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The SLC39 family of zinc transporters

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Abstract

Zinc is a trace element nutrient that is essential for life. This mineral serves as a cofactor for enzymes that are involved in critical biochemical processes and it plays many structural roles as well. At the cellular level, zinc is tightly regulated and disruption of zinc homeostasis results in serious physiological or pathological issues. Despite the high demand for zinc in cells, free or labile zinc must be kept at very low levels. In humans, two major zinc transporter families, the SLC30 (ZnT) family and SLC39 (ZIP) family control cellular zinc homeostasis. This review will focus on the SLC39 transporters. SLC39 transporters primarily serve to pass zinc into the cytoplasm, and play critical roles in maintaining cellular zinc homeostasis. These proteins are also significant at the organismal level, and studies are revealing their link to human diseases. Therefore, we will discuss the function, structure, physiology, and pathology of SLC39 transporters.

Keywords

SLC39; ZIP; zinc; transporter; uptake; homeostasis

1. Introduction

Zinc plays vital roles in cells. Zn^{2+} binds to ~10% of proteins in the human proteome, is a cofactor for over 300 enzymes, and is required for the function of over 2000 transcription factors. Zinc deficiency causes growth retardation, immune dysfunction, cognitive impairment, metabolic disorders, and infertility. However, excess zinc is toxic. Therefore, zinc levels are tightly regulated largely through the activity of specific zinc transporters. Mammalian zinc transporters come from two major families, the SLC30 (ZnT) family and the SLC39 (ZIP) family. ZIP transporters have now been identified at all phylogenetic levels, and there are 14 ZIP transporters encoded in the human genome. Their genes are designated *SLC39A1-SLC39A14* and they encode the proteins ZIP1–ZIP14 respectively (Figure 1). In this review, we will discuss functional and structural characteristics of SLC39/ZIP transporters. The physiology and pathology of these proteins will also be considered.

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2. Functional and structural aspects

ZIP transporters are responsible for zinc transport into the cytoplasm across cellular membranes, i.e. either influx from the extracellular space or efflux from intracellular organelles (Table 1, Figure 2). Most ZIP members have eight predicted transmembrane (TM) domains with their N- and C-termini facing the extracytoplasmic space (Figure 2, **inset**). A long loop region often harboring a histidine-rich domain is present between TM3 and TM4. TM4 and TM5 are particularly amphipathic and are thought to form a cavity through which metals are transported (Eide, 2006). Studies of Arabidopsis IRT1 have shown that conserved residues in this region are crucial for function (Rogers et al., 2000). Biochemical transport assays have shown that zinc uptake by ZIP1 and ZIP2 is energy independent (Gaither and Eide, 2000, 2001). HCO_3^- -stimulated zinc uptake by ZIP2 was detected suggesting a $\text{Zn}^{2+}/[\text{HCO}_3^-]_2$ symport mechanism (Gaither and Eide, 2000). Electroneutral $\text{Zn}^{2+}/[\text{HCO}_3^-]_2$ symport was also proposed for ZIP8 (Liu et al., 2008). In contrast, studies of a bacterial ZIP protein indicated that it formed a zinc-selective ion channel (Lin et al., 2010). X-ray crystal structures of ZIP transporters are not yet available.

ZIP proteins have been grouped into four subfamilies (Table 1). The LIV-1 subfamily has nine human ZIP members. These are distinct from other ZIPs by having a highly conserved putative metalloprotease motif (HEXPHEXGD) in addition to the histidine-rich loop domain (Taylor and Nicholson, 2003). Additional histidine residues on the N-terminal ectodomain and extracellular loop between TM2 and 3 are also found in LIV-1 subfamily members. Recently, bioinformatic analyses revealed similarities between LIV-1 subfamily members and prion genes (Schmitt-Ulms et al., 2009). It was shown that prion-like protein sequences were present in the N-terminal domains of ZIP5, ZIP6, and ZIP10, suggesting that prion proteins may be evolutionarily descended from ZIP proteins.

ZIP transporters play diverse roles in the physiology of cells and organisms. Their genes are expressed in various tissues and cell types, and their proteins are localized to distinct subcellular compartments (Table 1, Figure 2). Some ZIPs are regulated by dietary zinc, while others are responsive to hormonal signaling (Table 1). Such differential expression, subcellular localization, and transcriptional or post-transcriptional regulation provide clues to understand the unique physiological roles of each ZIP transporter. Studies with transgenic mice have also helped elucidate the functions of mammalian ZIPs (Table 2).

