

Different Zinc Sources Have Diverse Impacts on Gene Expression of Zinc Absorption Related Transporters in Intestinal Porcine Epithelial Cells

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Abstract

Intestinal zinc absorption is a complex process involving several transporters. In this study, we investigated the effects of different zinc sources on the gene expression of zinc absorption related transporters in intestinal porcine epithelial cells (IPEC-1). IPEC-1 cells were treated with zinc sulfate (ZnSO₄), zinc gluconate (Zn-Glu), zinc methionine (Zn-Met), and zinc citrate (Zn-Cit) at a concentration of 50 μg/L. The expression of SLC39A4, SLC30A1, and DMT1 was measured by quantitative real-time PCR (qRT-PCR). The results showed that ZnSO₄ treatment significantly increased the expression of SLC39A4 (IP4) and SLC30A1 (T1) ($P < 0.05$), while Zn-Glu, Zn-Met, and Zn-Cit treatments had no significant effect on the expression of these transporters. Additionally, ZnSO₄ treatment significantly increased the expression of DMT1 (DMT1) ($P < 0.05$), while Zn-Glu, Zn-Met, and Zn-Cit treatments had no significant effect on the expression of DMT1. These findings suggest that different zinc sources have diverse impacts on the gene expression of zinc absorption related transporters in intestinal porcine epithelial cells.

G

DMT1 RNA

. I

IPEC-1

SO₄. M, -G. -
M
T1, DMT1. IP4
-G. IP4
-M IP4.

Keywords

· RNA

Introduction

Zinc is an essential trace element for human health. It plays a crucial role in many biological processes, including enzyme activity, protein synthesis, and DNA replication. Zinc deficiency can lead to various health problems, such as growth retardation, immune deficiency, and neurological disorders. Intestinal zinc absorption is a complex process involving several transporters. The main transporters involved in zinc absorption are SLC39A4 (IP4), SLC30A1 (T1), and DMT1. SLC39A4 (IP4) is a zinc transporter that is expressed in the apical membrane of intestinal epithelial cells. SLC30A1 (T1) is a zinc transporter that is expressed in the basolateral membrane of intestinal epithelial cells. DMT1 is a zinc transporter that is expressed in the apical membrane of intestinal epithelial cells. The expression of these transporters is regulated by various factors, including zinc status, hormones, and diet. In this study, we investigated the effects of different zinc sources on the gene expression of zinc absorption related transporters in intestinal porcine epithelial cells (IPEC-1). IPEC-1 cells were treated with zinc sulfate (ZnSO₄), zinc gluconate (Zn-Glu), zinc methionine (Zn-Met), and zinc citrate (Zn-Cit) at a concentration of 50 μg/L. The results showed that ZnSO₄ treatment significantly increased the expression of SLC39A4 (IP4) and SLC30A1 (T1) ($P < 0.05$), while Zn-Glu, Zn-Met, and Zn-Cit treatments had no significant effect on the expression of these transporters. Additionally, ZnSO₄ treatment significantly increased the expression of DMT1 (DMT1) ($P < 0.05$), while Zn-Glu, Zn-Met, and Zn-Cit treatments had no significant effect on the expression of DMT1. These findings suggest that different zinc sources have diverse impacts on the gene expression of zinc absorption related transporters in intestinal porcine epithelial cells.

8–12. H

T, -G, -M, SO₄, IP4, IPEC-1

β- 2^{-ΔΔC} 15. ZIP4 siRNA Transfection

Materials and Methods

Cell Culture

IPEC-1 13. T DMEM/F12 10 % FBS, 2 /L L- (5 /L), (5 /L), (5 μ /L), (S) 37_C 5 % CO₂ . IPEC-1 30-50.

T IP4 I RNA (RNA) : IP4- , 5'-CUCAG UACUUCGUGGACUUTT-3', IP4- , 5'-AAGUCCACGAAGUACUGAGTT-3'. A , RNA (, 5'-UUC UCC GAA CGU GUC ACG UTT-3'; , 5'-ACG UGA CAC GUU CGG AGA ATT-3'). RNA. IPEC-1

Cell Viability Assay

IPEC-1 96- 10⁴. W 80 % DMEM -G, -M, SO₄, 0, 50, 100, 150, 200 μ /L. A 6, 12, 24 , 3-(4,5- -2-)-2,5- -2H- (MTT) . F (-G, -M , SO₄), 20 μL MTT (5 / L). 4 37_C. A 150 μL DMSO 570 (B. -R). C 14.

