Zinc Deficiency and Epigenetics

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Abstract

Zinc (Zn) is an essential micronutrient element. This element in relation with the structure and function of many proteins and enzymes is important for a variety of biological activities, including epigenetic regulations. Zinc deficiency is common in many parts of the world and particularly in poor populations. Accumulating evidence has demonstrated that several key enzymes and zinc finger proteins with zinc atom(s) in the reactive center and binding site play important roles in DNA methylation and histone modifications. Therefore, zinc deficiency may disrupt the functions of these enzymes and proteins and result in epigenetic dysregulation. Furthermore, zinc deficiency may enhance inflammatory response and subsequently alter DNA methylation status of the genes involved in inflammation. In this chapter, we first describe zinc dietary sources and deficiency, and then discuss direct and indirect effects of zinc deficiency in DNA and chromatin methylation alteration. Finally, we prospect a new zinc biomarker and further investigation on the effects of zinc deficiency in epigenetics.

Keywords

Betaine homocysteine methyltransferase • DNA methylation • Epigenetics • Histone modification • Methionine synthase • Oocyte epigenetic programming •

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Zinc • Zinc deficiency • Zn-dependent methyltransferases • Zinc finger proteins • Zinc food sources

List of Abbreviation

AI	adequate intake
BHMT	betaine homocysteine methyltransferase
DGLA	dihomo-γ-linolenic acid
dTMP	thymidylate monophosphate
DV	daily value of foods
FAO	food and agriculture organization
IL	interleukin
LA	linoleic acid
MTR	methionine synthase
RDA	recommended dietary allowance
RNI	recommended nutrient intake
SAMe	S-adenosyl methionine
SLC	solute-linked carrier
WHO	World Health Organization
ZFP	zinc finger protein

Contents

Introduction

Zinc is a chemical element with the symbol Zn^{2+} and atomic number 30. This chemical element is present in all body tissues and fluids as an essential component for approximately 1000 proteins (Dreosti 2001; Ho 2004; Chasapis et al. 2012). Of them, more than 300 enzymes participate in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients (Frassinetti et al. 2006; Prasad 2012, 2013). Except these enzymes, the rest mainly includes the proteins with a zinc atom in the reactive center and zinc finger proteins (ZFPs) (Laity et al. 2001). Therefore, zinc, in relation with the structures and functions of these proteins, is of importance in a variety of biological activities such as apoptosis, signal transduction, transcription, differentiation, and replication in all organ

systems and during embryonic development. Since zinc is involved in such fundamental and extensive biological activities, it most likely accounts for the essentiality of zinc for all life forms.

As an essential mineral, zinc has been perceived by the public as being of "exceptional biologic and public health importance." However, many peoples particularly in the developing countries consume less than the recommended nutrient intakes (RNIs) for dietary zinc (WHO and FAO 2004). Epidemiological study has reported that zinc deficiency affects about 2.2 billion people around the world and has been ranked 11th among global risk factors for mortality and 12th for burden of disease (Lopez et al. 2006). Clinical observation has demonstrated that zinc deficiency is associated with pathologic changes in many diseases (Frassinetti et al. 2006; Prasad 2012, 2013). Dysregulation of epigenetics due to zinc deficiency may be involved in the pathogenesis of the diseases.

Epigenetics is involved in many cellular processes. Within cells, there are three systems that can interact with each other to silence genes: DNA methylation, histone modifications, and noncoding RNA-associated silencing (Du et al. 2015). Zinc has been found to affect the activities of some key enzymes such as methionine synthase (MTR, also known as MS) and betaine homocysteine methyltransferase (BHMT) in the reaction of DNA methylation (Castro et al. 2008; Jing et al. 2015). ZFPs are the most abundant proteins in eukaryotes and fundamentally contribute to multiple layers of epigenetic regulation such as DNA methylation and histone modifications (Laity et al. 2001; Shimbo and Wade 2016). Furthermore, zinc homeostasis is involved in the immune cell signaling and activation. Zinc deficiency may enhance inflammatory response and subsequently alter DNA methylation status of the genes involved in the induction of a pro-inflammatory response (Wong et al. 2015). In this chapter, we briefly describe zinc dietary sources and deficiency, and then intensively discuss direct and indirect influences of zinc deficiency in DNA methylation changes. Finally, we prospect a new zinc biomarker and further investigation on the effects of zinc deficiency in epigenetics.

Zinc Food Sources and Deficiency

Zinc deficiency can occur not only in humans but also in soil, plants, and animals. In general, zinc deficiency is defined either qualitatively as insufficient zinc to meet the requirements of the body and thereby causing clinical manifestations or quantitatively as a serum zinc level below the normal range. In humans, the most common cause of zinc deficiency is reduced dietary intake, while other reasons include inadequate absorption, increased loss, or increased use (Wessells and Brown 2012; Wessells et al. 2012). Therefore, it is necessary to know the sources of zinc from foods.

