Molecular Characterization of Zinc Transporter Proteins (ZnT) and the Roles in Diseases

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Abstract. As an essential trace element, zinc is required by the human body, and it is associated with various physiological activities. It can impact body development, brain function, bone growth, reproductive health and immune function. Zinc deficiency can cause many diseases, whereas excess zinc can lead to cytotoxicity as well. Thus, in the human body, it is significant to sustain zinc homeostasis. Zinc transporter protein (ZnT) is reported to take responsibility for zinc transport from cytoplasm to extracellular or organelle. It includes nine family members, and they are widely localized in diverse tissues. Furthermore, some ZnTs function not only in zinc transport but also in modulating as well. Besides, ZnTs are shown to be involved with the occurrence and development of several diseases, including transient neonatal zinc deficiency (TNZD), Parkinson's disease (PD) and others. This paper will review the molecular characteristics of ZnTs and their recent research progress on diseases, which may offer new insight into treatment.

Keywords: Zinc, Zinc transporter proteins, diseases.

1. Introduction

Zinc is a necessary micronutrient for humans since it participates in various biological processes, including cell growth, division and differentiation, commonly as a key cofactor for biomolecules, for example, proteins. Due to their predicted membrane topology, in mammalian, zinc transporters can be classified into two major types [1]: 1) the Zrt / Irt-like protein (ZIP) family named by Docter Eide in the University of Wisconsin in USA are encoded by the SLC39A gene family and ZIP proteins are related to the zinc transport from the outside cell area or intracellular compartments into the cytoplasm; 2) the Zn transporter protein (ZnT) family are encoded by the SLC30A gene family, one of the cation diffusion facilitator (CDF) family as well and they transport the zinc from the cytoplasm to outside cell area or organelles insides cell. Growing evidence supports that both of these Zinc transporters are not only directly involved in the steady-state metabolism of Zn^{2+} in cells, but also affect the occurrence and development of diseases [1].

The study of ZnT transporters has advanced further compared to ZIP transporters in terms of molecular characterization. Thus, this review will focus on the ZnT proteins, comprising their molecular characterization and their roles in related diseases.

2. ZnT Proteins

According to differences in structure and function, the ZnT family can be divided into three subfamilies (subfamilyI, II and III). Members of subfamily I mainly exist in prokaryotic cells, while members of subfamily II and III are widely found in prokaryotic cells and eukaryotes.

In mammalian, the ZnT protein family have ten members named from ZnT1 to ZnT10, except for ZnT9, which is not involved in zinc transport and has now been renamed GAC63 (GRIP1-associated coactivator 63) for its function in a signal transduction pathway [1]. ZnT transporters don't only function in transporting zinc, but also in modulating as well.

Most ZnT proteins form homodimers and are predicted to feature six transmembrane domains (TMDs) [1]. Additionally, the zinc-binding site is intramembranous, composed of four conserved residues which are hydrophilic in TMDs II and V [1]. Besides, both N-and C-terminal regions of amino acid are directed toward the cytosol. ZnT5 is special and it will be illustrated in detail as follows.

2.1. ZnT1

ZnT1 is the first ZnT family member to be discovered in mammals and is mainly localized to the plasma membrane. Whereas the cellular locations of ZnT1 are different based on different cell types. The flow of zinc through ZnT1 is contingent upon the concentration of free zinc within the cell, and the zinc gradient is needed. The increase of intracellular free zinc concentration will cause the upregulation of ZnT1 mRNA level [2]. ZnT1 is primarily expressed in the small intestine (localized at the basolateral membrane), kidneys and placenta etc. [1]. ZnT1 is associated with the zinc movement from the basolateral membrane of red blood cells to blood circulation, participating in the circulation of zinc ions in the small intestine, the reabsorption and secretion of zinc ions in the kidneys, and the transport of zinc between the mother and the fetus [2, 3]. Mice of homozygous ZnT1 knockout were embryologically lethal early in utero for developmental failures of embryos, whereas heterozygous ZNT1 mice were survival [1]. This indicates that ZnT1 plays a big part in early embryonic development. Besides, in Drosophila homozygous mutants of ZnT1 (dZnT1) knockdown were lethal, with abundant zinc accumulation in the posterior midgut and gastric caeca, etc., while expression of ZnT1 instead only in the midgut could rescue the fatality of dZnT1 mutant flies [3]. This proves that ZnT1 is essential for the survival of Drosophila, and it functions mainly in the gut. Recent research states that ZnT1 gets involved in modulating the transport of Zn^{2+} and Ca^{2+} in neurons, which may impact synaptic signalling and plasticity intensely [4].