3. Zinc uptake and its regulation

ZIP1 is ubiquitously expressed in human tissues (Gaither and Eide, 2001). It is the major zinc uptake transporter in K562 erythroleukemia cells and prostate cells, where it is localized to the plasma membrane (Gaither and Eide, 2001; Dufner-Beattie et al., 2003; Franklin et al., 2003). Interestingly, ZIP1 is mainly present in intracellular organelles when cells were cultured in zinc-replete media, but translocates to the cell surface when zinc is limiting (Gaither and Eide, 2001; Milon et al., 2001). The mouse orthologs of ZIP1 and ZIP3 also traffic between intracellular organelles and the cell surface in response to zinc status (Wang et al., 2004b). It was found that a di-leucine sorting signal was needed for endocytosis of ZIP1 under zinc-replete conditions (Huang and Kirschke, 2007).

ZIP2 and ZIP3 were also reported to be zinc uptake transporters (Gaither and Eide, 2000; Kelleher and Lonnerdal, 2003, 2005). Even though ZIP3 function was required for survival of mammary gland epithelial cells in culture (Kelleher and Lonnerdal, 2005), homozygous *Slc39a3* knockout mice did not show visible phenotypes when fed an adequate zinc diet (Dufner-Beattie et al., 2005). However, abnormal embryonic development did occur when zinc deficiency coincided with pregnancy (Dufner-Beattie et al., 2005). Similarly, no overt

phenotypes were seen in homozygous single knockout mice of *Slc39a1* or *Slc39a2*, double knockout of *Slc39a1* and *Slc39a3*, and *Slc39a1*, *Slc39a2*, *Slc39a3* triple knockout mice fed adequate zinc diets. However, embryonic development in these mutant animals was also sensitive to zinc deficiency (Dufner-Beattie et al., 2006; Kambe et al., 2008). These studies suggest that ZIP1, ZIP2, and ZIP3 play key roles during pregnancy.

ZIP4 is involved in zinc absorption in the small intestine and is dynamically regulated by both transcriptional and post-transcriptional mechanisms. *SLC39A4* mRNA levels increased during dietary zinc restriction and the ZIP4 protein localized to the enterocyte apical plasma membrane, where it takes up dietary zinc from the lumen of the gut (Cousins, 2010). Up-regulation of *SLC39A4* transcripts during zinc deficiency might be due to mRNA stabilization rather than increased transcription of its gene (Weaver et al., 2007). However, the Krüppel-like factor 4 (KLF4) transcription factor was suggested to be responsible for regulating ZIP4 expression (Liuzzi et al., 2009). KLF4 is abundant in the intestine, induced during zinc restriction in mice, and binds to the mouse *Slc39a4* promoter (Liuzzi et al., 2009). Knockdown of KLF4 limited *Slc39a4* induction by zinc restriction and ⁶⁵Zn uptake by intestinal epithelial cells (Liuzzi et al., 2009).

Upon zinc repletion, *SLC39A4* mRNA was degraded and endocytosis of plasma membrane-localized ZIP4 rapidly occurred (Weaver et al., 2007). Endocytosis of ZIP4 occurs at high zinc concentrations and under still higher zinc concentrations, ZIP4 is degraded following its ubiquitination. Mutational analysis showed that the histidine-rich region between TM3 and TM4 is required for the zinc-induced ubiquitination and degradation of ZIP4 (Mao et al., 2007).

Studies in mice revealed that the N-terminal ectodomain of ZIP4 is proteolytically removed during prolonged zinc deficiency, leaving a 37-kD peptide of ZIP4 as the predominant form (Weaver et al., 2007; Kambe and Andrews, 2009). Cells overexpressing ZIP4 with ectodomain truncations showed hypersensitivity to zinc (Kambe and Andrews, 2009) and *SLC39A4* mutations near the predicted cleavage site of ectodomain that blocked processing of ZIP4 were found in patients with disrupted ZIP4 function (see below) (Kambe and Andrews, 2009). These results suggest that proteolytic processing of the ectodomain may play an important role in modulating ZIP4 activity.