A wide variety of foods contains zinc and the selected food sources of zinc are listed in Table 1. To help the consumers for comparison of the nutrient contents of products within the context of a total diet, the daily values of foods (DVs) in this table have been developed by the U.S. Food and Drug Administration. Oysters

Table 1 Selected food sources of zinc

Food	Mg per serving	Percent DV
Oysters, cooked, breaded, and fried, 85.05 g	74.0	493
Beef chuck roast, braised, 85.05 g	7.0	47
Crab, Alaska king, cooked, 85.05 g	6.5	43
Beef patty, broiled, 85.05 g	5.3	35
Breakfast cereal, fortified with 25% of the DV for zinc, ¾ cup serving	3.8	25
Lobster, cooked, 85.05 g	3.4	23
Pork chop, loin, cooked, 85.05 g	2.9	19
Baked beans, canned, plain or vegetarian, ½ cup	2.9	19
Chicken, dark meat, cooked, 85.05 g	2.4	16
Yogurt, fruit, low fat, 226.80 g	1.7	11
Cashews, dry roasted, 28.35 g	1.6	11
Chickpeas, cooked, ½ cup	1.3	9
Cheese, Swiss, 28.35 g	1.2	8
Oatmeal, instant, plain, prepared with water, 1 packet	1.1	7
Milk, low-fat or non-fat, 1 cup	1.0	7
Almonds, dry roasted, 28.35 g	0.9	6
Kidney beans, cooked, ½ cup	0.9	6
Chicken breast, roasted, skin removed, ½ breast	0.9	6
Cheese, cheddar or mozzarella, 28.35 g	0.9	6
Peas, green, frozen, cooked, ½ cup	0.5	3
Flounder or sole, cooked, 85.05 g	0.3	2

Mg milligrams; *DV* daily value. DVs were developed by the U.S. Food and Drug Administration to help the consumers for comparison of the nutrient contents of products within the context of a total diet

contain more zinc per serving than any other food. Oysters are unusual and delicious mollusks that provide the human body with a number of unique nutrients and minerals, particularly zinc (Murphy et al. 1975). The edible components are the meat inside the oyster, and once the shells have been cracked, we can cook this meat in a variety of ways, but they can also be eaten raw. The valves in oysters can actually cleanse entire ecosystems of pollutants and are a major benefit to the environment. In recent years, however, the oyster population of the world has dropped significantly, resulting in weaker overall ecosystems in the areas where oysters once flourished (Páez-Osuna et al. 2002; Lacerda and Molisani 2006). Red meat and poultry provide the majority of zinc in the diet. Other food sources, including beans, nuts, certain types of seafood (such as crab and lobster), whole grains, fortified breakfast cereals, and dairy products, are also good (WHO and FAO 2004). Comparatively, the bioavailability of zinc from animal foods is higher than that from grains and plant foods, because phytates are present in whole-grain breads, cereals, legumes, and other foods from plants and bind zinc and inhibit its absorption in foods (Wise 1995; Sandstrom 1997).

Table 2 RDAs for dietary zinc (mg/day) and the normative storage requirements from diets differing in zinc bioavailability

Group	Age	Assumed body weight (kg)	High bioavailability	Moderate bioavailability	Low bioavailability
Infants and children	0–6 months	6	l.l[a]	2.8[b]	6.6[C]
	7–12 months	9	0.8[a], 2.5[d]	4.1	8.4
	1–3 years	12	2.4	4.1	8.3
	4–6 years	17	2.9	4.8	9.6
	7–9 years	25	3.3	5.6	11.2
Adolescents	10–18 years (F)	47	4.3	7.2	14.4
	10–18 years (M)	49	5.1	8.6	17.1
Adults	19–65 years (F)	55	3.0	4.9	9.8
	19–65 years (M)	65	4.2	7.0	14.0
	65+ years (F)	55	3.0	4.9	9.8
	65+ years (M)	65	4.2	7.0	14.0
Pregnant women	First trimester	–	3.4	5.5	11.0
	Second trimester	–	4.2	7.0	14.0
	Third trimester	–	6.0	10.0	20.0
Lactating women	0–3 months	–	5.8	9.5	19.0
	3–6 months	–	5.3	8.8	17.5
	6–12 months	–	4.3	7.2	14.4