2.2. ZnT2

ZnT2 can be present in various secretory vesicles and granules, and it can be found in secretory tissues, such as mammary glands, etc. [1]. However, the subcellular localization of ZnT2 can alter due to the single nucleotide polymorphisms (SNPs) of ZnT2, which indicates SNPs may have a significant influence on human health by affecting zinc transporters [1].

The overexpression of ZnT2 can lead to the accumulation of zinc in vesicles [5]. Besides, ZnT2 functions in preventing the cells from zinc toxicity by collecting zinc in lysosomes and endosomes [5]. To support the growth and development of newborns, breast milk contains higher levels of zinc compared to the amount present in maternal serum. It suggests the epithelial layer from the mammary gland contains a special transport mechanism for zinc which causes the high concentration of zinc in milk during the period of lactation. Studies have shown that expression of ZnT2 in the breast gland decreases with the decrease of zinc content in breast milk, indicating that ZnT2 plays a crucial part in the process of zinc metabolism in the breast. Infants fed on maternal milk of ZnT2 gene-mutated mothers will occur serious zinc deficiency symptoms [5]. Moreover, recent research also shows that ZnT2 is related to gut dysbiosis and health in humans [6].

2.3. ZnT3

The amino acid sequence of ZnT3 is 50% homologous to ZnT2. ZnT3 is localized to the synaptic vesicles of certain glutamatergic neurons in the neocortex and hippocampal regions [1]. The concentration of zinc in vesicles is influenced by the expression level of ZnT3. In the hippocampus, the ZnT3 mRNA level is very high, and ZnT3 is associated with three-dimensional memory formation and behavioural shaping [5]. The ZnT3 knockout mice show cognitive impairment with ageing [7]. ZnT3 plays a protective role in preventing toxicities caused by endoplasmic reticulum (ER) stress [5]. Furthermore, ZnT3 is related to insulin secretion. ZnT3 exists in insulin-containing granules located. Additionally, ZnT8 mRNA expression will be impacted by the expression of ZnT3. Recent research indicates that ZnT3, expressed by Type II and III taste cells, may contribute to mediating taste signalling [8].

2.4. ZnT4

ZnT4 can be found in intracellular vesicles, endosomal compartments and the trans-Golgi network (TGN) [1]. In some cells, ZnT4 is located in the cell membrane as well [1]. In the mammary gland, ZnT4 has a similar function to ZnT2. ZnT4 can alter the amount of zinc in breast milk secreted by

the mammary glands [5]. In prostate epithelial cells exposed to high glucose, ZnT4 can transport zinc into lysosomal and ZnT1 can control zinc efflux, both of which co-work to maintain zinc homeostasis [9].

2.5. ZnT5 and ZnT6

Unlike other ZnTs forming homodimers, ZnT5 and ZnT6 form heterodimers [1]. Besides, in ZnT5, there is a particularly long amino-terminal region compared with other ZnTs, assumably consisting of fifteen TMDs in total [1]. As for ZnT6, it is unique as well. Most ZnTs have a histidine-rich loop in their sequences, however, in ZnT6, two histidines are replaced by leucine and phenylalanine respectively [10]. Thus, ZnT6 itself can't transport zinc due to a lack of conserved hydrophilic residues. In the heterodimer, ZnT5 is in charge of transporting zinc, while ZnT6 functions in modulating [1, 10].