RNA, 1 4, (T 2). T RNA (100 L TM2000 R (I ,C ,CA,USA) RNA 24 48 , IP4 RNA (RNA) PCR IP4 β-

MT1, DMT1, ZIP4, and ZnT1 mRNA Expressions in IPEC-1 Cells

IPEC-1 (10⁵) 50 μ /L -G, -M, SO₄, 6 , 0 μ /L T RNA IPEC-1 TRI (I ,C ,CA, USA), 0.5 μ RNA - DNA 10 μL P S TM RT (T ,T ,J). T , 30_C 10 , 42_C 30 , 70_C 15 R - PCR. QTM 5 R -T PCR D S (B. -R I , H , CA, USA). T : 1 95_C, 40 (10 95_C 25 63_C). P MT1, DMT1, IP4, T1, β- P E 2.0 (T 1). R

Transfection with ZIP4 siRNA on Cell Viability

C , IP4- RNA- (10⁵ / L). 96- . T 500 μL DMEM + 1.5 L DMEM/F12. T RNA. A 24- 48- MTT

MT1 and DMT1 Expression and Zinc Absorption Rate

N IP4 RNA A 24- , 50 μ /L -G, -M , SO₄ 6 , T , MT1 DMT1 I 16. IPEC-1 (PBS), 1 /L EDTA PBS, H₂O 10 L (ICP-MS).

Statistical Analysis

D SPSS (19.0) ANOVA. D T ' .G

Table 1 P

PCR	I	N . G	P	(5' . 3')	L
β-		XM 003124280.3	CCTGCGGCATCCACGAAAC		123
			TGTCGCGCATGCCTGGGTA		
MT1		NM 001001266.2	CTGTGCCTGAAGTCTGGGGAA		115
			CACAGAAAAAGGGATGTAGCATG		
DMT1		NM 001128440.1	GCTCTCATACCCATCCTCACGTTT		142
			GGACGTAAACCACGACGAAGTACA		
4		XM 001925360.3	CAGGGTCATCTGGGAAAGGAAGC		101
			CCGGCACTCAGGCACATCGTG		
T1		NM 001139470.1	AAAATGTGAAGACCCGACATCGTA		94
			AGGTTGAATGGTGGTAGCGTGAA		

M 1
D 1
SLC39A4
SLC30A1

t . D
P < 0.05
-M
IP4 RNA
RNA
(P < 0.05)
MT1
T1 RNA
(P < 0.05)
-G
SO₄
-M
(P < 0.05)
E
DMT1

Results

Cell Viability Assay

MTT
(P < 0.05)
6, 12, 24 (F . 1).
-G
SO₄
50 μ /L
-M

Cell Viability Assay in ZIP4 siRNA Cells

IPEC1
RNA
IP4 RNA 24 48
(P < 0.05), 64.60 % (P < 0.05) 78.43 %
RNA 1 (F . 3). M
IP4- RNA1 IPEC1
24 48 (P > 0.05) F . 3. S IP4 RNA1
24

MT1, DMT1, ZIP4, and ZnT1 mRNA Expressions

F 2
MT1
(P < 0.05),
IP4
T1 RNA
(P < 0.05)