ZIP5 is constitutively expressed in enterocytes and acinar cells, is localized to the basolateral surface of these cells, and specifically transports zinc (Dufner-Beattie et al., 2004; Wang et al., 2004a). Therefore, ZIP5 is thought to play a unique role in polarized cells by sensing zinc status via serosal-to-mucosal transport of zinc (Dufner-Beattie et al., 2004; Wang et al., 2004a). While *SLC39A5* mRNA is unresponsive to zinc status, translation of this mRNA is zinc regulated (Weaver et al., 2007). During zinc deficiency, ZIP5 protein translation was repressed (Weaver et al., 2007). However, *SLC39A5* mRNA remained associated with polysomes and could be rapidly translated when zinc was resupplied. A recent study has shown that the 3'-untranslated region (UTR) of the *SLC39A5* mRNA is involved in its post-transcriptional regulation in response to zinc availability (Weaver and Andrews, 2011). Interestingly, the 3'-UTR of *SLC39A5* mRNA is well conserved among mammalian orthologs, and forms a stem-loop structure flanked by potential seed sites for microRNA binding. Using wild type and mutant 3'-UTRs, these features were confirmed and shown to increase translation of ZIP5 when zinc is replete (Weaver and Andrews, 2011).

4. ZIPs and immune system function

Zinc deficiency is known to cause defects in immunity, abnormalities in T-cells, natural killer cells and monocytes, and reduction in antibody formation. However, mechanisms by which zinc affects the immune system are not well understood.

During LPS-induced dendritic cell (DC) maturation, dynamic changes in the expression of zinc transporters were observed, i.e. ZIP6 and ZIP10 were suppressed while several ZnTs were up-regulated (Kitamura et al., 2006). LPS-stimulation was shown to decrease free zinc in DCs, and increase expression of MHC class II and co-stimulatory molecules on DCs. Interestingly, treatment with a zinc chelator mimicked the LPS effect, while zinc supplementation or overexpression of ZIP6 inhibited up-regulation of MHC class II and co-stimulatory molecules (Kitamura et al., 2006). These results suggest that ZIP6 affects DC maturation events via regulating zinc homeostasis.

ZIP8 is highly expressed in T cells and induced upon in vitro activation (Aydemir et al., 2009). Knockdown of ZIP8 function resulted in reduced secretion of IFN- γ and perforin, signatures of T cell activation critical for immunity against pathogens and tumor suppression, while transient overexpression of ZIP8 caused enhanced activation of T cells (Aydemir et al., 2009). ZIP8 was localized to lysosomes and lysosomal labile zinc level was shown to decrease upon T cell activation. This suggests that ZIP8 is involved in IFN- γ regulation via lysosomal zinc transport (Aydemir et al., 2009).

ZIP8 also plays a critical role at the onset of inflammation, facilitating cytoprotection within the lung. Human *SLC39A8* was originally referred as *Bacillus calmette-guerin*-induced gene in monocyte clone 103 (BIGM103) due to its induction in primary monocytes following exposure to *Bacillus calmette-guerin* cell wall lipopolysaccharide (Begum et al., 2002). Inflammatory mediators such as LPS and TNF- α induce *SLC39A8* expression in the lung, and expression of glycosylated ZIP8, which localizes to plasma membrane and mitochondria (Besecker et al., 2008). Knockdown of ZIP8 reduced cellular zinc content, impaired mitochondrial function in response to TNF- α and increased cell death (Liuzzi et al., 2009). These results show that ZIP8 plays a critical role at the onset of inflammation, facilitating cytoprotection within the lung.

ZIP14, which is most closely related to ZIP8 evolutionarily, is also involved in inflammation. IL-6 is the main proinflammatory cytokine that regulates the response of acute-phase genes (Siewert et al., 2004). By screening *Slc30* and *Slc39* transcripts from wild type and *Il6*^{-/-} mice injected with an acute-phase inducer, *SLC39A14* was identified as an acute-phase gene responsive to IL-6 (Liuzzi et al., 2005). ZIP14 localizes to the plasma membrane of hepatocytes and contributes to hypozincemia in the liver (Liuzzi et al., 2005). Hypozincemia and hypoferremia are classic acute-phase responses (Moshage, 1997), and it is thought that decreasing zinc or iron levels in the serum would reduce availability of those minerals to pathogens, and this might help the host defense system.

