cells, both normal and pathological. It is evident that normal cells have evolved with conditions that permit the maintenance of zinc levels that are required for their normal existence in their natural environment, as Vallee and Falchuk described.

Normal mammalian cells contain and maintain total cellular zinc levels in the range of approximately 200–800 μM , depending on the cell type. Under these conditions, the normal cells possess mechanisms/factors that protect the cells from the potential adverse effects of zinc. Within the same *in situ* environment, malignancy develops under conditions in which the malignant cells are susceptible to the potential cytotoxic effects of the cellular zinc levels that exist in the normal cells. This suggests that the protective mechanisms/factors that exist in normal cells do not exist in malignant cells. Some *in vitro* studies also provided evidence that similar zinc accumulation from exposure to zinc treatment is more cytotoxic in malignant cells than in nonmalignant cells [26,27]. Therefore, the evolution and development of malignant cells probably involved a selection process in which those neoplastic cells with adaptive mechanisms that prevent zinc cytotoxicity are the surviving cells that become malignant cells. To achieve this, the malignant cells exhibit lower levels of zinc than the normal cells. This adaptive change prevents manifestation of zinc cytotoxic effects and also maintains the levels of zinc that are essential for the survival and activities of the malignant cells. This is an important distinction as a complete or near-complete loss and absence of zinc should not be expected in malignant cells; that is, a condition under which no cells would survive. In fact, this consideration impacts the reliability of zinc studies that commonly employ TPEN (N,N,N',N'-tetrakis[2-pyridylmethyl]ethylenediamine) treatment of cells. TPEN is a strong cell-permeant chelator of zinc ($\log K_f \sim 15$) which results in an unphysiological cellular zinc-deficient status that will induce cell death. Treatment of such cells with zinc will first restore minimal required zinc levels, which, in itself, will prevent cell death. From this, a conclusion is often reached that an important effect of zinc is the prevention of apoptosis, which can be in conflict with the demonstrated effect of zinc as an apoptogenic agent.

Factors & conditions that protect cells from the cytotoxic effects of zinc

The preceding discussion raises two related and highly relevant questions. The first question is, “what are the mechanisms and factors that protect the normal cells from the cytotoxic effects of zinc, and do not exist in the malignant cells?”. This key question has not been adequately addressed and remains largely unresolved. One potential mechanism relates to an

important identified cytotoxic effect of zinc – which is its apoptogenic effect. This effect results from zinc-induced mitochondrial Bax pore formation, increased cellular Bax and increased Bax/Bcl-2 ratio [28–31]. This leads to the possibility that in the natural environment of the prostate gland, the normal glandular epithelial cells may incorporate increased Bcl-2 production and/or antiapoptotic responses, which could prevent the potential apoptotic cytotoxic effect of zinc. The report of Park *et al.* provided evidence for a protective effect of increased HIF1 α against cytotoxicity resulting from high zinc levels in prostate cells [32]. Pursuant studies identified the anti-apoptogenic agent, survivin, as a mediator of HIF1 α protection of prostate cells from zinc cytotoxicity [33]. Such studies need to be expanded to elucidate the factors and mechanisms that protect normal cells from cytotoxic effects of zinc and which of these protective factors are absent in the development of malignant cells.

Cells can also employ zinc transporters to sequester zinc as a mechanism for prevention of cytotoxic effects. Once zinc enters the cell, the cytosolic zinc is subject to intracellular distribution. Although the cellular distribution of zinc varies for different cells, a reasonable estimate is that approximately 35% of the total amount of zinc resides in the nucleus and 65% in the cytoplasm [14]. Of the cytoplasmic zinc, an estimated 50% resides between the cytoplasmic organelles and the cytosol. The distribution between cytosol and organelles is dependent upon intracellular zinc transporters, mainly *ZnT* (SLC30A) family transporters. This can provide a zinc sequestering mechanism that will decrease the cytosolic concentration of the mobile reactive pool of zinc, thereby reducing the potential cytotoxic effects of zinc, although the total cellular zinc concentration is elevated. In addition, the upregulation of metallothionein in response to increased cellular zinc can also facilitate the sequestering of zinc.

