

## Transporter Research Yields New Discoveries in Life Sciences

### Review

## Zinc Transporters and the Progression of Breast Cancers

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**Zinc (Zn) is an essential heavy metal utilized in numerous biological processes in mammals, including its recently described role as a signaling mediator. The movement of Zn in and out of cells, across membranes, is regulated by two protein families: the zinc-regulated transporter (ZRT), iron-regulated transporter (IRT)-like protein (ZIP) and the Zn transporter (ZnT) families. ZIPs and ZnTs maintain intracellular Zn homeostasis and control important cellular functions through Zn signaling. Recent studies have highlighted the role of Zn transporters and Zn in disease. ZIP6, 7, and 10 contribute to human breast cancer progression. ZIP6 is associated with breast tumor grade, size, and stage, suggesting that it is a potent driving force toward malignancy; ZIP7 plays an important role in tamoxifen-resistant breast cancer cells, and ZIP10 is involved in invasion and metastasis of breast cancer cells. These Zn transporters are key molecules in the malignant process; thus, understanding Zn transporters will lead to novel diagnostic and therapeutic strategies for breast cancer. This review discusses the emerging functional roles of Zn and Zn transporters in breast cancer.**

**Key words** zinc; zinc transporter; breast cancer; progression

### INTRODUCTION

Zinc (Zn) is both a catalytic and metabolic cofactor for more than 300 enzymes, coordinating biological processes. Zn is also required by >2000 transcription factors to maintain their structural integrity and bind to DNA.<sup>1)</sup> In contrast, Zn has been reported to function as an intracellular signaling molecule to recruit extracellular stimuli and thus determine the final outputs of various physiological pathways, such as cell differentiation, proliferation, survival, and migration<sup>2,3)</sup> (Fig. 1). Intracellular Zn levels are regulated by zinc-binding proteins and Zn transporters.<sup>2,3)</sup> The bi-directional transport of Zn across cell membranes is tightly maintained by two families of proteins: zinc-regulated transporter (ZRT), iron-regulated transporter (IRT)-like proteins (ZIPs; solute carrier (SLC)39A) facilitate the influx of Zn into the cytosol from the extracellular media and the intracellular compartments, whereas Zn transporters (ZnT; SLC30A) mediate Zn efflux from the cytosol into extracellular media and the intracellular compartments. The mammalian ZIP family contains 14 members that are divided into four subfamilies based on the extent of sequence conservation: ZIP subfamily I (ZIP9/SLC39A9), ZIP subfamily II (ZIP1/SLC39A1, ZIP2/SLC39A2, ZIP3/SLC39A3), the LIV-1 subfamily (ZIP4/SLC39A4, ZIP5/SLC39A5, ZIP6/SLC39A6, ZIP7/SLC39A7, ZIP8/SLC39A8, ZIP10/SLC39A10, ZIP12/SLC39A12, ZIP13/SLC39A13, ZIP14/SLC39A14), and the GufA subfamily (ZIP11/SLC39A11).<sup>2,3)</sup> It is important to reveal the role of each of these subgroups in Zn transport and how they are associated with managing cellular Zn balance.

The results of previous studies indicate that specific Zn transporters spatiotemporally regulate intracellular and extracellular Zn levels and distributions in a tissue-specific manner.

Defects in Zn transporters have been linked to specific diseases,<sup>2–4)</sup> such as Alzheimer's disease,<sup>5)</sup> diabetes,<sup>6–8)</sup> cancers,<sup>9)</sup> and other pathological processes<sup>10,11)</sup> (Fig. 1). The mechanism underlying the action of Zn in cancers depends on the cancer type. Low levels of Zn have been measured in serum and tumor samples of men with prostate cancer (Table 1), and decreased expression of ZIP1, 2, and 3 has been demonstrated<sup>9,12)</sup> (Table 2). Breast tissue and serum Zn levels in patients with breast cancer are higher and lower than breast tissue and serum Zn levels in healthy subjects, respectively<sup>13,14)</sup> (Table 1). Moreover, both clinical and *in vitro* studies have demonstrated the importance of the LIV-1 subfamily, such as ZIP6, 7, and 10 in breast cancer progression<sup>14)</sup> (Table 2). These results suggest that studies aimed at understanding the functions of Zn and ZIPs in breast cancer, and findings regarding zinc biology, are necessary to facilitate the development of additional effective therapeutic strategies. In this review, we discuss current research on the role of ZIPs in breast cancer progression.