The early secretory route, which includes the ER, Golgi apparatus, and cytoplasmic vesicles, is where ZnT5 and ZnT6 are both located [1]. Some research states that ZnT5 can uniquely recruit ZnT6 to the Golgi apparatus and then the functional heterodimer is formed [11]. Endogenous ZnT5 can be found intensively in COP II- coated vesicles and it is related to the insulin granule membrane [1]. Although ZnT5 mRNA can be expressed in various tissues, it is comparatively abundant in the pancreas, prostates, ovaries and testicles. Concerning ZnT6, it is mainly localized to the TGN [1]. All of the above may imply that when ZnT5 and ZnT6 do not exist as a heterodimer, they have different functions individually. Furthermore, the transcription product ZnT6 gene is more found in the liver, brain, and kidneys, but the protein of ZnT6 is only expressed in the brain and lung. It can be assumed that the specificity of tissue expression of the ZnT6 protein is regulated by a post-transcriptional regulatory mechanism.

ZnT5 gene expression can be regulated by zinc concentration. Some research showed that with zinc concentration increasing in cell culture medium, the mRNA level of ZnT5 increased. Besides, ZnT5 mRNA can be induced by high levels of glucose as well [5]. In this regard, ZnT5 commonly co-works with ZnT7. Overexpression of ZnT5 and ZnT7 can lead to noticeable reductions in caspase-3, caspase-8, BAX and apoptosis-inducing factor (AIF) and then survive cells in a high glucose concentration medium [5]. Additionally, ZnT5 is also associated with alkaline phosphatases (ALPs). In DT40 cells, the breakdown of the ZnT5 gene resulted in reducing ALP activity by 45 per cent, compared to normal cells [5]. The recent study also shows that ZnT5-ZnT6 dimers are crucial in the activation of ALPs by providing zinc to ALPs like placental ALP (PLAP) and tissue-nonspecific ALP (TNAP) [11]. ZnT5-ZnT6 dimers and ZnT7 homodimers both promote the homeostatic maintenance of secretory pathway functions and are crucial for glycosylphosphatidylinositol-anchored protein expression [12].

2.6. ZnT7

ZnT7 is located in the cytoplasmic vesicles and Golgi apparatus, predominantly in the cis-face of the Golgi apparatus [1]. It is reported that ZnT7 can transport cytoplasmic zinc to the Golgi apparatus or vesicles to accumulate [5]. In mice, ZnT7 mRNA expresses in various tissues such as the liver, kidney, heart, brain, lung, spleen and small intestine, highly expressed in liver, small intestine and spleen. Whereas ZnT7 proteins are detected in the small intestine and lung. ZnT7 protein is high in the duodenum and jejunum, which suggests ZnT7 functions in zinc absorption of the small intestine.

ZnT7 gene expression is regulated by zinc level. Research shows that when Hela cells are supplemented with different concentrations of zinc, there is no effect on ZnT7 mRNA content. However, when cells lack zinc, the expression of ZnT7 mRNA increases significantly. The study also shows that ZnT7 knockout mice have zinc deficiency phenotypes, and they will not recover if zinc is provided. A large number of immunological studies prove that ZnT7 is involved in maintaining zinc homeostasis metabolism in the brain. Recent study shows that the amount of labile zinc ions in the mitochondrial matrix can be raised b ZnT7 overexpression and histone modification in hyperinsulinemic cardiomyoblasts can be caused [13].

