Insight to Physiology and Pathology of Zinc(II) Ions and Their Actions in Breast and Prostate Carcinoma

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Abstract: Zinc(II) ions contribute to a number of biological processes e.g. DNA synthesis, gene expression, enzymatic catalysis, neurotransmission, and apoptosis. Zinc(II) dysregulation, deficiency and over-supply are connected with various diseases, particularly cancer. 98% of human body zinc(II) is localized in the intracellular compartment, where zinc(II) is bound with low affinity to the intracellular matrix. Zinc carries its own molecules to and from cells or organelles. Imbalance of their regulation is described in cancers, particularly prostate (down-regulated zinc transporters ZIP1, 2, 3 and ZnT-2) and breast, notably its high-risk variant (up-regulated ZIP6, 7, 10). As a result, intracellular and even blood plasma zinc(II) levels are altered. MT protects cells against oxidative stress, because it cooperates with reduced glutathione (GSH). Recent studies indicate elevated serum level of MT in a number of malignancies, among others in breast, and prostate. MT together with zinc(II) affect apoptosis and proliferation, thus together with its antioxidative effects it may affect cancer. To date, only little is known about the influence of zinc(II) and MT on cancer, while these compounds may play an important role in pathogenesis. This review concludes current data regarding the impact of zinc(II) on the pathogenesis of breast and prostate cancers with potential outlines of new, targeted therapy and prevention. Moreover, blood plasma zinc(II) and MT levels and dietary zinc(II) intake are discussed in relation to breast and prostate cancer risk.

Keywords: Zinc, Cancer, Prostate Carcinoma, Breast Carcinoma, Homeostasis, Low Molecular Mass Thiols, Metallothionein, Glutathione, Zinc Intake, Apoptosis, ZnT and ZIP transporters.

1. INTRODUCTION

Zinc(II) is involved in numerous key intra- and extracellular processes including proliferation, differentiation and apoptosis [1,2], its regulation is important not only in development and proliferation of tissues, but also in neoplastic transformation [3,4]. To date, nearly 5,000 studies meeting the criteria of keywords “zinc” and “cancer” are indexed in Web of Knowledge portal, while the amount of papers increased 2.5-fold during the last decade. The effects of zinc(II) are both cancerogenic and protective and seems to be very complex and manifest in cancer-dependent manner. This review aims to summarize current associations between zinc(II) and cancers of prostate and breast, most common cancers, whose association with zinc has been studied most extensively.

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008. Incidence rates vary by more than 25-fold worldwide, with the highest rates recorded primarily in the developed countries of Oceania, Europe, and North America, largely because of the wide utilization of prostate-specific antigen testing that detects clinically important tumours as well as other slow-growing cancers that might otherwise escape diagnosis [5]. The prevalence of prostate cancer increases with age [6].

Hence, prostate cancer is a disease that predominantly affects older men. It does, however, usually respond to treatment and, if localized, may be curable. The rate of tumor growth varies from very slow to moderately rapid, and some patients may have prolonged survival, even after the cancer has metastasized to distant sites.

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008. About half the breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries. In general, incidence rates are high in Western and Northern Europe, Australia/New Zealand, and North America; intermediate in South America, the Caribbean, and Northern Africa; and low in sub-Saharan Africa and Asia [5]. Breast cancer is an acquired or inherited genetic disorder influenced by environmental, behavioral, and reproductive factors. The most significant risk factors are gender (being a woman) and age (growing older) [7]. Two distinct forms of the disease are identified as hereditary forms of cancers, which are often related to mutations in two high-penetrance susceptibility genes referred to as BRCA-1 and BRCA-2 [8] and sporadic forms account for 90–95% of cases and are consequences of a somatic mutation over the lifetime without any hereditary predisposition [9-11].

The major challenge in the management of prostate, breast and other types of cancers is the understanding biochemical pathways, where zinc metabolism is very interesting. In general, relation to zinc(II) and tumours can be regarded from the perspective of
dysregulation of their intracellular and blood serum zinc(II) levels and from the perspective of abundant or deficient dietary zinc(II) income and thus resulting alteration of its biochemical roles. Thus, we believe, it is important to study the role of zinc(II) in relation to cancer.

2. MOLECULAR AND CELLULAR BIOLOGY OF ZINC(II)

Most of the zinc (II) in human body, approx. 98%, is localized in the intracellular compartment [12]. Its total intracellular concentration is in a range within hundreds of micromoles, thus it is approx. 10-fold higher compared to serum levels [13-16]. Most of the intracellular zinc(II) is bound to or at least associated with proteins and peptides [17]. Thereof, app. 90% is tightly bound with the rest (10 %) is bound with relatively low affinities, forming reactive zinc(II) pool able to interact with other intracellular substances and compartments [18]. The last, very small fraction (approx. < 0.01 % of total cellular zinc(II), ranging from pM to single digit nM) includes free zinc(II) ions [16]. The tightly bound zinc(II) ions occur mainly in metalloproteins, metalloenzymes and nucleoproteins and acts as a structural component of these biomolecules or as an enzyme cofactor. This fraction can be considered as an immobile nonreactive zinc(II) pool [1,16]. The rest of zinc(II) ions fraction, which acts as a mobile reactive form [19,20], is bound to low molecular weight compounds (amino acids as cysteine, histidine, proline), protein metallothionein or organic acids (citrate, oxalate) [21]. If there is a focus on cellular functions of zinc(II), determination of total cellular zinc(II) concentration is not of such importance as the determination of mobile reactive zinc(II) [18].