### 1. ZIPS IN BREAST CANCER CELL PROGRESSION

#### 1.1. ZIP6 in Estrogen-Receptor Positive Breast Cancer

About 70% of all breast cancers are estrogen-receptor positive. ZIP6 was first identified in a genetic screen of estrogen-responsive factors in breast cancer tissue<sup>15)</sup> and thus has been associated with estrogen-receptor positive breast cancer and its subsequent spread to the regional lymph nodes.<sup>16,17)</sup> ZIP6 expression levels are downregulated in high-grade primary breast tumors.<sup>16,17)</sup> Moreover, high ZIP6 protein expression levels correlate with a longer relapse-free survival period in patients with breast cancer, suggesting that ZIP6 levels in primary breast tumors have the potential to be a poor prognostic factor in patients with breast cancer.<sup>16)</sup> To clarify the role of

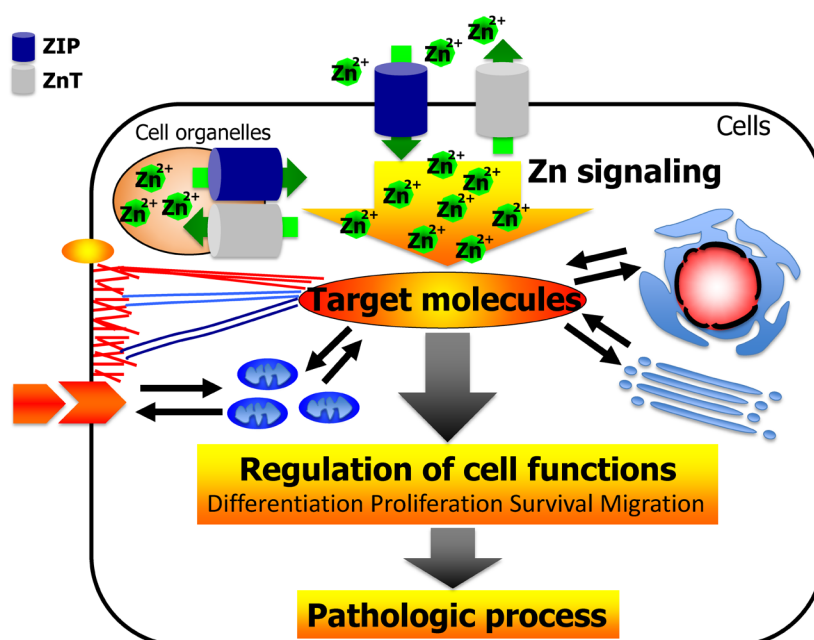


Fig. 1. Cell Functions and Pathologic Process Regulated by Zn Transporters

Zn has functions as an intracellular signalling molecule and thus determines the final outputs of various physiological pathways, such as cell differentiation, proliferation, survival, and migration. Aberrations in Zn transporters link to specific diseases. (Color figure can be accessed in the online version.).

Table 1. Malignant Tissue and Serum Zn Levels in Various Cancer Patients Compared with Healthy Subjects

	Prostate cancer	
	Digestive tracts carcinoma	Breast cancer
	Gallbladder carcinoma	
Serum	Low	Low
Malignant tissues	Low	High

The levels of Zn in the serum and malignant tissues decrease in patients with carcinoma such as prostate, digestive tracts or gallbladder. In patients with breast cancer, the Zn levels in the serum decrease and the Zn levels in malignant tissues increase.

ZIP6 in the progression of breast cancer, we produced ZIP6-knockdown cells in MCF-7 adherent estrogen-receptor positive breast cancer cells.<sup>18)</sup> A ZIP6 deficiency disturbs intracellular Zn homeostasis, leading to increased cell survival under hypoxic conditions, as well as promotion of the epithelial–mesenchymal transition (EMT)<sup>\*1</sup>, resulting in decreased ZIP6 expression, which is strongly associated with resistance to hypoxia<sup>\*11 18)</sup> (Fig. 2). ZIP6 knockdown in T47D human ductal breast epithelial tumor cells also causes resistance to apoptosis and tumorigenesis and promotes the EMT.<sup>19)</sup> Furthermore, there is an association between ZIP6 expression and less aggressive tumors due to high E-cadherin expression.<sup>18–20)</sup> On the other hand, ZIP6 promotes cell detachment, which allows cells to migrate and become metastatic.<sup>21)</sup> This result corroborates the high levels of ZIP6 found in patients with estrogen receptor-positive breast cancer and lymph node metastasis.<sup>15)</sup> These experimental and clinical observational studies indicate that the EMT process involves ZIP6-mediated progression of breast cancer, although the role of ZIP6 may differ between metastatic foci and primary tumors. The downstream target of ZIP6 regulates expression of signal transducer and activator of transcription 3 (STAT3), which was linked to the EMT in a zebrafish embryo study.<sup>22)</sup> These findings suggest that the

ZIP6-mediated zinc signal controls differently, depending on the cell type. Thus, elucidating the biochemical roles of Zn and ZIP6 in breast cancer will contribute to improvements in the understanding of breast cancer and facilitate the development of accurate diagnostic and effective therapeutic strategies that may be used to combat the disease.