Source: Adapted from WHO and FAO 2004. Unless otherwise specified, the interindividual variation of zinc requirements is assumed to be 25%. Weight data interpolated from FAO, *Food and Nutrition Series,* No. 23, 1988). RNIs: recommended nutrient intakes; *Mg*: milligrams; *Kg*: kilograms; *F*: females; *M*: males; a: Exclusively human-milk-fed infants. The bioavailability of zinc from human milk is assumed to be 80%; assumed co-efficient of variation 12.5%. b: Formula-fed infants. Applies to infants fed whey-adjusted milk formula and to infants partly human-milk-fed or given low-phytate feeds supplemented with other liquid milks; assumed coefficient of variation 12.5%. c: Formula-fed infants. Applicable to infants fed a phytate-rich vegetable protein-based formula with or without whole-grain cereals; assumed coefficient of variation 12.5%. d: Not applicable to infants consuming human milk only

The current recommended dietary allowance (RDA) is the average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals. The RDAs for zinc are summarized in Table 2. For infants aged

0–6 months, the food nutrition board at the Institute of Medicine of the National Academies established an adequate intake (AI) that is equivalent to the mean intake of zinc in healthy, breastfed infants (Institute of Medicine 2001; WHO and FAO 2004; Ackland and Michalczyk 2016). AI is generally established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy.

One previous report has demonstrated that zinc deficiency affects about 2.2 billion people around the world and is often caused due to poor diet consumption (Prasad 2001). Another recent study based upon on WHO growth standards has indicated that an estimated 17.3% of the world's population is at risk of inadequate zinc intake (Wessells et al. 2012). Country-specific estimated prevalence of inadequate zinc intake is negatively correlated with the total energy and zinc contents of the national food supply and the percent of zinc obtained from animal source foods (Wessells and Brown 2012; Wessells et al. 2012). Pregnant and nursing women are considered at higher risk of zinc deficiency, as are those with gut problems, babies born prematurely, or those who have consumed a high-grain or vegetarian diet (especially for a long period of time) (Ackland and Michalczyk 2016). The symptoms of zinc deficiency can vary and is often associated with the following problems: poor memory, weakened immune system or constant minor illnesses like colds, loss of taste or smell, sleep problems (zinc is needed to make melatonin), hair loss, loss of appetite, low libido, diarrhea, brain fog, slow wound healing, white spots on fingernails, and growth retardation in children (WHO and FAO 2004). However, most severe symptoms of zinc deficiency result from other factors including excessive alcohol use, liver diseases, malabsorption syndromes, renal disease, enteral or parenteral alimentation, administration of sulfhydryl-containing drugs, and sickle cell diseases (Evans 1986). Moreover, zinc deficiency is an important factor in the development and progression of cancers (Dhawan and Chadha 2010; Gumulec et al. 2011; Sharif et al. 2012**)** and metabolic disorders (Lin and Huang 2015; Wilson et al. 2016; Grüngreiff et al. 2016). Recent studies have provided evidence that zinc deficiency and zinc transports are involved in the pathogenesis of diabetes and diabetic complications (Gu 2015; Zhang et al. 2016a; Maret 2017). Taking together, the symptoms resulting from zinc deficiency are as diverse as the enzymes with which the element is associated. Although clinical observation of the symptoms caused by zinc deficiency is well documented, our knowledge concerning the mechanisms of zinc deficiency in relation with epigenetics is still limited. Herein, we mainly discuss the proteins, which are related with zinc in biological structure and function and possible problems in epigenetics caused by zinc deficiency.

Zinc Deficiency and the Oocyte Epigenetic Programming

It is well known that human pregnancy outcome is significantly influenced by the nutritional status of the mother and an optimal uterine environment. Quality of the maternal epigenome significantly contributes to promoting optimal embryonic development and postnatal health (Corry et al. 2009; Hales et al. 2011).

The epigenome of the oocyte is dramatically remodeled during oogenesis. As the oocyte nears ovulation, major changes in chromatin structure and biochemistry take place to prepare for fertilization and embryonic development (Debey et al. 1993; Zuccotti et al. 1995). Chromatin methylation is an important component of epigenetic programming during oogenesis.

Zinc deficiency during pregnancy causes abnormal embryo and fetal development and poor progeny health (Apgar 1985; Keen et al. 2003; Uriu-Adams and Keen, 2010). Several studies have implicated that zinc is an important factor necessary for regulating the meiotic cell cycle and ovulation (Kim et al., 2010; Bernhardt et al. 2011; Tian and Diaz, 2013). To investigate the effects of acute in vivo zinc deficiency before ovulation on oocyte epigenetic programming and embryonic development, Tian and Diaz (2013) have developed an animal model with zinc deficiency. Newly weaned 18-day-old female CD1 mice were given the zinc-deficient diet (zinc omitted from the mineral mix <1 mg zinc/kg), while the control mice have the diet of 29 mg zinc/kg. Diets are given for 3 or 5 days before ovulation. Results demonstrate that feeding a zinc-deficient diet (ZDD) for 3–5 days before ovulation (preconception) dramatically disrupts oocyte chromatin methylation and preimplantation development. Histone H3K4 trimethylation and global DNA methylation in zinc-deficient oocytes are significantly decreased. Furthermore, the H3K4 trimethylation can be restored by using supplementation of a methyl donor (s-adenosylmethionine) during in vitro maturation in oocytes from zinc-deficient mice. Methyl donor supplementation partially restores fertilization potential of zinc-deficient oocytes (Tian and Diaz 2013). Therefore, this study provides evidence that zinc deficiency leads to decreased oocyte chromatin methylation, and implicates that oocyte epigenetic programming during the period of final oocyte growth is important to perturbation in whole body zinc homeostasis.