The second question is “what are the mechanisms that the malignant cells adapted to prevent the cytotoxic effects of zinc?”. As already described, one mechanism is the reduction of cellular zinc to levels that are not cytotoxic to the malignant cells. This is achieved in the case of prostate cancer by the downregulation of *ZIP1*; that is, the functional zinc uptake transporter of the normal glandular epithelial cells. This does not imply that other zinc transporters are not also involved. Nor does this imply that other protective mechanisms are not adapted by the malignant cells.

Currently, there is insufficient information regarding normal cell and malignant cell zinc cytotoxic protective mechanisms and factors. It is also important to emphasize that these protective mechanisms exist and are influenced by the *in vivo* conditions of the natural environment of the normal and malignant cells. *In vitro* conditions and isolated cells do not represent the *in vivo* status and so, such studies for identification of the protective mechanisms might not be representative of the *in situ* cells. As an example that has been previously described, the *ZIP1* status in malignant prostate cell lines, such as PC-3, is not representative of the *in situ* status of the malignant cells from which the cell line was derived. Despite these challenges, the elucidation of the mechanisms and factors of the zinc cytotoxicity protective mechanisms in normal and malignant cells is critical to understanding the implications of zinc in the development of prostate cancer and other cancers.

These considerations are critical for a rational and efficacious zinc treatment approach for cancer. Any such zinc treatment must also consider the potential adverse effects on the normal cells. The systemic CQ ionophore approach described above will have the likely effect of increasing the cellular incorporation of circulating zinc in many tissues throughout the body. CQ has been employed for topical and systemic applications in humans. The extensive reviews of CQ toxicity in humans and animals by Mao and Schimmer [34] and

Bareggi and Cornelli [35] essentially indicate that, at effective therapeutic concentrations (generally in the range of ~20–50 mg/kg/day), CQ exhibits little or no toxic effects. Toxic effects are observed with a severalfold higher dosage of CQ. Seemingly, the normal cell protective mechanisms as described above are effective in minimizing potential zinc cytotoxicity. Another consideration is that the malignant cells are more susceptible than normal cells to zinc cytotoxicity, so that a relative low treatment dose may be effective. Even with some tolerable level of adverse effects and contraindications, the effective treatment of the highly morbid and deadly cancer can provide the best option.

Are the prostate cancer–zinc relationships applicable to other cancers?

It is becoming evident that some other cancers exhibit zinc relationships that are similar to prostate cancer. Several reports have consistently demonstrated that zinc levels in hepatocellular cancer are markedly decreased (~60% decrease) compared to normal liver tissue [4–10]. We recently found that the hepatoma cells in hepatocellular cancer exhibit a major decrease in zinc compared to the hepatocytes in normal liver [11], which further corroborated other reports of decreased zinc in liver cancer [4–10]. Concurrently with the decrease in zinc, we identified ZIP14 as the functional zinc uptake transporter in the normal hepatocytes, which is downregulated in the hepatoma cells. These simultaneous changes exist in early-stage well-differentiated malignancies and persist in advanced stage malignancies. In addition, zinc treatment of hepatoma cell lines has been shown to inhibit cell proliferation [11,36,37]. We also found the same relationships in pancreatic adenocarcinoma; in which zinc levels of ductal and acinar epithelium are markedly decreased in adenocarcinoma [12]. However, ZIP3 is the functional zinc transporter in pancreatic ductal and acinar epithelium that is downregulated in malignancy concurrently with the decrease in zinc. Additionally, zinc treatment of malignant pancreatic cells results in inhibition of cell proliferation [12,26,38]. In both hepatocellular and pancreatic cancers, the decrease in zinc and ZIP is evident in early-stage malignancy [11,12]. Thus, prostate, hepatocellular and pancreatic cancers exhibit very similar zinc relationships in which the functional transporters differ, but in each cancer the functional transporter is markedly decreased in the malignant cells concurrently with the decrease in zinc. Lightman *et al.* reported a significant decrease in zinc levels of ovarian tumors when compared with benign tissue [13]. Surprisingly, no pursuant studies to corroborate and extend this potentially important observation have been reported. However, zinc treatment of malignant ovarian cells has been shown to exhibit cytotoxic effects [24,39,40]. Although more research is necessary, ovarian cancer appears to exhibit the zinc relationships as described previously for the other cancers. Therefore, the present information would suggest that a zinc treatment approach may be applicable to these cancers.