2.7. ZnT8

ZnT8 is mainly found in the insulin granule membrane [1]. Not only can be found in pancreatic β cells but ZnT8 also can be found in pancreatic α cells as well [1]. ZnT8 collects zinc from the cytoplasm to intracellular vesicles, so zinc can be accumulated in vesicles and then bonded by insulin to form zinc-insulin crystals [1, 5]. Therefore, ZnT8 is important for insulin maturity, storage and secretion by transporting zinc. In ZnT8 knockout cells, insulin granules are replaced by immature insulin progranules [5]. Interestingly, the lack of ZnT8 expression doesn't seem to change insulin biosynthesis or content [5]. Previous research shows that overexpression of ZnT8 insulinoma cells upgrades insulin secretion stimulated by glucose [1]. A recent study also shows that mice carrying the human SLC30A8 (gene encode ZnT8) R138X loss-of-function mutation increases the ability of β cells for insulin secretion when facing high blood sugar conditions [14].

2.8. ZnT10

ZnT10 is located in the early/recycling endosomes in the cells and can be found in several tissues such as the brain, liver and retina [1, 5]. There is a little difference in the histidine-rich loop of the ZnT10 sequence——histidine is replaced by asparagine. Thus, in addition to transporting zinc ions, ZnT10 is more inclined to transport manganese ions [10].

It was reported that the downregulation of ZnT3 and ZnT10 can mediate the zinc-dependent pathway of Angiotensin (Ang) II-induced senescence, both of which co-work to decrease cytosolic zinc. Zinc increases Ang II induced senescence, while overexpression of ZnT3 or ZnT10 can decrease reactive oxygen species (ROS) and prevent senescence [5]. Vitamin D3 has a positive effect on ZnT8 protein expression [5].

3. Related Diseases

3.1. Transient neonatal zinc deficiency (TNZD)

Infants will have several symptoms of zinc deficiency such as erythematous, erosive dermatitis, and transient growth retardation. Sometimes, zinc deficiency is caused by acrodermatitis enteropathy (AE). The mutation of ZnT4 is accountable for AE. In other cases, TNZD is an outcome of infants fed with breast milk of low zinc concentration, which only exists during the period of breastfeeding period. Symptoms of TNZD can be relieved by zinc supplementation to the infant [15].

Recent studies have shown that mutations in the ZnT2 gene lead to TNZD and there are four mutations having been identified. Although as the previous context stated, ZnT4 is associated with zinc concentration in breast milk as well, so far, no evidence shows the breast milk from mothers of ZnT4 gene mutations leads to TNZD. However, it is documented that mRNA expression of ZnT5 and ZnT6 decreases strongly in fibroblasts and lymphoblasts of females whose milk is at low zinc concentration [15]. It indicates ZnT5 and ZnT6 may be potential triggers of TNZD.

3.2. Diabetes mellitus (DM)

There are two primary subgroups of this complicated metabolic disease, DM: Type 1 and Type 2. [16]. The former belongs to one of the autoimmune diseases. In type 1 diabetics, pancreas islet β cells which can produce insulin are destroyed by self-reactive immune cells, whereas, the latter is characterized by insulin resistance [16].

For the SLC30A8 gene, the most significant finding is the gene polymorphisms which are related to the changed risk of Type 2 diabetes [17]. Based on SNP, in ZnT8, the W325R variant is concerned with an increased risk of developing T2DM [18]. Previous studies have described that 12 mutants which are loss-of-function of ZnT8 are altogether associated with a 65 per cent reduction in Type 2 diabetes risk [17]. On the other hand, ZnT8 can regulate liver insulin levels as well. Consequently, ZnT8 is a prospective therapeutic target for treating Type 2 diabetes.

For Type 1 diabetes, ZnT8 also attracts attention as an autoimmune marker for most patients and occurs in the serum of pre-diabetics and diabetics [19]. It may be due to the structure of ZnT8 that it might cause Type 1 diabetes autoimmunity. ZnT8 protein also has immunogenicity and can cause Type 1 diabetes autoimmune responses. It might be assumed as the aetiology of Type 1 diabetes. Besides, ZnT8 is enabled to be regarded as a potential immunobiological target for pediatric Type 1 diabetes [19].