Zinc Deficiency and the DNA Methylation Pathway

DNA methylation is one of epigenetic marks that regulates gene expression and suppresses transposon activity. The DNA methylation pathway is a process by which carbons are added onto folic acid from amino acids and redistributed onto other compounds throughout the body. Figure 1 illustrates the homocysteine recycling for DNA methylation. In the cycle, several key enzymes, including MTR and BHMT, are responsible for the formation of methionine, S-adenosyl methionine (SAMe), and thymidylate monophosphate (dTMP), and subsequently facilitate virtually every DNA methylation reaction in the body (Matthews and Goulding 1997; Smith and Denu 2009; Shimbo and Wade 2016). Zinc deficiency may disrupt their biological activities and consequently result in the decreased DNA methylation levels.

BHMT is mainly present in kidneys and liver (http://www.genecards.org/cgi-bin/carddisp.pl?gene=BHMT&keywords=BHMT). This enzyme and BHMT2 are the only enzymes that can catalyze the conversion of betaine and homocysteine to dimethylglycine and methionine, respectively. This reaction is considered the alternate or short route for methylation. BHMT uses zinc as a cofactor to catalyze the

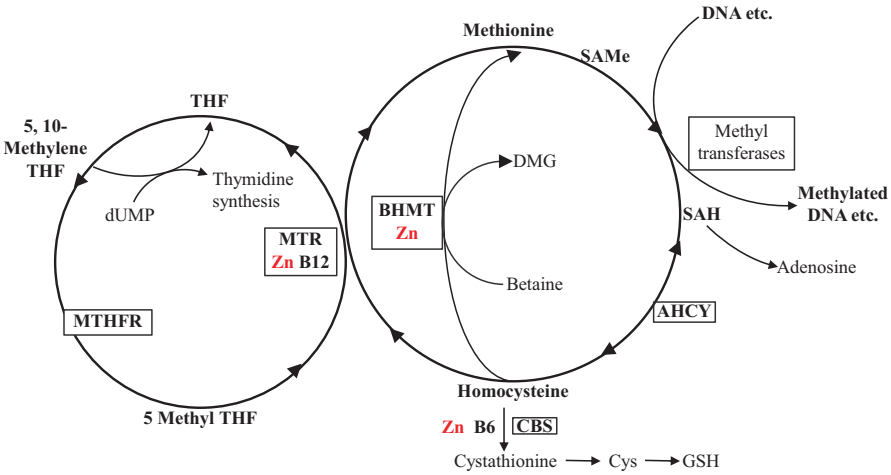

Fig. 1 Homocysteine recycling: involvement of zinc in MTR and BHMT for DNA methylation. This figure illustrates that methionine synthase (*MTR*) and betaine homocysteine methyltransferase (*BHMT*) are responsible for the formation of methionine, S-adenosyl methionine (*SAMe*), and thymidylate monophosphate (*dTMP*), and subsequently facilitate virtually every DNA methylation reaction in the homocysteine recycling. These two key enzymes are zinc-dependent methyltransferases. Zinc deficiency may disrupt their biological activities and consequently result in the decreased DNA methylation levels

transfer of a methyl group from betaine to homocysteine. Several studies have demonstrated that mutations in the BHMT gene may decrease the activity of this enzyme in the homocysteine recycling and DNA methylation. BHMT mutations in mothers may increase the risk of Down syndrome for their children. BHMT mutations may result in fatty liver and hepatocellular carcinomas (Matthews and Goulding 1997; Castro et al. 2008; Shimbo and Wade 2016).

MTR (also known as 5-methyltetrahydrofolate-homocysteine methyltransferase) is widely expressed in different organs and tissues. This enzyme facilitates the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine using zinc, cobalamin (B12), and MTRR enzyme as a catalyst. The end products of this reaction are the amino acid methionine and the vitamin tetrahydrofolate. Dysfunction of this enzyme due to mutations in the gene or disruption of zinc deficiency may lead to a lack of methionine and the accumulation of homocysteine in the body (hyperhomocysteinemia) (Matthews and Goulding 1997; Castro et al. 2008; Shimbo and Wade 2016). The pathological consequence of the gene mutation depends on how profoundly these methylation pathways are affected and the degree of homocysteine accumulation in the body.