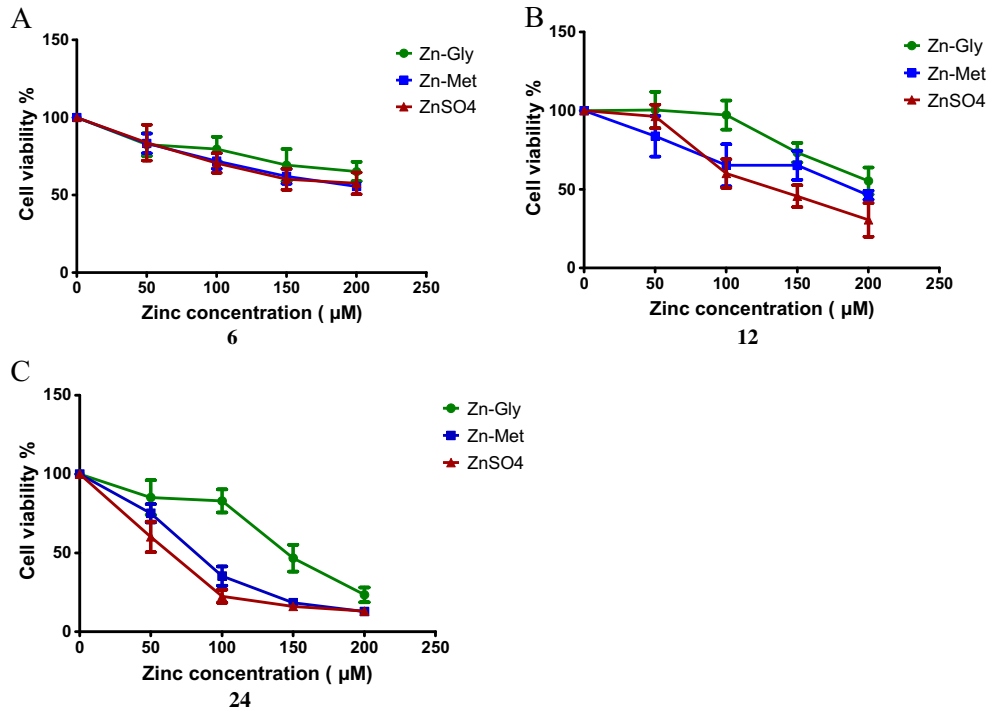
MT1 and DMT1 mRNA Expression and Zinc Absorption Rate in ZIP4 siRNA Cells

A F . 4, 16.94 % (P < 0.05)
33.62 % (P < 0.05)
MT1 RNA

Table 2 P
IP4 RNA

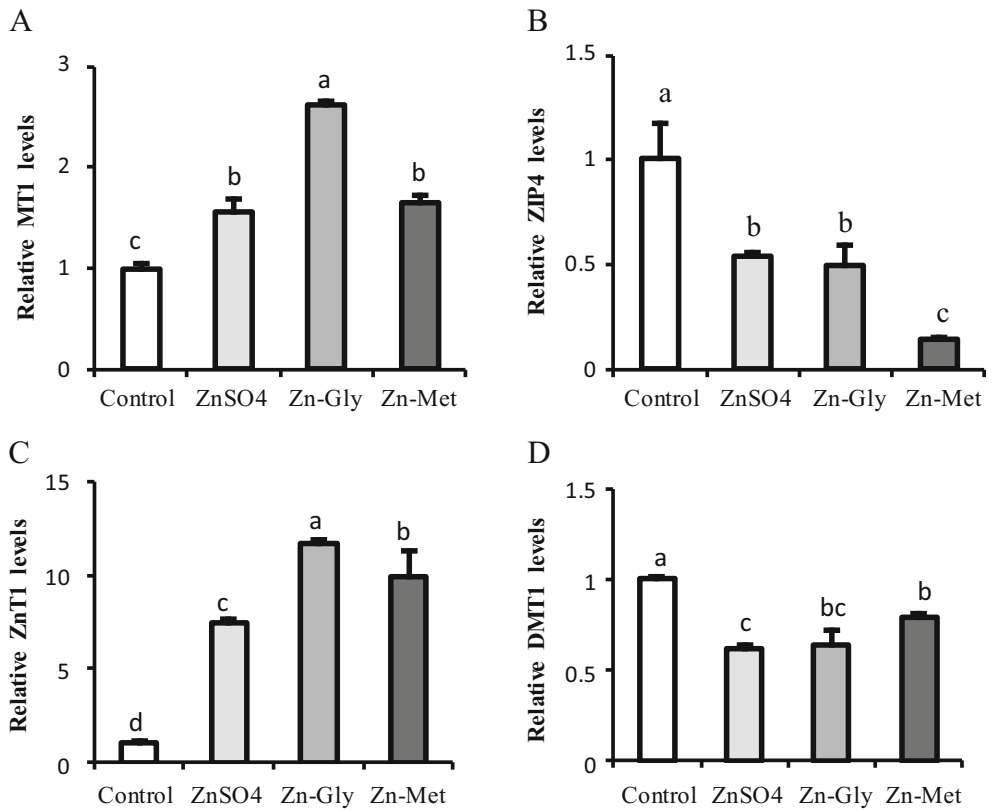
RNA	P	(5' . 3')
IP4 RNA-663	S	CUC AGU ACU UCG UGG ACU UTT
	A	AAG UCC ACG AAG UAC UGA GTT
IP4 RNA-711	S	CCA ACA UCA CAC UGG CUG ATT
	A	UCA GCC AGU GUG AUG UUG GTT
IP4 RNA-1583	S	GUG CAC AAC UUC GCU GAU GTT
	A	CAU CAG CGA AGU UGU GCA CTT
IP4 RNA-1978	S	GCU GUC UCU GUA UGA GGA UTT
	A	AUC CUC AUA CAG AGA CAG CTT