From the point of view of cell compartments, zinc(II) is not uniformly distributed. Approximately 30–40 % is in the nucleus, about 50 % in the cytoplasm and in organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, endosomes and lysosomes, and the rest is bound to cell membranes [12]. In addition to the ubiquitous conventional organelles, zinc(II) ions were also found in the specialized organelles such as synaptic vesicles, secretory granules and lysosome like structures [22].

Zinc(II) concentration varies within the range 200–400 nmols/gram wet weight tissue in most tissues [23]. However, tissues with physiologically significantly higher zinc(II) content have been described. These include beta cells of the pancreas, secretory cells of the pineal gland, lymphocytes, cells of the salivary glands, cells of retina and epithelial cells of prostate [24]. In prostate, particularly in its peripheral zone, which is the major site of malignancy initiation, zinc(II) is accumulated in approx. tenfold higher concentration compared to other tissues [25], within 3,000–4,500 nmols/gram wet weight tissue [23]. This is because of tissue-specific zinc(II) functions in prostate (Fig. 1). First, prostatic tissue produces zinc-rich portion of semen; zinc(II) is necessary for proper movement of sperms [12,26]. Second, prostate contributes to high content of citrate in semen [27]. Prostate-specific citrate production is zinc-mediated: Zinc(II) inhibits mitochondrial enzyme aconitase, responsible for conversion of citrate to isocitrate, following its utilization in Krebs cycle. Due to this inhibition citrate is not utilized in Krebs cycle, is accumulated in prostatic cells and may be released into the seminal fluid [28]. Its prostatic intracellular concentration is 12 μmol/g wet tissue, more than 30-fold higher compared to other tissues [23]. However, this causes prostatic tissue less efficient in ATP production: one mol of glucose generates 14 moles of ATP compared to 38 moles in case of complete citrate oxidation [18].

In addition to zinc(II) levels in normal prostatic tissue, its levels are increased in benign prostate hypertrophy, and radically decreased in prostate cancer. In peripheral zone of neoplastic prostate, its levels ranges within 400–800 nmols/gram wet weight tissue, thus 5-fold lower compared to healthy prostate tissue, however still 2-fold higher compared to most other tissues [23]. In terms of zinc(II) level in breast cancer tissue, results of studies are inconsistent. Based on study by Cui et al. on 500 US participants, significantly higher zinc(II) content was found in breast cancer tissue compared to controls. In addition, high levels of zinc(II) may be associated with a modest increase of risk of subsequent breast cancer in benign breast tissue [29]. Similarly, based on a study on 36 participants in India, significant elevation (> 2-fold) of tissue zinc(II) was observed in tumor tissue [30]. However, in study presented by Majewska et al., significant differences in tissue samples obtained from breast cancer and benign lesions were not observed [31]. Thus, it can be expected that zinc(II) may play an important role in prostate cancer pathogenesis and apparently also in breast cancer, however probably by other mechanisms due to the fact that its level is higher compared to healthy tissue.

Based on the involvement of zinc(II) in the complex regulatory network, precise mechanisms to maintain intracellular zinc(II) level exist (Fig. 1). Zinc(II) pool is maintained by two types of proteins: (a) zinc-binding proteins (mostly by metallothionein), which act as buffer and donor of zinc for intracellular processes, and (b) zinc transporters, which are responsible for zinc fluxes into/from cells and organelles. A key regulator of intracellular free zinc level is metal regulatory transcription factor 1 (MTRF-1, also called MRE-binding factor) [32]. This 753 amino acids transcription factor directly responds to the elevated zinc(II) level and induces the transcription of metallothionein and main zinc transporter responsible for its export, ZnT-1 (discussed below) [33-35]. This autoregulatory loop maintains narrow optimal limits of intracellular zinc(II): when the level of metallothionein and ZnT-1 is elevated, more free zinc(II) may be buffered (i.e. bound to metallothionein) and more zinc may leave cells (through larger amount of membrane transporters).

2.1. Zinc Transport

Zinc(II) cannot freely pass through membranes, thus, special membrane transporters have developed in a cell. Zinc(II) transporters are transmembrane proteins, which transfer zinc(II) ions through cellular membranes [36]. Most of them are localized both on plasmatic and on organelle’s membranes. There are two zinc transporter families: Zinc-Iron Permease transporter (ZIP), also called Zrt-Irt-like protein, or solute-linked carrier 39 (SLC39) family and Zinc transporter (ZnT, SLC30) family. ZIP transporter family is responsible for the influx of zinc(II) ions to the cytoplasm, in other words for transporting zinc(II) ions from extracellular compartments or from intracellular organelles to the cytoplasm. ZnT family is responsible for opposite action, i.e. the efflux of zinc(II) ions from cytoplasm (transport from cytoplasm to the organelles or to the extracellular matrix) [37].