Interestingly, Jing et al. (2015) have investigated the effects of dietary zinc (Zn) supply on homocysteine levels and expression of these two enzymes in the growing rats. Male weanling rats Sprague-Dawley are randomly assigned to four dietary groups for 3 weeks: Zn deficient (ZD; <1 mg Zn/kg); Zn control (ZC; 30 mg Zn/kg); pair fed (PF; 30 mg Zn/kg), and Zn supplemented (ZS; 300 mg Zn/kg). As expected,

several parameters, including feed intake, body weight gains, kidney weights, and serum and femur Zn concentrations, in the ZD rats are lower than those of ZC rats but not significantly different from the PF controls. The mRNA expression levels of the MTR gene are lower in liver and kidney of ZD rats compared to PF rats. However, hepatic and renal BHMT mRNA expression levels in ZD rats are not altered compared to controls. This study provides evidence suggesting that homocysteine homeostasis is disturbed by zinc deficiency but not zinc supplementation, and elevated serum homocysteine may be due to reduced expression of MTR but not BHMT during zinc deficiency.

Zinc Deficiency and Zinc Finger Proteins

Three classes of mammalian proteins recognize methylated DNA: MBD proteins, SRA proteins, and ZFPs. ZFP are a massive, diverse family of proteins and serve a wide variety of biological functions (Laity et al. 2001; Blattler et al. 2013). It is difficult to simply define what unites ZFPs due to their diversity. However, the common character of all ZFPs is that the functional domains of ZFPs require the coordination by at least one zinc ion. In general, the second structures (α-helix and β-sheet) of a ZFP, which are referred as "fingers" are joined by the zinc ion. For example, the Cys2His2 was the first domain discovered (also known as Krüppel-type). Figure 2 represents the structure of the Cys2His2 zinc finger motif consisting of α-helix and antiparallel β-sheet. The zinc ion (green) is located in the center and coordinated by two histidine residues and two cysteine residues. The Cys2His2 zinc fingers that bind DNA tend to have 2–4 tandem domains as part of larger protein. Cys2His2 proteins are the biggest group of transcription factors in most species. Lack of the zinc ion in the ZFP will result in the structural damage and functional loss (Wu 2002). Therefore, zinc serves to stabilize the integration of ZFP itself but is

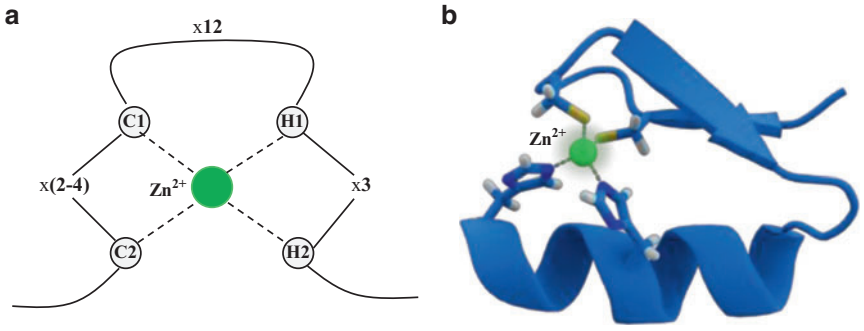

Fig. 2 A cartoon representation of the Cys2His2 zinc finger motif. The Cys2His2 zinc finger motif consists of α-helix and antiparallel β-sheet. Two histidine residues and two cysteine residues are coordinated by the zinc ion (in the *center* and labeled in *green*). (**a**): a schematic outline of the Cys2His2 type zinc finger domain. The number of x shows the intervals between the zinc binding residues. (**b**): a crystal of structure of the Cys2His2 zinc finger motif

Table 3 Zinc finger proteins, their recognition of methylated and specific DNA sequences

Protein name	Size molecular mass	Gene symbol	Aliases	DNA-binding specificity	
				Methylated sequence	Nonmethylated consensus
Zinc finger and BTB domain containing 33	672 AA 74484 Da	ZBTB33	KAISO	5′-CGCG-3’	5′-TCCTGCNA-3’
Zinc finger and BTB domain containing 4	1013 AA 105114 Da	ZBTB4	KAISO-L1	5′-CGCG-3’	5′-CTGCNA-3’
Zinc finger and BTB domain containing 38	1195 AA 134257 Da	ZBTB38	Croz	5′-CGCG-3’	5′-CATGTG-3’

not involved in binding targets. Subsequently, zinc finger-containing domains typically act as interactors, binding DNA, RNA, protein, or small molecules (Laity et al. 2001; Wu 2002; Blattler et al. 2013).