In contrast to the information above, several reports have established that zinc levels are markedly increased in breast cancer tissue as compared with noncancerous tissue [41–44]. However, the role of zinc and its effects on breast cells are conflicting. For example, recent evidence indicates that accumulated zinc in breast cancer cells is compartmentalized by overexpression of *ZnT2* transporter expression [45]. This compartmentalization protects the cancer cells from the cytotoxic effects of high cytosolic levels of zinc. Therefore, even in breast cancer, where zinc accumulates in malignancy, the effect of elevated cytoplasmic zinc is possibly cytotoxic. This raises a host of issues regarding the role of zinc and its specific effects and actions in different cancers.

The possible involvement of zinc in other cancers is, for the most part, poorly defined. Some indications of decreased zinc levels and cytotoxic effects of zinc do exist in esophageal cancer, lingual cancer, other head and neck cancers, colon cancer and choriocarcinoma (reviewed in [46,47]). However, in most instances the zinc levels in the malignant tissue

versus normal tissue has not been established; and the role of zinc transporters and other zinc factors remain unknown.

The ultimate enigma: what is the meaning of the decreased zinc relationship that is common in many cancers & the opportunities it presents?

Until recently, we considered that the implications and role of zinc as identified in prostate cancer were highly specific for prostate cancer. However, it is now evident that the zinc relationships and role in prostate cancer is a more 'general phenomenon' that is applicable to a variety of cancers; although there exists specific differences and nuances for each specific cancer. This is an important revelation. What is the underlying commonality for this role of zinc in cancer? Should we now consider a consistent implication of zinc as an important event in the carcinogenesis process applicable to many cancers? If so, what is the common oncogenetic event that leads to altered zinc in the transformation of normal cells to malignant cells? Thus, a host of issues and questions now surface regarding the implication of zinc in the development and progression of cancer. This leads to the opportunities that can be derived from this new understanding. The early events and factors involved in the alterations in zinc provide new opportunity for the identification of biomarkers for early detection of specific cancers. The cytotoxic/tumor suppressor effects of zinc provide the opportunity for the development of new chemotherapeutic agents for treatment and prevention of specific cancers. More research regarding the implications of zinc in cancer is required to resolve the enigmas and to take advantage of the potential opportunities.

Expert commentary

Despite enormous investments in research and development, the search for efficacious chemotherapeutic agents for many, if not most, cancers has been largely disappointing. For example, the 5-year survival rate for pancreatic adenocarcinoma has remained at approximately 3% over the past 40 years. The incidence of hepatocellular cancer has been rising over the past two decades. Advanced-stage prostate cancer still results in death. For such examples, there exists no effective treatment and no efficacious chemotherapeutic agents. Thus, new approaches are required for the development of effective anticancer agents. This is dependent upon improved identification and understanding of the factors and mechanisms associated with the development of malignancy and the carcinogenic process involved in the transformation of normal cells to malignant cells. Evolving clinical and experimental evidence has implicated an important role for zinc as a cytotoxic/tumor suppressor agent in the development of prostate, pancreatic, hepatocellular, and possibly other cancers. An understanding of the mechanisms of altered zinc regulation that occurs in the transformation of normal cells to malignant cells provides a new opportunity for development of zinc-related chemotherapeutic agents for treatment and possibly prevention of these cancers. Further understanding and research that focuses on the role of zinc in specific cancers is essential to achieve this goal.

Five-year view

First, there must be a change in the view of the clinical and biomedical research community regarding the role and implication of zinc in the development and progression of cancers. This requires that viewpoints are developed from the scientifically-based existing clinical and experimental evidence of zinc relationships in cancers; and not based on subjective 'impressions or beliefs'. This will then result in the scientifically credible review of programs and grants for the essential research that is required to elucidate a better