5. Cadmium toxicity

ZIP8 was also identified by characterizing the *Cdm* gene locus, which is responsible for resistance to Cd-induced testicular toxicity in some inbred mouse strains (Dalton et al., 2005). Studies with metallothionein-null (A7) cells or mice showed that Cd-sensitivity correlated with high ZIP8 expression (Fujishiro et al., 2009b; He et al., 2009b; Himeno et al., 2009). Repression of *SLC39A8* expression in Cd-resistant cells was possibly due to epigenetic silencing, because hypermethylation of the CpG island of *SLC39A8* was detected in A7 cells (Fujishiro et al., 2009a). ZIP14-mediated Cd²⁺ uptake has also been detected (Girijashanker et al., 2008).

6. ZIPs and human iron metabolism

In addition to zinc and cadmium, ZIP14 has also been shown to transport transferrin-bound and non-transferrin-bound iron (NTBI) (Liuzzi et al., 2006; Zhao et al., 2010). Plasma iron is normally bound to transferrin but during iron overload, as occurs in hereditary hemochromatosis, the concentration of NTBI increases dramatically (Hentze et al., 2004). Hereditary hemochromatosis is caused by a mutation in the *HFE* gene (Feder et al., 1996). Interestingly, expression of *HFE* in HepG2 cells was shown to inhibit iron uptake by down-regulating the ZIP14 protein. *HFE* expression did not affect iron uptake when ZIP14 was knocked down (Gao et al., 2008). These results suggested that HFE controls ZIP14 activity, which consequently influences the iron loading of hepatocytes.

7. SLC39 proteins and human disease

Mutations in ZIP transporters are currently known to be associated with two genetic diseases in humans. Loss of function mutations in *SLC39A4*, encoding ZIP4, cause acrodermatitis enteropathica (AE) (Kury et al., 2002; Wang et al., 2002). This disorder disrupts intestinal zinc absorption and causes systemic zinc deficiency. AE is readily treated by oral zinc supplements indicating the presence of additional zinc transporters on the apical surface of enterocytes that are responsible for zinc absorption from the lumen of the gut. Mutations in *SLC39A13*, encoding ZIP13, cause a novel subtype of Ehlers-Danlos Syndrome (EDS) (Fukada et al., 2008; Giunta et al., 2008). EDS is a spectrum of connective tissue disorders caused by mutations affecting collagen synthesis and modification (Yeowell and Pinnell, 1993). The type associated with loss of ZIP13 function is referred to as spondylocheiro dysplastic-EDS (SCD-EDS), and involves skeletal dysplasia especially in the spine and hands in addition to the classical EDS symptoms (Fukada et al., 2008; Giunta et al., 2008). Collagens of SCD-EDS patients were underhydroxylated although activity of the lysyl and prolyl hydroxylases was normal (Giunta et al., 2008). *Slc39a13* knockout mice also showed phenotypic abnormalities that resembled EDS-symptoms, and it was found that ZIP13 was involved in BMP/TGF- β signaling pathways in connective tissues and nuclear translocation of Smad proteins (Fukada et al., 2008). How loss of ZIP13 affects these signaling pathways and causes SCD-EDS are still open questions.

Changes in intracellular zinc levels are associated with tumor growth and progression as zinc is a cofactor of enzymes involved in angiogenesis, cell proliferation, and metastasis of cancer, such as matrix metalloproteases and carbonic anhydrase. Altered expression of zinc transporters has been observed in many cancers and these changes may play causal roles in cancer progression. Breast cancer is associated with zinc hyperaccumulation in breast tissue and increased expression of ZIP6, ZIP7, and ZIP10 has been found associated with these changes (Taylor et al., 2007). Similarly, increased ZIP4 expression in pancreatic cells is associated with pancreatic cancer (Li et al., 2007; Li et al., 2009; Zhang et al., 2010; Zhang et al., 2011). In contrast, decreased zinc accumulation is associated with progression of prostate cancer (Huang et al., 2006; Golovine et al., 2008b; Johnson et al., 2010). Reduced accumulation of zinc in tumorigenic prostate epithelial cells might be due to decreased expression of ZIP1 protein or redistribution of ZIP3 to lysosomal vesicles (Huang et al., 2006; Johnson et al., 2010). Interestingly, expression of ZIP1 correlates with the zinc levels in prostate glands implying that ZIP1 may be the major regulator of prostate gland zinc (Golovine et al., 2008a; Johnson et al., 2010). Table 1 also provides references to studies linking ZIP function to other cancers and disease states such as neurodegeneration.