Kaiso, ZBTB4, and ZBTB38 are three ZFPs can bind either methylated DNA in a sequence-specific manner or unmethylated consensus sequences. They may use a mode of binding common to other zinc-finger proteins, suggesting that many other sequence-specific methyl-binding proteins may exist. Table 3 represents the methylated DNA and unmethylated consensus sequences of Kaiso, ZBTB4, and ZBTB38.

Kaiso is originally described as a BTB/POZ zinc finger transcription factor and a p120-catenin-binding partner. This protein functions as a DNA methylation-dependent transcriptional repressor by binding to sequence-specific Kaiso-binding sites or to methyl-CpG dinucleotides (Blattler et al. 2013). Kaiso represses the expression of glucocorticoid receptor via a methylation-dependent mechanism and attenuates the antiapoptotic activity of glucocorticoids in breast cancer cells. Kaiso promotes cell migration and invasiveness through regulation of miR-31 expression in prostate cancer cells (Wang et al. 2016; Zhou et al. 2016). ZBTB4 (also known as Kaiso-L1) is found to be downregulated in breast cancer patients, and that its expression is significantly correlated with relapse-free survival. ZBTB4 functions as a novel tumor-suppressor gene with prognostic significance for breast cancer survival. CIBZ, was discovered to implicate in the spinal cord injury process for the first time. Further studies indicate that CIBZ is extensively distributed in various tissues, and the expression level is highest in muscle, followed by spinal cord, large intestine, kidney, spleen, thymus, lung, cerebrum, stomach, ovary, and heart, respectively. CIBZ gene is involved in secondary injury process and triggers the activation of apoptotic caspase-3 and bax genes independent of p53 (Cai et al. 2012). To date, the precise factors contributing to ZFPs-related zinc deficiency remain poorly defined.

Zinc Deficiency and Inflammation

As an essential micronutrient, zinc is required for many cellular processes, especially for the normal development and function of the immune system. There are remarkable similarities between the hallmarks of zinc deficiency and age-related immunological dysfunction because both are characterized by impaired immune responses and systemic chronic inflammation (Mocchegiani et al. 2000; Foster and Samman 2012; Prasad 2014). Moreover, zinc has anti-inflammatory properties and low zinc status is associated with increased susceptibility to infections and exaggerated inflammatory responses.

Interleukin 6 (IL-6) is an interleukin that acts as both a proinflammatory cytokine and an anti-inflammatory myokine. Wong et al. (2015) have recently demonstrated that zinc deficiency induces a progressive demethylation of the IL6 gene promoter in human monocytic cell line THP-1. The decreased IL6 gene methylation levels are correlated with increased IL6 expression. Evidence from this study suggests that zinc deficiency may have the effects in promoting inflammation and subsequently disrupt DNA methylation of the genes involved in the inflammation process. IL6 promoter methylation may also be reduced with age in human population. Most likely, there is a potential epigenetic link between zinc deficiency and age-related chronic inflammation (Bonaventura et al. 2015). However, it is necessary to include other proinflammatory cytokines such as IL1β, IL8, and tumor necrosis factor alpha (TNFα) for analysis before a conclusion is drawn.

Zinc Deficiency and Epigenetics

The effects of zinc deficiency in epigenetics are summarized in Fig. 3. *Zinc deficiency directly affects the key enzymes involved in the reaction of DNA methylation such as MTR and BHMT, and the proteins, in which zinc iron are constructed, including ZFPs, and consequently disrupts the DNA methylation and histone modification. Zinc deficiency also indirectly induces inflammation response and subsequently causes epigenetic alteration.*

Zinc transporters play an important role in regulating cellular zinc homeostasis. Members of Zip and ZnT zinc transporter families exhibit tissue- and cell-specific expression and possess differential responsiveness to dietary zinc, as well as to physiologic stimuli, including cytokines (Liuzzi and Cousins 2004; Bonaventura et al. 2015). Zinc transporters may also be involved into the indirect influence in epigenetics under the condition of zinc deficiency. Intrauterine growth retardation causes hypomethylation and hyperacetylation of genomic DNA while zinc deficiency is found to accompany fetal growth retardation in the patients with diabetes (Miao et al. 2013; Lin and Huang 2015). In alpha- and beta-cells of the islets of Langerhans, zinc has specific functions in the biochemistry of insulin and glucagon. When zinc ions are secreted during vesicular exocytosis, they have autocrine, paracrine, and endocrine roles. Solute carrier family 30 member 8 (SLC30A8, also known as ZnT8) is a zinc efflux transporter in the cell membrane and transports zinc