Transport mechanisms of those proteins are not fully understood, however, it is expected there is ATP-independent facilitated diffusion, secondary active transport or symport mechanism [38]. Regulation of expression of both transporter families is not yet fully clear, however, in general, zinc(II) has antagonistic effect on transporters: whereas low zinc(II) load induces expression of zinc importers – ZIPs, high zinc(II) level induces expression of zinc exporters – ZnTs [37]. In the following chapter, transporters associated with breast and prostate cancers or transporters expected to play a role in those cancers are displayed only.

2.1.1. ZIP Transporter Family

ZIP transporter family is responsible for energy-independent zinc(II) influx [39]. In humans, fourteen members of these proteins have been found and denominated ZIP1 – 14 [36]. The proteins are introduced in Table 1. This family can be divided into subfamilies I, II, LIV-1 and gufA [40]. Most members of the ZIP family consist of 220–650 amino acids residues with eight putative
Fig. (1). Zinc in healthy prostate. ZIP and ZnT transporters maintain intracellular zinc transport (1); ZIP1 is a major zinc(II) importer, ZnT-1 is the only export transporter [41,67]. Intracellular free zinc(II) induce metallothionein (MT) expression through metal regulatory transcription factor-1 (MTF-1), which binds to metal regulatory element (MRE) region of MT gene [32] (2). Consequently, zinc(II) is bound by MT [16] (3) (white MT represents reduced/metal-free form, grey MT represents oxidized/metal-bound form). High zinc(II) load induces oxidative stress (ROS), (5), which is reduced by glutathione system in cooperation with MT (4) [82]; MT convert glutathione to its reduced form while being oxidized [83-86]. Endoplasmic reticulum may serve as the intracellular zinc(II) pool and regulates cytoplasmic free zinc(II) level by ZIP7 transporter, which increase zinc(II) content in cytoplasm (7) [43]. Free zinc(II) affect gene expression through mitogen-activated protein kinase cascade (MAPKs, 9) [3,4]. In prostate, high zinc(II) level inhibit mitochondrial aconitase (mAC), thus citrate is accumulated (6) and released in high level [25]. Zinc induces a prostate-specific BAX pore formation (8) causing cytochrome C (CytC) release from mitochondria and subsequent caspase-mediated apoptosis [18].

Fig. (2). Zinc in prostate cancer. (A) ZIP1 zinc(II) transporter is down-regulated due to up-regulated Ras-Raf-MEK-ERK cascade in prostate cancer [44]. This causes (B) lower zinc(II) influx and thus lower intracellular zinc(II) in prostate cancer cells [25]. Lower zinc(II) (C) abolish mitochondrial aconitase (mAC) inhibition, causes citrate utilization in Krebs [25]. (D) Abolished formation of BAX pores on outer mitochondria membrane together with (E) upregulated Bcl-2 decrease cytochrome-C release that causes reduced apoptosis [18]. MT has tumor cell-protective mechanisms through (F) NF-κB activation and (G) reactive oxygen species reduction. (F,G,D). Therefore, MT may be a prostate cancer-specific tumor-protective mechanism (I). Similarly to prostate specific antigen (PSA), MT (H) leaves tumor cells to circulation (thus possible tumor marker) [83].
transmembrane units. Long and variable-in-composition loop rich in histidine, which is probably responsible for metal binding is placed between units 3 and 4. Based on actual studies, six of these fourteen ZIP transporters are reported to be associated with breast or prostate tumors: ZIP1, 2, 3, 6, 7 and ZIP-10.

ZIP1 transporter, the major zinc(II) importer [41], belongs to subfamily II. ZIP1 is encoded by SLC39A1 gene, which is located on a chromosome 1 and the protein itself consists of 324 amino acids (Table 1). This transporter is ubiquitously and consistently expressed at the plasma membrane of most cells [41]. It is known that the prostate, mainly epithelial cells, accumulates very high levels of zinc(II), up to 4,500 nmols/gram wet weight tissue [25]. Accumulated zinc(II) is then secreted into the seminal fluid. ZIP1 contributes significantly to this accumulation because it is responsible for zinc uptake from the circulation [42]. Prolactin and testosterone induces expression of ZIP1. Therefore, administration of prolactin and testosterone results in enhanced expression ZIP1 and, therefore, increased capacity of zinc(II) accumulation by these cells [21]. This has been demonstrated on the prostate cell lines in the study by Costello et al. Authors found that ZIP1 was elevated in androgen-responsive cell line LNCaP, not in androgen nonresponsive PC-3, when exposed to testosterone [21]. One may speculate that testosterone administration may be beneficial in androgen-responsive prostate tumors. However, this topic needs further research. High levels of zinc(II) in diet leads to the reduction of the expression of this protein. In prostate cancer, the ZIP1 expression is reduced. Due to the fact that ZIP1 serves as a main zinc(II) importer, prostate tumor cells have a reduced ability to accumulate zinc(II) ions [23,43]. Reduced intracellular zinc(II) level causes a series of metabolic transformations such as increased proliferation and altering of sensitivity to apoptotic signals (Fig. 2). It is known that decreased expression of ZIP1 is not caused by deletion or mutation, but by down regulation which is caused by “Ras-responsive element-binding protein 1” (RREB-1) [44]. RREB-1 is a transcription factor containing zinc(II) finger domains with both activation and inactivation potential, whereas activation or inactivation preference is driven by promoter preference [45]. RREB-1 is activated by cascade RAS-RAF-MEK-ERK [46]. Notably, this cascade is up-regulated in prostate cancer [47]. However, RREB-1 is not the only transcription factor with ability to regulate ZIP1 expression. There have been detected other regions in ZIP1 promoter with ability to down-regulate ZIP1 expression [44]. One may speculate that restoration of the expression of ZIP1 or increase of zinc(II) levels in prostate cancer cells might be the new possible therapeutic approach in prostate cancer [25]. Cultivation of prostate cancer cell line LNCaP in medium with high zinc(II) content reduced their proliferation. Intracellular zinc(II) increase go along with increase of VHR phosphatase, ZAP-70 kinase, and p-ERK 1 and 2 [48,49]. Selective zinc(II) deficiency induced by chelator activate NF-xB causes overexpression of the proangiogenic and prometastatic cytokines VEGF, IL-6 and IL-8 in prostate cancer cell lines [50]. Similar effect was described in androgen-independent prostate cancer cell line PC-3, where addition of 50 μM of zinc(II) to medium (approx. 3 times higher compared to blood plasma level) significantly reduce the proliferation compared to PNT1A cell line derived from healthy prostate cells [51].