3.3. Alzheimer's disease (AD)

AD is a kind of chronic neurodegenerative disorder caused by multiple factors, and its common outcome is dementia. Studies show that zinc ions are thought to be associated with various neurodegenerative diseases including AD. Thus, ZnTs in AD pathology cannot be ignored.

The level of ZnT3 will decrease with age in Alzheimer's patients, which results in the loss of cognitive function [1]. However, other ZnTs are upregulated. In AD postmortem brain, the mRNA levels of ZnT1, ZnT4 and ZnT6 are increased in the cortex, and the ratios of the mRNA levels of them / mRNA level of neuron-specific enolase are also increased with AD progressing and Braak stage increasing [7]. It is worth mentioning that for ZnT1, the situation is complex. ZnT1 protein significantly increases in AD inferior parietal lobule (IPL), but decreases in AD superior and middle temporal gyrus (SMTG) [7]. In patients with mild cognitive impairment, the situation is similar [7]. Additionally, in fulminant AD patients, ZnT1 is also elevated [7]. Recent research states that ZnT10 mRNA is downregulation strongly in the frontal cortex of patients with AD [15].

3.4. Parkinson's disease (PD)

PD is a type of progressive neurodegenerative disorder that is commonly characterized by symptoms such as tremors and bradykinesia. It is a prevalent neurological condition. A great amount of evidence has implied zinc ions may function in the pathogenesis of PD [20]. Furthermore, recent studies have shown that ZnT10 gene mutations including frameshift, nonsense and missense can lead to PD [5, 15]. ZnT10 functions in transporting manganese, and in patients, a high accumulation of manganese is detected in their tissues such as the liver and brain, where ZnT10 highly express as well. Patients with symptoms caused by ZnT10 gene mutations can be treated by iron supplementation [5].

3.5. Other diseases

Some human skin disorders can be caused by ZnTs. Epidermodysplasia verruciform (EV) is an uncommon skin disorder belonging to autosomal recessive diseases which can develop skin cancers for the vulnerability to tumorous human papillomaviruses (HPVs). The mutations of either EVER1 or EVER2 occur in EV patients [21]. In keratinocytes, ZnT1 can be formed as s complex with EVER1 and EVER2, which exists mostly in the ER, whereas less in the Golgi apparatus and nuclear membrane [21]. It may assume that ZnT1 is associated with EV pathology. It is also reported that in various human tumour tissues, the ZnT7 protein expression is downregulation. Thus, it can be assumed that ZnT7 is associated with tumour development.

Furthermore, zinc deficiency (ZnD) can lead to various diseases such as necrolytic migratory erythema (NME), pellagra and alopecia. As zinc transporter family, ZnTs have a potential role in these diseases.

4. Conclusion

Zinc is a necessary nutrient both for humans and animals, which is widely involved in a range of metabolic activities in the body, as a crucial modulator or activator. ZD can cause a series of abnormal symptoms, while the high level of zinc concentration will produce cytotoxicity. Therefore, it is necessary for the human body to keep zinc homeostasis. ZnT family play a key role in it.

This paper simply reviews the molecular characteristics of ZnTs and their roles in several diseases. Most ZnT genes are ubiquitously but some of them are cell or tissue specific. Their subcellular

localizations are diverse. Their structures are similar except for ZnT6. They function not only in transport but also in modulating as well. Therefore, it is promising to focus on the cooperation among ZnT family, even with ZIP family. For example, ZnT3 and ZnT8 are involved in insulin mature and secretion, but research of these two protein functions in DM is still abscent. Besides, ZnT family is a member of the CDF family, so it is worth discussing the association between ZnT family and the rest members of CDF family.

According to the roles of ZnTs in different diseases, they may provide prospective thinking and targets for the prevention and treatment of diseases, contributing to human health.

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