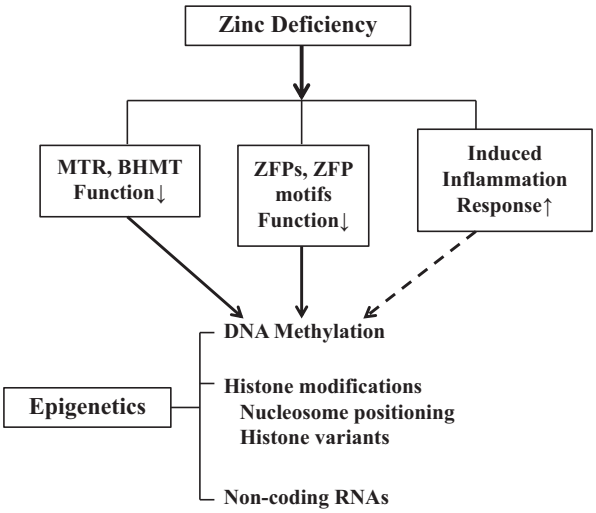

Fig. 3 Effects of zinc deficiency in epigenetics. Zinc deficiency can directly affect the activities of key enzymes such as MTR and BHMT and zinc finger proteins and consequently disrupt the DNA methylation and histone modification. Moreover, zinc deficiency can also indirectly induce inflammation response and subsequently cause epigenetic alteration. Other proteins such as zinc efflux transporters may also be involved into the indirect influence in epigenetics under the condition of zinc deficiency

ions into the insulin and glucagon granules and the subsequent crystallization of hexameric insulin. Pathological studies have demonstrated that the SLC30A8 gene expression levels are downregulated in pancreatic islets of diabetic mice (Fu et al. 2009). The downregulation of the SLC30A8 gene expression results in the reduction of insulin content and glucose-inducible insulin secretion (Chimienti et al. 2004; Gu 2016; Katsarou et al. 2017). Seman et al. (2015) have analyzed SLC30A8 DNA methylation with a bisulfite pyrosequencing protocol and found that DNA methylation levels of the SLC30A8 gene in type 2 diabetes patients are significantly higher compared to nondiabetic subjects. Interestingly, the average DNA methylation levels of the SLC30A8 gene in all studied subjects are high (~81.4%). However, the relationship between DNA methylation changes in this gene and zinc deficiency is still unknown. Furthermore, the recent studies have provided evidence that zinc deficiency enhanced albuminuria and extracellular matrix protein expression, associated with diabetic renal interstitial fibrosis by activation of renal interstitial fibroblasts and regulation of the expression of fibrosis-associated factors, which may be mediated by the activation of fibroblasts via the TGF-β/Smad signaling pathway (Zhang et al. 2016a). Zinc supplementation significantly inhibited the pathway (Zhang et al. 2016b). Therefore, zinc is one of the many factors in multiple gene-environment interactions that cause the functional demise of pancreatic beta-cells and renal interstitial fibrosis. Further epigenetic study is necessary to better understand between nutritional or conditioned zinc deficiency, environmental

exposures, and pathobiochemistry of the diabetes and diabetic complications including diabetic nephropathy.

Currently, the serum zinc concentration is used for evaluation of zinc deficiency. This biomarker for zinc status, however, may not be reliable because a decrease in serum concentration is only detectable after long-term or severe depletion. Therefore, it is necessary to develop new biomarker and protocol to more accurately detect dietary zinc deficiency. Dietary zinc deficiency affects blood linoleic acid: dihomo-γ-linolenic acid (LA:DGLA) ratio. The erythrocyte LA:DGLA ratio as a new zinc biomarkers has shown promise in preclinical and clinical trials and can be developed to more accurately detect dietary zinc deficiency (Knez et al. 2016). Consequently, the precise determination of zinc status will improve the basic and translation medicine researches on the effects of zinc deficiency in epigenetics.

Zinc is one of the many factors in multiple gene-environment interactions that cause the epigenetic alteration. Further investigation is of importance to better understand the inactive mechanisms of nutritional or conditioned zinc deficiency with zinc metabolism, environmental exposures and epigenetic regulation. Based upon the advanced knowledge concerning the relationship between zinc deficiency and epigenetic alteration, the possible interventions may include personalized nutrition, bioactive food, and pharmaceuticals targeting the control of cellular zinc in precision medicine.

Key Facts

Zinc is a chemical element with the symbol Zn^{2+}. The atomic number of zinc in periodic table of elements and chemistry is 30.

Zinc is publically considered as an essential micronutrient element because it exists in all body tissues and fluids and is included in the reactive centers and/or binding sites of many proteins and enzymes.

Zinc deficiency is defined either qualitatively as insufficient zinc to meet the requirements of the body and thereby causing clinical manifestations or quantitatively as a serum zinc level below the normal range.

An estimated global prevalence of inadequate zinc intake is 17.3% according to the data analysis based upon WHO growth standards. Zinc deficiency is actually common in many parts of the world and particularly in poor populations.