EMT<sup>\*1</sup>: The EMT is considered to be an important feature of malignancy. During this transition, cellular adhesion of epithelial cells is lost, allowing cells to gain migratory and invasive properties to transform to mesenchymal-like cells.

Hypoxia<sup>\*11</sup>: Hypoxia occurs in internal solid cancer structures, including breast cancer, as a result of rapid tumor cell growth. Tumor hypoxia leads to acquisition of the EMT phenotype, resulting in aggressive malignant tumor phenotypes (e.g., cell mobility and metastasis, cell survival, and therapeutic resistance).

**1.2. ZIP10 in the Breast Cancer Invasion and Metastasis** An association between ZIP10, the family member closest to ZIP6, and invasion and metastasis of human breast cancer cells was reported by Kagara *et al.* who quantified the expression of ZIP10 mRNA in 177 surgical samples derived from patients with breast cancer. Using the real-time quantitative PCR, the authors demonstrated that ZIP10 mRNA expression in the breast cancer tissues of patients with lymph node metastasis is significantly higher than that in patients without metastasis.<sup>23)</sup> Similarly, ZIP10 mRNA is more highly expressed in a metastatic than in a non-metastatic breast cancer cell line.<sup>23)</sup> ZIP10 knockdown in the metastatic cell line decreased both zinc intake and cell migration.<sup>23)</sup> ZIP10 has recently been shown to form a heteromeric complex with ZIP6, resulting in an interaction with neural cell adhesion molecule 1 (NCAM1) to regulate the EMT paradigm.<sup>24,25)</sup> We demonstrated that high glucose-induced cell migration is inhibited by ZIP6 or ZIP10 knockdown and by zinc chelation, indicating Zn transported via ZIP6 and ZIP10 has essential functions for increased motility of MCF-7 cells in a high glucose envi-

Table 2. Zn Transporters and Specific Cancers

Zn transporter	Human gene name	Subfamily	Cancer
ZIP1	SLC39A1	ZIP Subfamily II	Prostate cancer
ZIP2	SLC39A2	ZIP Subfamily II	Prostate cancer
ZIP3	SLC39A3	ZIP Subfamily II	Prostate cancer
ZIP4	SLC39A4	LIV-1 subfamily	Pancreatic carcinoma
ZIP6	SLC39A6	LIV-1 subfamily	Estrogen-receptor positive breast cancer
ZIP7	SLC39A7	LIV-1 subfamily	Tamoxifen-resistant breast cancer
ZIP10	SLC39A10	LIV-1 subfamily	Breast cancer invasion and metastasis

Some ZIP family members involve in cancer progression. The expression levels of ZIPs in human tumors associate with malignancy and the alteration of intracellular Zn homeostasis can contribute to the severity of cancer.

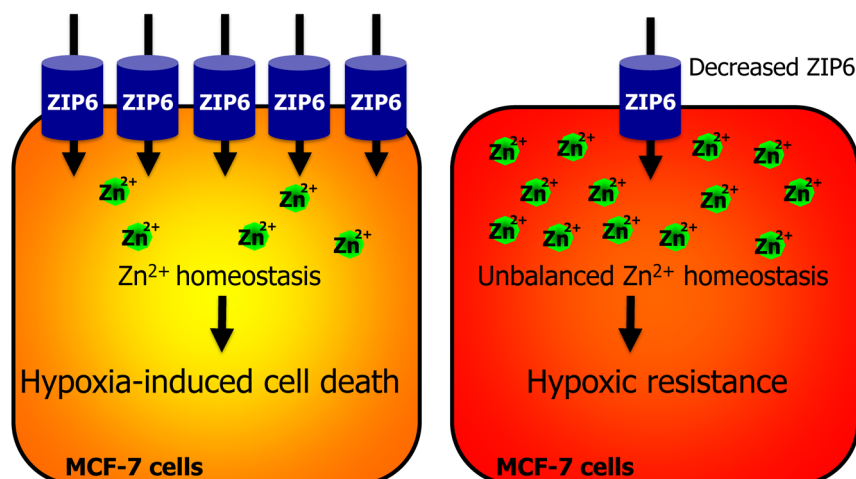


Fig. 2. Regulatory Role of ZIP6 in Hypoxic Stress Condition

ZIP6-attenuation disturbs intracellular Zn homeostasis, leading to increased cell survival activity against hypoxic stress in MCF-7 cells. ZIP6 is a key transporter in adaptability to hypoxic stress condition linked with malignant progression. (Color figure can be accessed in the online version.)