<table>
<thead>
<tr>
<th>Transporter (gene names)</th>
<th>Subfamily</th>
<th>Tissue localization</th>
<th>Cellular localization</th>
<th>Function</th>
<th>Association with disease</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIP1 (SLC39A1, IRT1, ZIRTL, CGI-08, CGI-71)</td>
<td>II</td>
<td>ubiquitous</td>
<td>plasma membrane</td>
<td>Zn uptake</td>
<td>decreased expression in PCs</td>
<td>[20]</td>
</tr>
<tr>
<td>ZIP2 (SLC39A2)</td>
<td>II</td>
<td>uterus, prostate, monocytes, optic nerve</td>
<td>plasma membrane</td>
<td>Zn, Co Cd, Cu, Mn</td>
<td>uptake</td>
<td>decreased expression in PCs</td>
</tr>
<tr>
<td>ZIP3 (SLC39A3)</td>
<td>II</td>
<td>ubiquitous, higher in bone marrow, spleen</td>
<td>plasma membrane</td>
<td>Zn uptake, mammary gland Zn secretion pathway</td>
<td>decreased expression in PCs</td>
<td>[55-57]</td>
</tr>
<tr>
<td>ZIP6 (SLC39A6, LIV1)</td>
<td>LIV-1</td>
<td>ubiquitous</td>
<td>plasma membrane</td>
<td>Zn uptake, positively regulated by estrogen</td>
<td>highly expressed in high-risk BCa, highly expressed in cervix ca and lung ca</td>
<td>[58,65]</td>
</tr>
<tr>
<td>ZIP7 (SLC39A7, HKE4, RING5)</td>
<td>LIV-1</td>
<td>ubiquitous</td>
<td>ER, Golgi</td>
<td>transport Zn from organelles to cytosol, Zn signalling</td>
<td>elevation in tamoxifen-resistant BCa</td>
<td>[43]</td>
</tr>
<tr>
<td>ZIP10 (SLC39A10, KIAA1265)</td>
<td>LIV-1</td>
<td>ubiquitous</td>
<td>plasma membrane</td>
<td>imports Zn to Sertoli cells and spermatocytes</td>
<td>highly expressed in high-risk BCa, reduced level associated with impaired spermatogenesis</td>
<td>[60,149]</td>
</tr>
</tbody>
</table>

Table 1. ZIP Transporters Associated with Cancers

Description of the localization and function of ZIP transporter family genes associated or expected to be associated with prostate or breast tumours. Association with cancers described with particular trend. The table was prepared according to Kambe et al. [37] and Hogstrand et al. [43]. Gene and protein names and variants according to uniprot.org. Abbreviations: PCs – prostate cancer, BCa – breast cancer, ca – cancer.
product. ZIP3 is ubiquitously localized, however higher expression was found in bone marrow and spleen and lower levels were in small intestine and liver [55]. This transporter plays important role in mammary gland in secretion of zinc(II) into the milk [56]. Like ZIP1 and ZIP2, its expression is also decreased in prostate carcinoma [43,57].

Another ZIP transporter, ZIP6, alternatively named Estrogen-regulated protein LIV-1 (LIV1) or Solute carrier family 39 member 6 (SLC39A6) is a 755 amino acid protein localized on a cell membrane. ZIP6 is highly expressed in the breast, prostate, placenta, kidney, pituitary and corpus callosum and weakly expressed in heart and intestine [58]. Above that, elevated expression of ZIP6, together with ZIP7 and ZIP10 were demonstrated in the high-risk breast cancer with metastatic potential [43,59-61]. In addition, ZIP6 is positively regulated by estrogen suggesting that aberrant estrogen receptor signaling might modulate zinc(II) metabolism [59,62].

ZIP7 (also called Histidine-rich membrane protein Ke4 or Solute carrier family 39 member 7) is a 496 amino acid protein. Although ZIP7 is localized ubiquitously, elevated expression was described in placenta, lung, kidney and pancreas [58]. ZIP7 transporter is localized on the endoplasmic reticulum (ER) membrane [63], which may serve as reservoir of intracellular zinc(II) [43]. ZIP7 has, thus, an important role in zinc signaling. Release of zinc(II) from ER induces zinc-based signals with following signal transduction via protein-tyrosine phosphatases signaling [43]. Based on this mechanism, antiapoptotic or cell proliferation mechanisms can be influenced. Tamoxifen-resistant breast cancer cells has increased intracellular zinc(II) levels compared with sensitive cancer cells. This observation corresponds with the high expression of ZIP7 and epidermal growth factor receptor (EGFR) activation that is sign of worse prognosis [64,65]. Together with the finding that elevated expression of ZIP7 was found in high risk breast cancer one may suggest that ZIP7 play important role in its pathogenesis.