Fig. 1 V IPEC-1
6-, 12-, 24-
SD
(n = 6)



SO4 -G IP4 28.95 % ($P < 0.05$)
RNA H, DMT1 RNA IP4 26.44 % ($P < 0.05$), S IP4
IP4- RNA -G -M 73.33 % ($P < 0.05$)

Fig. 2 R RNA IPEC-1
1 6-
50 μ /L SO4, -G,
-M, V
SD (n = 3). L
 $P < 0.05$. MT1,
1; IP4,
SLC39A4; T1,
SLC30A1; DMT1,
1



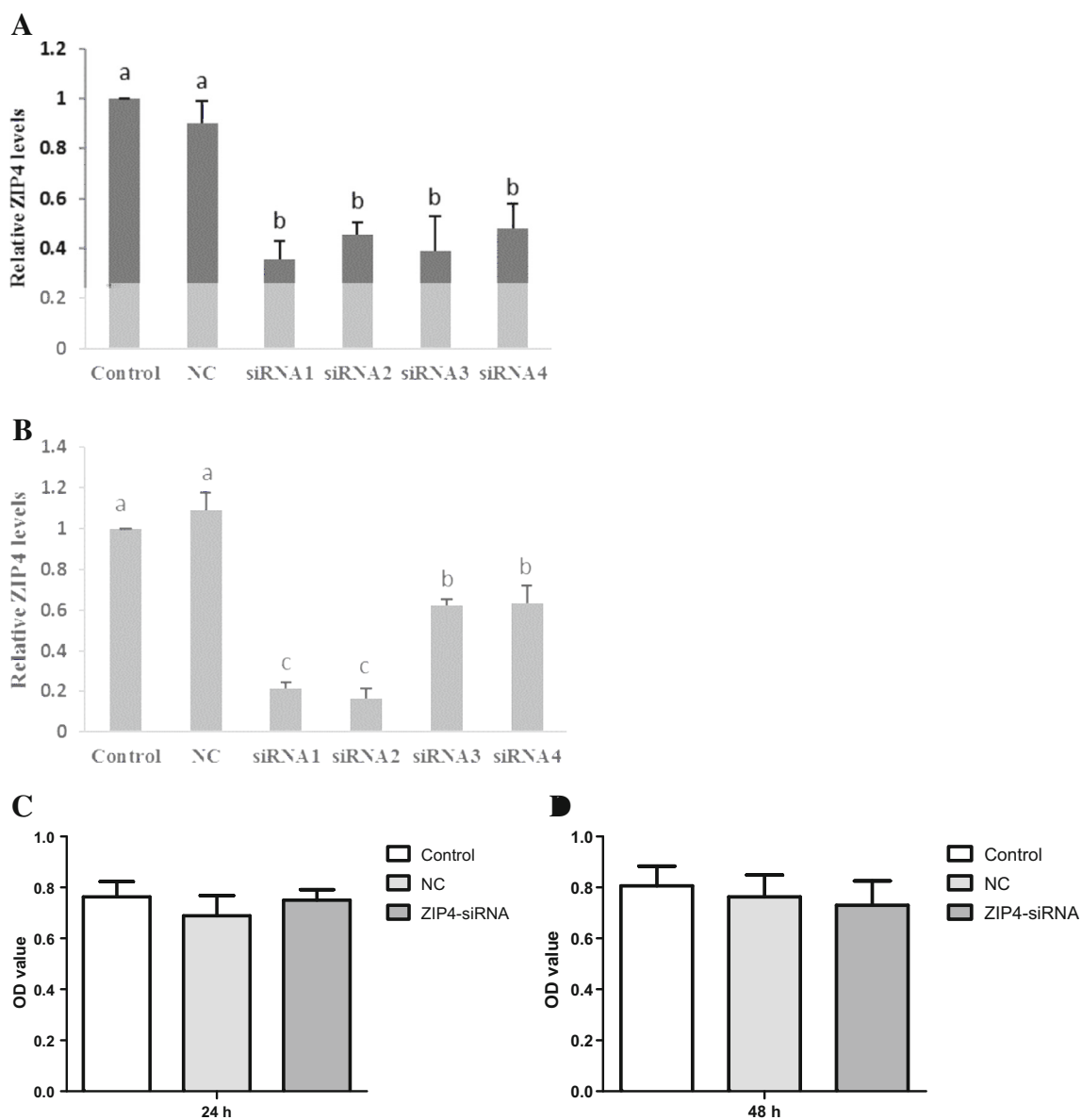
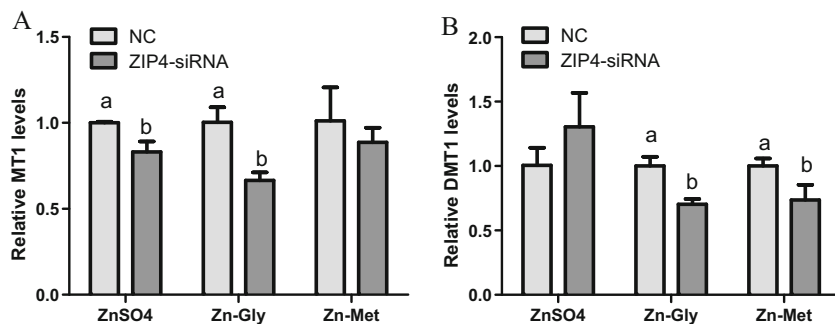