8. Perspectives

This review briefly summarizes our current knowledge of the ZIP transporters and their roles in cellular function, human physiology, and pathology. It should be noted that many

important questions remain to be resolved. For example, little work has been done on some ZIP family members, such as ZIP9, ZIP11 and ZIP12, and their biological roles are unclear. In addition, we still do not know the precise biochemical mechanisms used by ZIP family members to mediate zinc transport. How ZIP family members and other transporters or trafficking systems coordinate to regulate zinc homeostasis is also yet unknown. Although there has been a clear correlation established between ZIP transporters and cancer and other human diseases, the actual role of these transporters on the target genes or proteins involved often remains to be understood. Once these questions are resolved, it will greatly contribute to understanding the biology of ZIP transporters and improving human health.

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Figure 1.

Phylogenetic relationships of the human ZIP transporters. Protein sequences were retrieved from NCBI, and the dendrogram was generated from the amino acids sequences aligned by ClustalW2 and the phylogenetic graphics program TreeDyn (Chevenet et al., 2006). Among the 55 human SLC families, the SLC39 family is one of the six families involved in metal ion transport (He et al., 2009a). SLC39 proteins are members of the 2.A.5.2.1, 2.A.5.3.1, and 2.A.5.4.1 categories of the Transporter Classification Database (<http://www.tcdb.org/>).

Figure 2. Subcellular localization of human ZIP transporters. Directions of zinc transport are shown as arrows. Note that this figure only provides a very simplified view, as expression of some ZIP proteins are tissue- or cell type-specific, and regulated by zinc status or other signals. The inset shaded in *yellow* depicts the predicted topology of a ZIP transporter.

Summary of ZIP transporters

Table 1

Gene/ Subfamily	Protein/ Alias	Substrates	Tissue distribution in humans/ Response to Zn or hormones ^a	Subcellular localization	Disease/ Pathology	Human gene locus	Sequence accession ID
SLC39A1/ subfamily II	ZIP1/ ZIRT1	Zn	Widespread/ Prolactin (+), testosterone (+), IL-6 (+), cell differentiation (+)	Plasma membrane, intracellular vesicles	Prostate cancer, neurodegeneration ^b	1q21	NM_01443
SLC39A2/ subfamily II	ZIP2/ Eti-1, 6A1	Zn	Widespread	Plasma membrane		14q11.1	NM_014579.2
SLC39A3/ subfamily II	ZIP3	Zn, not specific	Widespread, mammary cells, testis/ High Zn (-), prolactin (+)	Plasma membrane, lysosomes	Neurodegeneration ^b	19p13.3	NM_144564 NM_213568
SLC39A4/ LIV-1	ZIP4	Zn	Gastrointestinal tract, kidney hippocampal neurons/ Low Zn (+)	Plasma membrane Apical surface of enterocytes, lysosomes	AE, pancreatic cancer, liver cancer ^c	8q24.3	NM_017767.2 NM_130849
SLC39A5/ LIV-1	ZIP5/ LZT-Hs7	Zn	Pancreas, kidney, liver, stomach, intestine/ IL-6 (+)	Plasma membrane Basolateral surface of enterocytes		12q13.13	NM_173596.2 NM_001135195
SLC39A6/ LIV-1	ZIP6/ LIV-1	Zn	Widespread/ IL-6 (+), IL-1 (+), LPS(+)	Plasma membrane	Breast, pancreatic ^d , cervical ^e , prostate cancer ^f , neuroblastoma ^g	18q12.1	NM_012319.3 NM_001099406
SLC39A7/ LIV-1	ZIP7/HKE4 , RING5	Zn, Mn	Widespread	ER, Golgi, intracellular vesicles	Breast cancer	6p21.3	NM_006979.2 NM_001077516
SLC39A8/ LIV-1	ZIP8/ BIGM103, LZT-Hs6	Zn, Cd, Mn	Widespread, T-cells, erythroid, testis/ LPS (+), immune activation (+), TNFα (+)	Plasma membrane, Lysosomes, mitochondria	Inflammation, breast cancer	4q22-q24	NM_022154 NM_001135146 NM_001135147 NM_001135148
SLC39A9 subfamily I	ZIP9			trans-Golgi ^h		14q24.1	NM_018375.3
SLC39A10/ LIV-1	ZIP10/ LZT-Hs2	Zn	Brain, liver, erythroid, kidney/ Low Zn (+), thyroid hormone (+), regulated by MTF-1 ⁱ	Plasma membrane	Breast cancer	2q33.1	NM_020342.2 NM_001127257
SLC39A11 gufA	ZIP11					17q25.1	NM_139177.3