understanding of the issues, factors, and mechanisms of the role and effects of zinc in normal and malignant cells. Coupled with this is the requirement for an improved education and understanding among clinicians and biomedical researchers regarding the biochemistry, physiology and pathophysiology of zinc in mammalian cells. This will lead to development and testing of promising zinc-delivery agents in appropriate experimental systems such as *in vitro* cell studies and animal models. The promising agents will then be subjected to clinical trials to establish the efficacy and the contraindications of zinc treatment in specific cancers.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1•• Costello LC, Franklin RB. The clinical relevance of the metabolism of prostate cancer; zinc and tumor suppression: connecting the dots. *Mol Cancer*. 2006; 5:17. Review of important zinc relationships in prostate cancer, which will also serve as a background for other cancers. [PubMed: 16700911]
2. Franklin RB, Milon B, Feng P, Costello LC. Zinc and zinc transporter in normal prostate function and the pathogenesis of prostate cancer. *Front Biosci*. 2005; 10:2230–2239. [PubMed: 15970489]
3. Costello LC, Franklin RB, Feng P, Tan M, Bagasra O. Zinc and prostate cancer: a critical scientific, medical and public interest issue. *Cancer Causes Contr*. 2005; 16:901–915.
4. Danielsen A, Steinnes E. A study of some selected trace elements in normal and cancerous tissue by neutron activation analysis. *J Nucl Med*. 1970; 11:260–264. [PubMed: 4316187]
5. Ebara M, Fukuda H, Hatano R, et al. Relationship between copper, zinc and metallothionein in hepatocellular carcinoma and its surrounding liver parenchyma. *J Hepatol*. 2000; 33:415–422. [PubMed: 11019997]
6. Gurusamy K. Trace element concentration in primary liver cancers – a systematic review. *Biol Trace Elem Res*. 2007; 118:191–206. [PubMed: 17916922]
7. Liaw KY, Lee PH, Wu FC, Tsai JS, Lin-Shiau SY. Zinc, copper, and superoxide dismutase in hepatocellular carcinoma. *Am J Gastroenterol*. 1997; 92:2260–2263. [PubMed: 9399766]
8. Tashiro H, Kawamoto T, Okubo T, Koide O. Variation in the distribution of trace elements in hepatoma. *Biol Trace Elem Res*. 2003; 95:49–63. [PubMed: 14555799]
9. Tashiro-Itoh T, Ichida T, Matsuda Y, et al. Metallothionein expression and concentrations of copper and zinc are associated with tumor differentiation in hepatocellular carcinoma. *Liver*. 1997; 17:300–306. [PubMed: 9455736]
10. Al-Ebraheem A, Farquharson MJ, Ryan E. The evaluation of biologically important trace metals in liver, kidney and breast tissue. *Appl Radiat Isot*. 2009; 67:470–474. [PubMed: 18675548]
11. Franklin RB, Levy BA, Zou J, et al. ZIP14 zinc transporter downregulation and zinc depletion in the development and progression of hepatocellular cancer. *J Gastrointest Cancer*. 2011 (Epub ahead of print). 10.1007/s12029-011-9269-x
12. Costello LC, Levy B, Desouki M, et al. Decreased zinc and downregulation of ZIP3 zinc uptake transporter in the development of pancreatic adenocarcinoma. *Cancer Biol Ther*. 2011; 12:297–303. [PubMed: 21613827]
13. Lightman A, Brandes JM, Binur N, Drugan A, Zinder O. Use of the serum copper/zinc ratio in the differential diagnosis of ovarian malignancy. *Clin Chem*. 1986; 32:101–103. [PubMed: 3940688]
- 14•• Costello LC, Fensaleu C, Franklin RB. Evidence for operation of the direct zinc ligand exchange mechanism for trafficking, transport, and reactivity of zinc in mammalian cells. *J Inorg Biochem*. 2011; 105:589–599. Review and understanding of zinc relationships in mammalian systems. [PubMed: 21440525]
15. Mawson CA, Fischer MI. The occurrence of zinc in the human prostate gland. *Can J Med Sci*. 1952; 30:336–339. [PubMed: 14954495]