ZIP10 (SLC39A10) is 831 amino acid protein. Its high levels of expression are correlated with invasiveness in several breast cancer lines. Significantly higher levels were found in MDA-MB-231 and MDA-MB-435S breast cancer cell lines with high metastatic potential compared to MCF7, T47D, ZR75-1 and ZR75-30 breast cancer cell lines with lower metastatic potential [60]. Expression of ZIP10 mRNA in breast cancer samples positively correlated with the presence of node metastasis [60]. These data suggest that ZIP10 is a potential marker of breast cancer invasivity and potential target of therapeutic strategy [60]. In conclusion ZIP 6, 7 and 10 are overexpressed in breast cancer particularly in those with high metastatic potential and ZIP 1 and 2 are downregulated in prostate cancer.

2.1.2. ZnT Transporter Family

This transporter family, also called “cation diffusion facilitator” (CDF), takes the opposite function compared to ZIP transporters.

Table 2. ZnT Transporters Associated with Cancers

<table>
<thead>
<tr>
<th>Transporter (gene names)</th>
<th>Subfamily</th>
<th>Tissue localization</th>
<th>Cellular localization</th>
<th>Function</th>
<th>Association with disease</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnT-1 (SLC30A1)</td>
<td>II</td>
<td>ubiquitous</td>
<td>plasma membrane</td>
<td>Zn removal from the cytoplasm</td>
<td>decreased in AD, increased in lung ca</td>
<td>[67,68,70]</td>
</tr>
<tr>
<td>ZnT-2 (SLC30A2)</td>
<td>III</td>
<td>small intestine, kidney, testes, placenta, prostate, mammary gland</td>
<td>endosomes</td>
<td>transport Zn vesicles and lysosomes; Zn secretory pathway in prostate, breast</td>
<td>role in PCa</td>
<td>[75,150,151 109]</td>
</tr>
<tr>
<td>ZnT-4 (SLC30A4)</td>
<td>III</td>
<td>ubiquitous, especially mammary gland</td>
<td>endosome, Golgi apparatus</td>
<td>transport Zn to vesicles, Zn secretion pathway in breast</td>
<td>increased in AD, lung ca</td>
<td>[70]</td>
</tr>
</tbody>
</table>

Description of the tissue and cellular localization and function of ZnT transporter family genes associated or expected to be associated with prostate or breast tumours. Association with cancers described with particular trend. The table was prepared according to Kambe et al. [37] and Hogsstrand et al. [43]. Gene and protein names and variants according to uniprot.org. Abbreviations: AD – Alzheimer’s disease, ca – cancer.
2.2. Thiol Compounds

Metallothioneins are a group of mostly intracellular proteins belonging to the metalloprotein family. They are widespread in the animals, however, similar types of proteins have been found in bacteria, fungi and plants. Mammalians metallothioneins were firstly found in horse kidney by Margoshes and Valleé in 1957 [76]. They are low molecular weight (app. 6 kDa) and consist of 60–62 amino acids. In organism, metallothioneins occur in four isozymes; MT-1, MT-2, MT-3 and MT-4 [77]. MTs have atypical primary structure with lack of aromatic amino acids and with un-obviously high content of amino acids, because this amino acid is almost one third of all amino acids. Due to cysteine residues, metallothioneins have an ability to bind metals. One molecule of metallothionein consists of two domains, α and β, whereas both of these include cysteine clusters capable to bind up to 7 divalent or 12 monovalent ions [19,78]. Experiments on MT found significant differences in zinc-binding affinities of cysteine clusters [79]. Affinity varies in nanomolar to picomolar range of zinc(II) concentration, i.e. in at least three orders of magnitude [16]. In addition, there were published papers studying the influence of oxidation on polymerizing of metallothionein and its capacity to bind zinc(II) ions [80,81].

Metallothioneins play a key role in metabolism, transport and storage of heavy metals, particularly zinc(II) [16,82]. When zinc(II) ions get into a cytosol through zinc transporters, it is immediately buffered by metallothioneins, thus the free zinc(II) ion level is maintained on very low level, in picomolar to nanomolar range [16]. Due to signaling roles of free zinc(II) ions, metallothioneins play an important role in this process because of its level regulation (Fig. 1). Whether zinc(II) level exceeds the buffering capacity of metallothioneins, it is being eliminated out of cells by ZnT-1 exporter and subsequently, zinc(II) induces expression of metallothionein and ZnT-1 by binding to transcription factor MTF-1 that binds to metal responsive elements (MREs) which regulate metallothionein expression (Fig. 1).