Zinc deficiency may lead to decreased oocyte chromatin methylation. The subsequent oocyte epigenetic programming during the period of final oocyte growth is important to perturbation in whole body zinc homeostasis.

Zinc deficiency may cause decreased DNA and chromatin methylation mainly due to dysfunction of MTR and BHMT in the reaction of DNA methylation.

Some zinc finger proteins (ZFPs) such as Kaiso, ZBTB4, and ZBTB38 bind either methylated DNA in a sequence-specific manner or unmethylated consensus sequences. These ZFPs may have the effects in epigenetic regulations under the condition of zinc deficiency.

Zinc deficiency is associated with immunological dysfunction, and may induce inflammation response and subsequently cause epigenetic alteration in the related genes.

Zinc transporters play an important role in regulating cellular zinc homeostasis. DNA methylation changes of some genes encoded for zinc transporters for example SLC30A8 has been found to be involved in the pathogenesis of diabetes.

Dietary zinc deficiency affects blood LA:DGLA ratio. This ratio can be used as a new zinc biomarker to more accurately detect dietary zinc deficiency.

Dictionary of Terms

- **Zinc deficiency** – It is defined either qualitatively as insufficient zinc to meet the requirements of the body and thereby causing clinical manifestations or quantitatively as a serum zinc level below the normal range. Zinc deficiency can occur in soil, plants, and animals. In humans, the most common cause of zinc deficiency is reduced dietary intake, while other reasons include inadequate absorption, increased loss, or increased use.
- **Homocysteine recycling** – Homocysteine is a sulfhydryl-containing amino acid, an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine. In the methylation cycle, homocysteine is methylated to methionine, which undergoes S-adenosylation and forms S-adenosylmethionine (SAM). SAM is the principal methyl donor for all methylation reactions in cells. The reactions are controlled by two key enzymes named as methionine synthase (MTR) and betaine homocysteine methyltransferase (BHMT). MTR and BHMT are both zinc-dependent methyltransferases. Zinc deficiency may disrupt the biological activities of these two enzymes in the reaction of DNA methylation and consequently result in the decreased DNA methylation levels.
- **Zinc finger proteins** – They are a massive, diverse family of proteins that serve a wide variety of biological functions. These types of proteins are structured with a zinc finger, which is a small protein structural motif and characterized by the coordination of one or more zinc ions in order to stabilize the fold.
- **Zinc transports** – These proteins are encoded by two solute-linked carrier (SLC) gene families: SLC30 (ZnT) and SLC39 (Zip), and all have transmembrane domains. There are at least 9 ZnT and 15 Zip transporters in human cells. ZnT transporters reduce intracellular zinc availability by promoting zinc efflux from cells or into intracellular vesicles, while Zip transporters increase intracellular zinc availability by promoting extracellular zinc uptake and, perhaps, vesicular zinc release into the cytoplasm. Both ZnT and Zip transporter families exhibit unique tissue-specific expression, differential responsiveness to dietary zinc deficiency.
- **LA:DGLA ratio** – Linoleic acid (LA) is a polyunsaturated omega-6 fatty acid. Dihomo-γ-linolenic acid (DGLA) is a 20-carbon ω-6 fatty acid. The LA:DGLA in blood samples is closely responsible for dietary zinc deficiency. Thereby, this ratio is a potential new zinc biomarker for determination of dietary zinc deficiency.

Summary Points

- Current studies concerning the effects of zinc deficiency in diseases and in the relation with proteins or enzymes are extensive but our knowledge regarding the relationship between zinc deficiency and epigenetic dysregulation is still limited. Further basic and clinical investigation is necessary to better understand the roles of zinc and effects of its deficiency in epigenetic regulations. To summarize what we have discussed above, several points are listed as below: Zinc is an essential micronutrient element for health. Zinc deficiency is common in the developing countries and in the poor populations of the developed countries. The global prevalence of zinc deficiency is estimated by approximately 17.3%.
- MTR and BHMT are two key enzymes in the reaction of DNA methylation. Both are zinc dependent. Therefore, zinc deficiency leads to decreased DNA and chromatin methylation zinc finger proteins (ZFPs), mainly including Kaiso, ZBTB4, and ZBTB38, bind either methylated DNA in a sequence-specific manner or unmethylated consensus sequences. All these proteins are structured with a zinc finger, which is a small protein structural motif and characterized by the coordination of one or more zinc ions. Zinc deficiency may influence the function of ZFPs and subsequently result in epigenetic alteration.
- Zinc deficiency is found to induce inflammation response and subsequently cause epigenetic alteration in the genes encoded for inflammatory factors.
- Zinc transporters, including 9 members of SLC30 family and 15 members of SLC39 family in human play the important roles in regulating cellular zinc homeostasis. DNA methylation levels of these zinc transporter genes may be associated with dietary zinc deficiency.

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