Gene/ Subfamily	Protein/ Alias	Substrates	Tissue distribution in humans/ Response to Zn or hormones ^a	Subcellular localization	Disease/Pathology	Human gene locus	Sequence accession ID
SLC39A12 LIV-1	ZIP12/ LZT-Hs8	Zn	Brain, lung, testis, retina			10p12.33	NM_152725.3
SLC39A13 LIV-1	ZIP13/ LZT-Hs9	Zn	Widespread	Intracellular vesicles, Golgi	SCD-EDS	11p11.12	NM_152264.3 NM_001128225
SLC39A14 LIV-1	ZIP14/ LZT-Hs4	Zn, Fe, Mn, Cd	Widespread, liver/ IL-1 (+), IL-6 (+), NO (+)	Plasma membrane	Asthma, inflammation, colorectal cancer ^k	8p21.2	NM_015359.4 NM_001128431 NM_001135153 NM_001135154

^a (+), up-regulation; (–), down-regulation. References not cited in the manuscript:

^b (Qian et al., 2010),

^c (Weaver et al., 2010),

^d (Unno et al., 2009),

^e (Zhao et al., 2007),

^f (Lue et al., 2011),

^g (Chovanadisai et al., 2008),

^h (Matsuura et al., 2009),

ⁱ (Wimmer et al., 2005),

^j (Bly, 2006),

^k (Thorsen et al., 2011).

Table 2

Transgenic mouse studies on ZIP transporters

Gene	Alteration	Phenotype
<i>Slc39a1</i>	Knockout; homozygous	No visible phenotype with adequate zinc diet; abnormal embryo development under zinc deficiency
<i>Slc39a2</i>	Knockout; homozygous ^a	No visible phenotype with adequate zinc diet; abnormal embryo development under zinc deficiency
<i>Slc39a3</i>	Knockout; homozygous	No visible phenotype with adequate zinc diet; abnormal embryo development under zinc deficiency
<i>Slc39a1, Slc39a3</i>	Knockout; homozygous	No visible phenotype with adequate zinc diet; abnormal embryo development under zinc deficiency; reduced passive zinc uptake into CA1 neurons ^b
<i>Slc39a1, Slc39a2, Slc39a3</i>	Knockout; homozygous	No visible phenotype with adequate zinc diet; abnormal embryo development under zinc deficiency
<i>Slc39a4</i>	Knockout; homozygous ^c	Embryonic lethal; not rescued by excess zinc supplementation
<i>Slc39a4</i>	Knockout; heterozygous ^c	Hypersensitive to zinc deficiency; Growth retardation, morphological defects, abnormal neurogenesis
<i>Slc39a8</i>	Increased gene number ^d	Cd sensitivity
<i>Slc39a8</i>	<i>Slc39a8(neo)</i> ; hypomorph ^e	Dramatically decreased ZIP8 expression in embryo, fetus and visceral yolk sac
<i>Slc39a13</i>	Knockout; homozygous	Phenotypes similar to SCD-EDS symptoms
<i>Slc39a14</i>	Knockout; homozygous ^f	Growth retardation, impaired gluconeogenesis, impaired GPCR-signaling

References not included in the listed references:

^a (Peters et al., 2007),

^b (Qian et al., 2010),

^c (Dufner-Beattie et al., 2007),

^d (Wang et al., 2007),

^e (Wang et al., 2011),

^f (Hojyo et al., 2011).