Fig. 3 IP4 RNAi efficiency and cell growth. (a) IP4 RNAi efficiency in IPEC-1 cells at 24 h. (b) IP4 RNAi efficiency in IPEC-1 cells at 48 h. (c) Cell growth (OD value) at 24 h. (d) Cell growth (OD value) at 48 h. Error bars represent standard deviation (SD) (n = 3). L, P < 0.05.

RIP4 RNAi efficiency in IPEC-1 cells. IP4 RNAi was performed in IPEC-1 cells for 24 h (a) and 48 h (b). Cell growth was measured by OD value at 24 h (c) and 48 h (d). Error bars represent standard deviation (SD) (n = 3). L, P < 0.05.

Fig. 4 DMT1 mRNA levels in IPEC-1 cells. IPEC-1 cells were treated with ZnSO₄, Zn-Gly, or Zn-Met for 24 h. DMT1 mRNA levels were measured by qPCR. Error bars represent standard deviation (SD) (n = 3). L, P < 0.05.



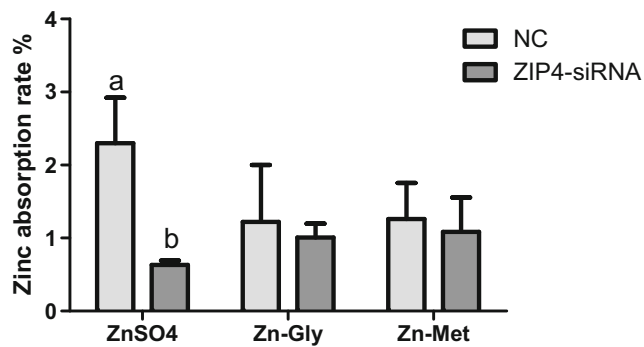


Fig. 5 D IPEC-1 IP4 RNA. V SD (n = 3). L , P < 0.05

Discussion

H RNA (P < 0.05) T1 RNA (2015) T1 RNA -G. L (2015) T1 RNA IP4 RNA T1 RNA MT1 T1 SO₄ 100 μ /L IP4 RNA T1 IPEC-J2 -G -M 18 . T MT1 SO₄ I SO₄ M 1 (MT1) (2008) IP4 RNA C -2 22 . I 19, 20 . I MT1 5, 21 , MT1 22, 23 . O MT1 RNA DMT1 -G (2015) 200 μ /L SO₄ 0 IPEC-J2 (2007) MT RNA 37 . I IP4 RNA (P < 0.05) SO₄ SO₄ H C T 25 . T 26 . G), G . A IP4 RNA SO₄ MT (MTF-1) RNA DMT1 SO₄ 27 . M (2014) DMT1 H . (2013) T . (2012) 38 . T G

IP4
 30. H , DMT1 RNA
 -G -M
 SO₄ , -G
 -M IP4- RNA
 .T IP4
 -G IP4 -M

Conclusions

I
 IPEC-1
 SO₄. M , -G -M
 MT1, IP4, T1,
 DMT1. IP4
 -G IP4
 -M IP4.

Acknowledgments T N N
 S F C (G N . 31472102), K S
 P "973" A N S T
 C (G N . 2012CB124705).

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NCIMB 10415
F U B , G , D