- 16•. Franklin RB, Feng P, Milon BC, et al. hZIP1 zinc uptake transporter downregulation and zinc depletion in prostate cancer. *Mol Cancer*. 2005; 4:32. First paper to show the important role of zinc and the zinc transporter in prostate cancer. [PubMed: 16153295]
17. Johnson LA, Kanak MA, Kajdacsy-Balla A, Pestaner JP, Bagasra O. Differential zinc accumulation and expression of human zinc transporter 1 (hZIP1) in prostate glands. *Methods*. 2010; 4:316–321. [PubMed: 20705137]
18. Cortesi M, Fridman E, Volkov A, et al. Clinical assessment of the cancer diagnostic value of prostatic zinc: a comprehensive needle-biopsy study. *Prostate*. 2008; 68:994–1006. [PubMed: 18386292]
- 19•. Franklin RB, Costello LC. The important role of the apoptotic effects of zinc in the development of cancers. *J Cell Biochem*. 2009; 106:750–757. Provides an understanding of the cytotoxic/apoptotic mechanism of zinc. [PubMed: 19160419]
20. Costello LC, Franklin RB. The genetic/metabolic transformation concept of carcinogenesis. *Cancer Metastasis Rev*. 2011 (Epub ahead of print). 10.1007/s10555-011-9334-8
21. Feng P, Li T, Guan Z, Franklin RB, Costello LC. Effect of zinc on prostate tumorigenicity in nude mice. *Ann NY Acad Sci*. 2003; 1010:316–320. [PubMed: 15033742]
22. Costello LC, Liu L, Zou J, Franklin RB. Evidence for a zinc uptake transporter in human prostate cancer cells which is regulated by prolactin and testosterone. *J Biol Chem*. 1999; 274:17499–17504. [PubMed: 10364181]
23. Franklin RB, Ma J, Zou J, et al. Human Zip1 is a major zinc uptake transporter for accumulation of zinc in prostate cells. *J Inorgan Biochem*. 2003; 96:435–442.
24. Ding WQ, Liu B, Vaught JL, Yamauchi H, Lind SE. Anticancer activity of the antibiotic clioquinol. *Cancer Res*. 2005; 65:3389–3395. [PubMed: 15833873]
- 25•. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev*. 1993; 73:79–118. Major review of the chemistry, biochemistry, physiology and pathology of zinc. [PubMed: 8419966]
26. Jayaraman AK, Jayaraman S. Increased level of exogenous zinc induces cytotoxicity and up-regulates the expression of the *ZnT-1* zinc transporter gene in pancreatic cancer cells. *J Nutr Biochem*. 2011; 22:79–88. [PubMed: 20392624]
27. Sliwinski T, Czechowska A, Kolodziejczak M, Jajte J, Wisniewska-Jarosinska M, Blasiak J. Zinc salts differentially modulate DNA damage in normal and cancer cells. *Cell Biol Int*. 2009; 33:542–547. [PubMed: 19254773]
28. Liang JY, Liu YY, Zou J, Franklin RB, Costello LC, Feng P. Inhibitory effect of zinc on human prostatic carcinoma cell growth. *Prostate*. 1999; 40:200–207. [PubMed: 10398282]
29. Feng P, Li L, Guan ZX, Franklin RB, Costello LC. Direct effect of zinc on mitochondrial apoptosis in prostate cells. *Prostate*. 2002; 52:311–318. [PubMed: 12210492]
30. Feng P, Li T, Guan Z, Franklin RB, Costello LC. The involvement of Bax in zinc-induced mitochondrial apoptosis in malignant prostate cells. *Mol Cancer*. 2008; 7:25. [PubMed: 18331646]
31. Ku JH, Seo SY, Kwak C, Kim HH. The role of survivin and Bcl-2 in zinc-induced apoptosis in prostate cancer cells. *Urol Oncol*. 2010 (Epub ahead of print). 10.1016/j.urolonc.2010.06.001
32. Park SE, Park JW, Cho YS, Ryu JH, Paick JS, Chun YS. HIF-1alpha promotes survival of prostate cells at a high zinc environment. *Prostate*. 2007; 67:1514–1523. [PubMed: 17683079]
33. Yun YJ, Li SH, Cho YS, Park JW, Chun YS. Survivin mediates prostate cell protection by HIF-1alpha against zinc toxicity. *Prostate*. 2010; 70:1179–1188. [PubMed: 20564420]
- 34•. Mao X, Schimmer AD. The toxicology of clioquinol. *Toxicol Lett*. 2008; 182:1–6. Excellent review of the clinical use and toxicology of clioquinol. [PubMed: 18812216]
35. Bareggi SR, Cornelli U. Clioquinol: review of its mechanisms of action and clinical uses in neurodegenerative disorders. *CNS Neurosci Ther*. 2010 (Epub ahead of print). 10.1111/j.1755-5949.2010.00231.x
36. Lemire J, Mailloux R, Appanna VD. Zinc toxicity alters mitochondrial metabolism and leads to decreased ATP production in hepatocytes. *J Appl Toxicol*. 2008; 28:175–182. [PubMed: 17582580]