Metallothioneins also protect cells against oxidative stress [82], because they cooperate with reduced glutathione (GSH) [83]. Due to these features it is not surprising that metallothioneins are overexpressed under conditions with increased risk of reactive oxygen species formation such as cell proliferation or embryonic development [83-86]. A lot of recent studies indicate elevated serum levels of metallothioneins in number of malignancies such as breast, bronchial, urogenital, colorectal, prostate carcinomas, melanoma and several lymphoma [82,87-91]. In prostate carcinoma, metallothionein serum levels are up to three times higher compared to healthy controls [83,92]. Metallothioneins that are detected by immunohistochemistry are localized in the nuclei of epithelial cells in a benign prostatic lesion, whereas in the adenocarcinoma they occurred mainly in the cytoplasm [82]. Methods for detection and determination of metallothioneins were recently reviewed [93,94].

As a consequence of higher zinc(II) content in breast cancer cells, metallothionein mRNA and protein levels are significantly increased [61]. Moreover, metallothionein level is positively correlated with more aggressive and higher-grade tumors [64]. Metallothioneins seem to be possible tumor markers in some tumors including breast cancer. More studies are needed to explore effects of anti-hormone therapy on zinc(II) transporter regulation, because these processes are endocrinologically regulated and hormonal therapy is the conventional treatment of breast cancer that express hormonal receptors [59].

Although the cause and the mechanism is not clear, it is considered that its increased level is responsible for protecting cancer cells from apoptosis, for increasing proliferation and ability to metastasize [82]. This finding leads to several outputs (Fig. 2). (A) If the serum level of metallothionein is elevated in the malignancies with the highest possible sensitivity, metallothionein could be used as a new tumor marker after further research, because many questions are still unanswered e.g. how metallothionein levels correlate with age, or how MT levels can be influenced by viral or bacterial infection, or how MT levels change with physical activity. (B) Elevated serum level reflects altered metallothionein metabolism of tumor. Zinc(II) fluxes are tightly bound with metallothioneins, also zinc(II) metabolism and zinc(II) transport mechanisms are altered in cancer patients [95]. Metallothioneins also play important role in resistance to cytostatic drugs, particularly platinum compounds. Transfer of platinum from cisplatin or carboplatin to MTs result in inactivation of these drugs [96]. Thus, pre-treatment of zinc(II) results in MT induction and thus reduction of cisplatin or carboplatin cytotoxicity [97] [82]. Therefore, targeted pharmacological inhibition of MTs may reverse resistance to such cytostatics. These findings however need to be confirmed on large data set.

2.3. Molecular Biology of Zinc(II)

Zinc(II) as the only transition metal lacking redox activity is an essential part of app. 10 % human proteins [1,2]. Zinc(II) acts mainly as a cofactor of these proteins. Due to great portion of the zinc-dependent proteins it is not surprising that zinc(II) is involved (through these proteins) in numerous key intra- and extracellular processes including proliferation, differentiation and apoptosis. Zinc-dependent enzymes can be found in all classes of enzymes, i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases [16-98]. These enzymes include so-called zinc fingers domains, repetitive sequences with ability to bind zinc(II) ions. Those domains consist of mostly histidine and/or cysteine. Zinc fingers are able to form complex with DNA based on interactions between α-helix of the zinc finger and DNA specific bases [99]. Function of zinc fingers consists not only in DNA recognition and transcriptional activation, but also in RNA packaging, protein folding and apoptosis, which regulation is important not only in development of tissues, but also in neoplastic transformation and proliferation [3,4].

Zinc(II) is necessary for all stages of cell cycle. Its higher level was described during G1 phase and G1/S phase transition [100]. There was identified influence of MT-1 on ATM/Chk2/cdc25A pathway during the G1/S phase transition [101]. Furthermore, induction of cyclin D3 and E is zinc-dependent, therefore, zinc(II) is required for proper S phase process and consequent G2/M transition [102]. It is expected, that high levels of zinc are required for nuclear functions during the early stage of differentiation of some cell systems [17].

Zinc(II) also play important role in regulation of apoptosis. Effects of zinc on apoptosis are cell specific and very complex. Depending on type of cells, concentration of other compounds and conditions zinc(II) ions can cause and/or counteract apoptotic effects [18]. Apoptotic effects of zinc(II) can be direct, through influence on nucleus/mitochondria, or indirect, through modulating cellular apoptotic signaling factors/pathways [18]. In general, zinc(II) acts as an antiapoptotic agent. It was demonstrated that zinc(II) deficiency induces apoptosis. Antiapoptotic properties of zinc(II) manifest in two ways [103]. First, it protects cells against oxidative damage [104]. Second, it directly inhibits activation of caspase 3 [105,106]. Its antioxidative properties may be mediated through activation of MTF-1 (and thus elevation of MTs and GSH) [19,33,35] and most likely also through activation of Cu/Zn superoxide dismutase (Cu/Zn SOD), a primary defence system involved in the antioxidant response [107-109]. On the other hand, zinc(II) may act as a pro-apoptotic agent in prostatic and neuronal cells [18,110,111]. High zinc(II) content affects apoptosis through the synthesis and formation of BAX pores on the outer mitochondrial membrane (Fig. 1). Cytochrome-C can further be
released from mitochondria and can trigger cascade of caspases resulting in apoptosis [111].