ronment.<sup>26,27)</sup> Thus, these findings support essential roles for zinc transported through ZIP10 in the migratory activity of highly metastatic breast cancer cells, and ZIP10 may cooperate with the ZIP6 transporter functions during cell migration, although further studies are necessary to clarify the involvement of a ZIP6 and ZIP10 heteromer.

### 1.3. ZIP7 in Tamoxifen-Resistant Breast Cancer Cells

About 70% of breast cancer cells express the estrogen receptor and are involved in growth and survival. Tamoxifen and other selective estrogen receptor modulators are now clinically being used to block the estrogen-receptor signaling pathway and show, initially, high therapeutic effectiveness.<sup>28,29)</sup> However, efficacy is limited because of the high rate of intrinsic and acquired endocrine resistance,<sup>30,31)</sup> and the molecular mechanisms underlying resistance to tamoxifen treatments are largely unknown. Thus, identifying the molecules and pathways responsible for resistance are crucial to develop novel strategies for breast cancer therapy.

Taylor *et al.* performed experiments focusing on the relationship between Zn and therapeutic resistance with tamoxifen and demonstrated that ZIP7, the subfamily member with ZIP6, is abundantly expressed in tamoxifen-resistant MCF-7 breast cancer cells.<sup>32)</sup> ZIP7 is located in the endoplasmic reticulum (ER) membrane and its activity depends on phosphorylation by casein kinase II (CK2), an enzyme that promotes cell division.<sup>33)</sup> CK2-stimulated ZIP7 promotes release of Zn from the ER into the cytoplasm and the released Zn inhibits protein

phosphatases and enhances the activity of tyrosine kinases as well as the Akt survival and extracellular regulated kinase 1/2 growth signaling pathways, which together increase cancer progression.<sup>33)</sup> As activation of ZIP7 by CK2 enhances proliferation and migration of breast cancer cells, ZIP7 may be a promising candidate molecular target for breast cancer chemotherapy.<sup>4,33)</sup> However, some questions remain to be answered, such as, are the above-described observations mimicked *in vivo*? Is release of Zn from the ER regulated only by ZIP7 activity? Hessels *et al.* demonstrated localization of two fluorescence resonance energy transfer-based Zn sensors in the cytosol and ER of MCF-7 and tamoxifen-resistant MCF-7 cells.<sup>34)</sup> In addition to revealing the cellular localization of Zn, these probes can aid in increasing our understanding of Zn transported *via* ZIP7.

## 2. ZINC AND ZINC TRANSPORTERS IN AUTOPHAGIC BREAST CANCER CELL DEATH

The pathways controlling cell survival and death are likely to be advantageous therapeutic targets for breast cancer. Autophagy, in which autophagosomes form around misfolded proteins and damaged organelles to induce their degradation, is crucial for maintaining cell homeostasis and controlling cell death; it has also been implicated in the development and progression of breast cancer.<sup>35,36)</sup> Hwang *et al.* reported that intracellular labile Zn accumulates in autophagosomes and