37. Xu J, Xu Y, Nguyen Q, Novikoff PM, Czaja MJ. Induction of hepatoma cell apoptosis by c-myc requires zinc and occurs in the absence of DNA fragmentation. *Am J Physiol.* 1996; 270:G60–G70. [PubMed: 8772502]
38. Donadelli M, Dalla PE, Scupoli MT, Costanzo C, Scarpa A, Palmieri M. Intracellular zinc increase inhibits p53^{-/-} pancreatic adenocarcinoma cell growth by ROS/AIF-mediated apoptosis. *Biochim Biophys Acta.* 2009; 1793:273–280. [PubMed: 18951928]
39. Bae SN, Lee YS, Kim MY, Kim JD, Park LO. Antiproliferative and apoptotic effects of zinc-citrate compound (CIZAR[®]) on human epithelial ovarian cancer cell line, OVCAR-3. *Gynecol Oncol.* 2006; 103:127–136. [PubMed: 16624386]
40. Ding WQ, Yu HJ, Lind SE. Zinc-binding compounds induce cancer cell death via distinct modes of action. *Cancer Lett.* 2008; 271:251–259. [PubMed: 18639975]
41. Rizk SL, Sky-Peck HH. Comparison between concentrations of trace elements in normal and neoplastic human breast tissue. *Cancer Res.* 1984; 44:5390–5394. [PubMed: 6488192]
42. Santoliquido PM, Southwick HW, Olwin JH. Trace metal levels in cancer of the breast. *Surg Gynecol Obstet.* 1976; 142:65–70. [PubMed: 1244691]
43. Margalioth EJ, Schenker JG, Chevion M. Copper and zinc levels in normal and malignant tissues. *Cancer.* 1983; 52:868–872. [PubMed: 6871828]
44. Farquharson MJ, Al-Ebraheem A, Geraki K, Leek R, Jubb A, Harris AL. Zinc presence in invasive ductal carcinoma of the breast and its correlation with oestrogen receptor status. *Phys Med Biol.* 2009; 54:4213–4223. [PubMed: 19521003]
45. Lopez V, Foolad F, Kelleher SL. ZnT2-overexpression represses the cytotoxic effects of zinc hyper-accumulation in malignant metallothionein-null T47D breast tumor cells. *Cancer Lett.* 2011; 304:41–51. [PubMed: 21353385]
46. Doerr TD, Prasad AS, Marks SC, et al. Zinc deficiency in head and neck cancer patients. *J Am Coll Nutr.* 1997; 16:418–422. [PubMed: 9322189]
47. Franklin RB, Costello LC. Zinc as an anti-tumor agent in prostate cancer and in other cancers. *Arch Biochem Biophys.* 2007; 463:211–217. [PubMed: 17400177]

Key issues

- Zinc is a cytotoxic/tumor suppressor agent in prostate and other cancers.
- Restoration of zinc levels in the malignant cells will restore the adverse effects of zinc in these cells.
- This relationship provides the opportunity for development of zinc-associated chemotherapeutic agents for the treatment of these cancers.
- The achievement of this potential requires additional resolution and understanding of the zinc relationship in malignant versus normal cells.
- The role of zinc transporters and other protective factors against the adverse effects of zinc need to be identified. This requires identification of those protective factors that exist in the normal cells as compared with the factors that exist in malignant cells.
- An efficacious zinc treatment regimen will require a zinc-delivery agent or mechanism, such as an ionophore approach, that will facilitate the uptake and accumulation of mobile reactive zinc into the malignant cells.
- Further research into these zinc issues will lead to achievement of effective zinc treatment of certain cancers.

Figure 1.
The concept of the oncogenetic and genetic/metabolic transformations in the carcinogenesis process, and the role of zinc and zinc transporters as represented in prostate cancer.