In terms of apoptosis and proliferation of prostate cancer cells, due to decreased zinc(II) level, BAX-Cytochrome C apoptotic cascade is significantly silenced [18]. In addition, overexpression of Bel-2 was observed. Antiapoptotic gene Bel-2 is overexpressed in 30–60% of prostate cancers within the luminal epithilium in high-grade PIN lesions, but is absent in most low-to-intermediate grade carcinomas [112,113]. Bel-2 inhibits caspase activity either by preventing the release of Cytochrome C from the mitochondria and/or by binding to the apoptosis-activating factor (APAF-1).

Compared to healthy prostate as a result of down-regulation of zinc(II) in prostate cancer cells, apoptotic/antiapoptotic balance and energetic metabolism is changed in favor of the reduction of apoptosis [25]. Furthermore, because zinc(II) level is decreased, the inhibitory effect of zinc(II) on aconitase is abolished, citrate can enter the Krebs cycle, and cancer cells can then become more energy-efficient compared to healthy “energy inefficient” prostate cells [25]. This energetic turnover can also support prostate cancer cells growth (Fig. 2). If so, it should lead to a speculation that the resumption of the inhibitory effect on mitochondrial aconitase by some inhibitors e.g. fluoroacetate (which is metabolized into fluorocitrate) may have tumor-suppressor effects. Unfortunately, fluoroacetate is not specific for prostate cells and therefore is toxic to most mammalian cells [25].

Furthermore, zinc(II) has a role as a signaling compound. It was demonstrated that concentration of free cytosolic zinc(II) ions could be increased by various intracellular or extracellular stimuli e.g. by reactive oxygen species or nitric oxide [37,43,114,115]. A pool of zinc(II) ions is released from cellular organelles such as endoplasmic reticulum, Golgi’s apparatus or special vesicles -- “zincosomes” [17,43]. Zincosomes play most likely role in endocytosis of zinc(II), however, their nature is not completely clear to date and needs further research [116]. Free zinc(II) level thereby fluctuates and has therefore downstream effects on cell signaling. All levels of signaling can be affected, from receptor level, through second messengers to transcription factors. Zinc(II) also interferes with the signaling cascades of cyclic nucleotides, tyrosine kinases and protein kinase C [117]. When dysregulated in cancers, activation of various oncogenic genes, e.g., Fos, Akt1, Jak3 and PI3K, was described [118].

3. WHOLE BODY ZINC(II) STATUS

3.1. Blood Plasma Level

Blood plasma zinc(II) concentration ranges within 12–20 μM [23,119,120]. In blood plasma, most of zinc(II) is bound with low affinity to albumin (approx. 60%). Approx. 10% is bound to transferrin and the remainder forms free fraction [121]. Albumin plays important role in blood plasma zinc(II) “buffering” [122]. It participates in the transport of newly absorbed zinc(II) from intestine to liver, it assist the transfer of zinc(II) to target sites [123], it promotes zinc(II) uptake by endothelial cells [124]. However, mechanisms of zinc(II) transfer from albumin are not completely understood. To date, only limited evidence on albumin zinc(II) binding sites exists [122].

Whereas zinc(II) is particularly intracellular ion, its serum level does not reflect total zinc status in humans [123]. However, it has been demonstrated in number of studies, that serum zinc(II) significantly differs in patients suffering from cancer. In United States Mortality Study of the Second national Health and Nutrition Examination Survey (NHANES II) was performed on 6,244 participants with complete history data. Except others, risk of serum zinc(II) and other metals was analyzed in terms of overall cancer mortality. It was concluded that the relation of serum zinc(II) and cancer mortality is nonlinear and the risk is significantly reduced for people with serum zinc between 13.2 to 15.8 μM compared to subjects with serum zinc 10.7 to 13.0 μM. This protective trend is more significant in men compared to women (RR = 0.56 for men vs. 0.79 for women) [125]. Based on similar follow-up study performed in Europe on 4,035 participants, subjects with a combination of low serum zinc(II) and high copper(II) values had increased cancer mortality risks [120].

In terms of serum zinc(II) level in prostate cancer patients, studies performed by Adaramoye et al. and Goel et al. revealed significantly lower (p < 0.05) level in patients of all PSA levels [126,127]. Similar reduction was observed in group of 41 participants using whole blood analysis of zinc(II) level [128]. However, Park et al. did not observe any difference and association between serum zinc level and prostate cancer risk in cancer and control group of 1,175 US participants [129]. In terms of serum level in breast cancer patients, decrease in zinc(II) was described by Borella et al. [130]. Gupta et al. found similar results, however, in the advanced stages of disease only [131]. Significant reduction of whole blood zinc(II) level was furthermore observed by Memon et al. on the 80 participants [132].