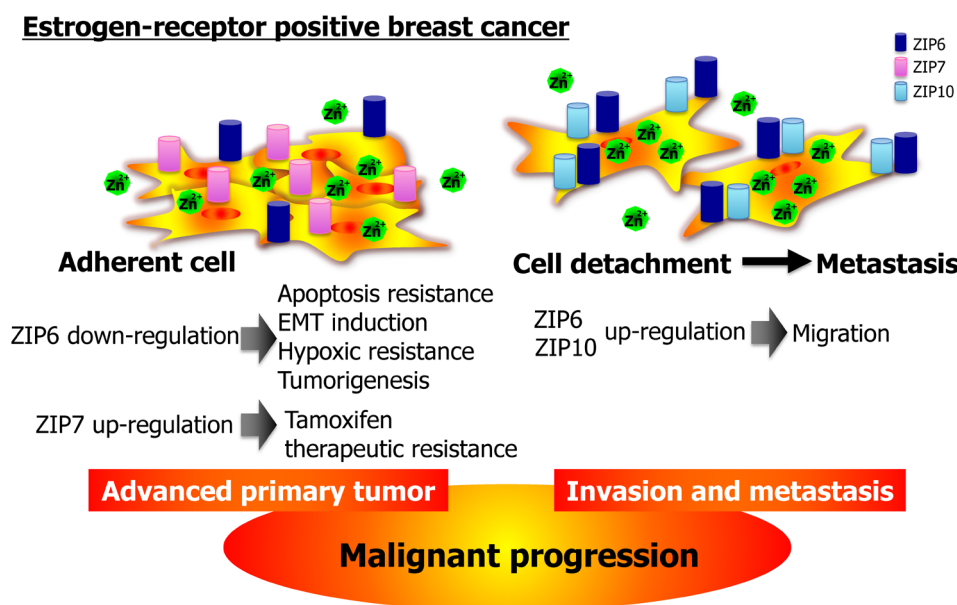


Fig. 3. The Specific Relationship between ZIPs and Breast Cancer

Zn and its transporters ZIPs play critical roles in malignant progression of estrogen-receptor positive breast cancer. The functions of ZIPs differ by pathological process. (Color figure can be accessed in the online version.).

is required for autophagosome-mediated tamoxifen-induced death of MCF-7 cells.<sup>37)</sup> One study indicated that Zn is an essential contributor to autophagy through the Zn transporters ZIP4, ZIP14, ZIP8, ZnT10, ZnT4, and ZnT2 in endosomes, lysosomes, and autolysosomes.<sup>38,39)</sup> Moreover, we have demonstrated that MCF-7 cells increase intracellular labile Zn levels using a Zn ionophore, Zn pyrithione, which changes the cell-death type from apoptosis to autophagic cell death during treatment with an apoptosis inducer, and that the change in cell-death type is controlled by the level of ZIP6 expression (*in preparation*). These results suggest that Zn and Zn transporters are closely related with autophagic cell death, but further studies regarding the mechanisms regulating zinc signaling are required.

### 3. POTENTIAL APPLICATION OF ZN AS A BREAST CANCER BIOMARKER

Previous studies have reported that breast cancer cells have high Zn levels compared with those in normal breast tissue, suggesting that Zn metabolism and the Zn network in or out of the cells differs according to the development and malignancy of breast cancer<sup>13,14)</sup> (Table 1). Zn has potential as a candidate biomarker for an early diagnosis of breast cancer.<sup>13)</sup> Larner *et al.* determined Zn isotopic compositions (<sup>66</sup>Zn/<sup>64</sup>Zn ratio) of various tissues in patients with breast cancer and age-matched controls ( $n=7-10$ ) using multiple-collector inductively-coupled plasma mass spectrometry, which is one of the most sensitive methods for stable isotope measurements.<sup>13)</sup> Breast cancer tumors were found to have a significantly lighter Zn isotopic composition than the blood, serum, and healthy breast tissue in both groups.<sup>13)</sup> The change in natural intrinsic Zn isotopic composition of breast tissue is possibly linked to mechanistic changes in Zn metabolism during breast tumor formation. A better understanding of Zn function using a highly precise isotopic analysis strongly indicates the potential to develop a novel early biomarker for breast cancer.

### CONCLUSION

Exhaustive studies on the role of Zn and ZIPs in breast cancer are likely to manifest novel Zn functions, as well as promising approaches for the efficacy of chemotherapy. This review has discussed several critical roles of Zn and its transporters associated with breast cancer malignant progression (Fig. 3).

Breast cancers are not comprised of a uniform group of cells. Rather, they are comprised of complex and heterogeneous aggregations of various types of cells, which are differentially affected by changes in environmental conditions according to the portion of the tumor that they occupy.<sup>40,41)</sup> Thus, it has been proposed that effective molecular targeted therapies for breast cancer can be selected by analyzing portions of tumors rather than by analyzing tumors as a whole. The results of studies on breast cancer are beginning to reveal that sophisticated regulation of specific ZIPs plays an important role in the acquisition of a malignant phenotype in response to specific types of stimulation. Some questions are not yet solved regarding the target molecules of Zn signaling, the mechanisms underlying the expression of ZIP genes, the relationships between Zn transported *via* ZIPs or ZnTs and Zn reservoirs (intracellular metallothionein or glutathione), the effects of other metal ions on Zn functions, and the roles of ZIPs and ZnTs in the cellular/extracellular Zn network of breast cancer. Moreover, elucidating the novel biochemical functions of Zn and their transporters within the cellular network in breast cancer will lead to improvements in the understanding of breast cancer and facilitate the development of accurate diagnostic and effective therapeutic strategies.

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