3.2. Zinc(II) in Diet

In terms of inadequate dietary zinc(II) income, on average, daily zinc(II) intake varies within the range from 8 to 14 mg/day in developed countries, and slightly less in developing countries, app. 5–11 mg/day [115]. The usually recommended zinc(II) intake is 15 mg/day [133], while its upper limit of daily intake is around 50 mg [134]. According to results from epidemiological study National Health and Nutrition Examination Study (NHANES III), zinc(II) intake may be critical in older population [135]. Based on more recent study, “Zinc and Health: Current Status and Future Directions” the zinc(II) intake is significantly inadequate in 12% of Americans [136] and one may speculate that similar situation is in other developed countries. Deficiency in such a daily dose, leading to a serum zinc(II) concentration is less than 10 μM [119], Zinc(II) deficiency could disrupt the function of both signaling molecules and proteins directly involved in DNA replication and repair [137,139]. Moreover, zinc(II) affect function of anti-tumor gene p53 – as demonstrated in the rat glioma C6 cells – zinc(II) deficiency up-regulates expression of p53. Furthermore, low intracellular zinc(II) impairs DNA-binding ability of p53, NF-kB and activator protein-1 transcription factor (AP-1) [140,141]. Zinc(II) deficiency may support tumorigenesis also through angiogenesis: endostatin, an angiogenesis inhibitor, require zinc(II) to be bound for its activity. In conditions of zinc(II) deficiency, its antiangiogenic potential is significantly reduced [142]. Such conditions may contribute to the development of cancer. More specifically, in terms of dietary zinc(II) intake and prostate cancer risk, several studies were performed. Kristal et al. found protective effect of zinc(II) supplementation on 697 US participants (relative risk 0.55 in higher zinc intake group) [143]. Conversely, based on a study performed on 46,974 US men participating in the Health Professionals Follow-Up Study during 14 years of follow-up from 1986 through 2000, no significant risk of risk prostate cancer was observed (relative risk 2.29 at p < 0.003) in patients with intake higher than 100 mg/day. However, significant increase of risk prostate cancer was observed (relative risk 2.37 p < 0.001) in patients with intake more than 100 mg/day. Similarly, men taking supplemental zinc for 10 or more years had a relative risk of 2.37 (p < 0.001) [144]. Although results are not consistent, data suggest, that there is an ambivalent effect of zinc supplementation: In recommended daily dosage 15 mg/day there might be a protective effect [133]. However, in tenfold higher dosage (higher than 150 mg/day) zinc(II) can promote tumorigenesis. In breast cancer, study performed by Adzersen et al. in Germany on 653 participants concluded significantly reduced breast cancer risk (Relative risk 0.35 at p = 0.01) in patients with dietary zinc(II) income higher than 13.2 mg/day [145]. However, short-term high levels of zinc(II) are
relatively non-toxic compared to other metals. Many effects of so-called “zinc toxicity” are mostly due to the interference of zinc(II)-copper(II) metabolism, which results in copper(II) deficiency [146]. This phenomenon is most likely due to a competitive mechanism in enterocytes with participation of metal-binding protein metallothionein (MT). Due to competition between zinc(II) and copper(II) and displacing of copper(II) by zinc(II) ions, MT forms with copper(II) complexes which are excreted. Therefore, long-term overdosing with zinc(II) causes reduced absorption of copper [146]. Copper deficiency can then cause, among others, decreased activities of superoxide dismutase and cytochrome-c [147,148]. Hence, studies describing an increased incidence of prostate cancer when long-term over-supplemented with zinc(II) are more likely related with secondary copper deficiency rather than with primary effects of zinc(II).

4. SUMMARY AND CONCLUSIONS

According to recent studies, zinc(II) ions are not only a passive structural component of different cellular compartments, but also have regulatory effects on important cellular and subcellular processes such as regulation of proliferation, differentiation, apoptosis, neurotransmission and many others. The regulation of zinc(II) level is therefore important and its aberrancies may lead to various diseases, particularly cancers. Zinc(II) fluxes are tightly bound with metallothionein and together with finding that both compounds have effects on apoptosis and proliferation, it is naturally predictable that the misbalance of their regulation could affect cancer pathogenesis. However, many questions still exist. Currently, it is known that zinc(II) misbalance in some cancers, particularly in prostate cancer, is caused by alteration of ZIP1 transport mechanism. It is known that RREB-1 down-regulates ZIP1 expression in prostate cancer. However, also other potential pathways for ZIP1 down-regulation in prostate cancer cells may exist. This condition is most likely reversible and, therefore, this pathway may have therapeutic effect on prostate cancer. However, even if ZIP1 play a key role in zinc(II) intake, also other transporters’ expression is somehow altered. Therefore, this prostate cancer-specific zinc(II) dysregulation is still not fully clarified and needs further research that leads to a more complex view on zinc(II) fluxes.

Similarly, only a little is known about zinc(II) status in breast cancer. ZIP6, 7 and 10 are identified to be highly expressed in high-risk forms of breast cancer. It has been also demonstrated that ZIP6 is positively regulated by estrogens and depletion of ZIP10 transporter inhibits the migration of highly metastatic breast cancer [60]. These data suggest a potential path to regulate metastatic potential of breast cancer. Further objectives are needed to be focused on (A) identifying of all other mechanisms leading to zinc(II) deregulation, (B) finding other mechanisms leading to zinc/metallothionein misbalance, (C) detailed identification of all effects on prostate and breast cancer pathogenesis. One may speculate that restoring of the expression of these transporters to the physiological levels and understanding of complex zinc(II) effects may lead to new, targeted therapy and prevention.

ACKNOWLEDGEMENTS

We highly acknowledge the support from grants GACR 301/09/P436, IGA MZ NS 10200-3, GA AV IAA40199070, NANOSEMED GA AV KAN208130801 and CEFETEC CZ.1.05/1.1.00/02.0068.

ABBREVIATIONS
AD = Alzheimer’s disease
ATZ = 3’-azido-3’-deoxythymidine

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Current Medicinal Chemistry, 2011 Vol. 18, No. 33 5049


