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

Danping  $\operatorname{Huang}^1 \cdot \operatorname{Zhao} \operatorname{Zhuo}^1 \cdot \operatorname{Shenglin} \operatorname{Fang}^1 \cdot \operatorname{Min} \operatorname{Yue}^1 \cdot \operatorname{Jie} \operatorname{Feng}^1$ 

R : 19 D 2015/A : 15 F 2016/P © S S +B M N 2 2016	: 19 M 2016
S S +B M N 2016  Abstract T  7 (IPEC-1). IPEC-1 ( -G )7 - ( -M ), 7 ( SO <sub>4</sub> ), , .  T , IPEC-1  7 (50 μ /ΙΖ ).  SLC39A4 ( IP4) - IP4 7 - SO <sub>4</sub> 7 - SO <sub>4</sub> 7 - IP4 (P < 0.05), IP4 (P < 0.05) RNA (RNA) (P < 0.05)	SO <sub>4</sub> M , -G , MT1, IP4, T1, DMT1, IP4, T , AT, AT, AT, AT, AT, AT, AT, AT, AT
7. ; IPEC-1 ; 7. ; 7. ;	A , DMT1  , , , , , , , , , , , , , , , , , , ,

326 H

# **Materials and Methods**

### **Cell Culture**

#### **Cell Viability Assay**

IPEC-1 96- $10^4$ . W 80 % . . DMEM \_ G -G , -M , , SO<sub>4</sub>, 0, 50, 100, 150, 200 μ . /L. A 3-(4,5--2H- 7... (MTT) 6, 12, 24 , 7 -2- )-2,5-. F  $^{\circ}$ ,  $^{\circ}$ ,  $^{\circ}$ ,  $^{\circ}$ ,  $^{\circ}$ ,  $^{\circ}$ ,  $^{\circ}$ SO<sub>4</sub>), 20 μL MTT (5 / L). 4 37 C. A - 150 μL DMSO (B<sub>2</sub> -R ). C 14.

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

#### ZIP4 siRNA Transfection

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 ATT3')

RNA. IPEC-1

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

 $2^{-\Delta\Delta C}$ 

15.

# Transfection with ZIP4 siRNA on Cell Viability

C , IP4- RNA-(10<sup>5</sup> / 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 ,

SO<sub>4</sub> 6 , .T ,

MT1 DMT1 
I 7

16 . IPEC-1

(PBS), 
1 /L EDTA PBS,

H2O 10 L

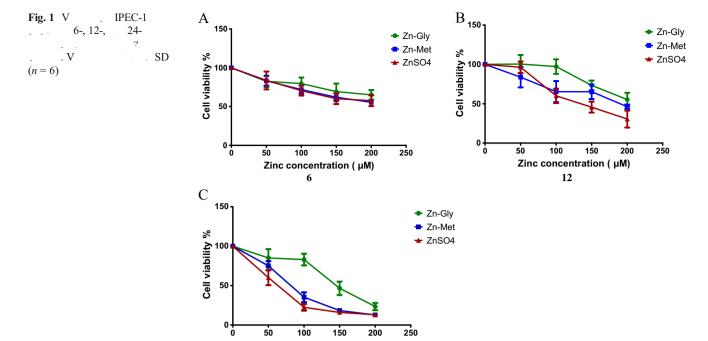
(ICP-MS).

# **Statistical Analysis**



Table 1 P - PCR	I	$N \cdot G = G$	P	(5' , 3')	
- PCR	β-	XM 003124280	3 CCTG	CGGCATCCACGAAAC	123
	MT1	NM 001001266.		GGCGATGCCTGGGTA GCCTGAAGTCTGGGGAA	115
	DMT1	NM 001128440.		GAAAAAGGGATGTAGCATC TCATACCCATCCTCACGTTT	
			GGAC	GTAAACCACGACGAAGTAC	CA
	4	XM 001925360.	CCGG	GTCATCTGGGAAAGGAAGC CACTCAGGCACATCGTG	
	; <b>T</b> 1	NM 001139470.	1 AAAA AGGT		
	M , , , , , ,	1 1			
		SLC39A4 SLC30A1			
P < 0.0	)5		IP4 RNA	MT1 T1 RNA $(P < 0.05)$ -M $(P < 0.05)$ . E	SO <sub>4</sub> , DMT1
Results				(P < 0.05)	
Cell Viability Assay			Cell Viability Assay in ZIP4 siRNA Cells		
6, 12, 24	(P < 0.05). 4 (F . 1) (SO 50 μ . /L . (O	G , , , , -M O <sub>4 -</sub> ,	IP4- RNA1	RNA 1 (F . 3). M	24 48 78.43 % ,
- · ·					
MT1, DMT1, ZIP4, and Zn	T1 mRNA Exp	ressions		Γ1 mRNA Expression and	Zinc Absorption
MT1, DMT1, ZIP4, and Zn	7_ ',		Rate in ZIP4 s	iRNA Cells	6 (P < 0.05)
MT1, DMT1, ZIP4, and Zn $F = 2$ MT1  T1 $P < 0.05$ ), IP4	Z., , , RNA ,		Rate in ZIP4 s	F .4, 16.94 % ( 0.05)	6 (P < 0.05)
MT1, DMT1, ZIP4, and Zn $P < 0.05$ , IP4  Table 2 P	P < 0.05		Rate in ZIP4 s	F .4, 16.94 % (5)	% (P < 0.05) MT1 RNA
MT1, DMT1, ZIP4, and Zn $ \begin{array}{ccccccccccccccccccccccccccccccccccc$	RNA $(P < 0.05)$ RNA $IP4 RNA-66$	33	A	P (5  CUC AGU ACU U  AAG UCC ACG A	MT1 RNA  "3' 3')  CG UGG ACU UTT AG UAC UGA GTT
MT1, DMT1, ZIP4, and Zn $ \begin{array}{ccccccccccccccccccccccccccccccccccc$	RNA $ (P < 0.05) $ RNA	33	Rate in ZIP4 s  A	P (5  CUC AGU ACU U  AAG UCC ACG A  CCA ACA UCA C	(P < 0.05) MT1 RNA  (' 3') CG UGG ACU UTT AG UAC UGA GTT AC UGG CUG ATT
MT1, DMT1, ZIP4, and Zn $ \begin{array}{ccccccccccccccccccccccccccccccccccc$	RNA $(P < 0.05)$ RNA $IP4 RNA-66$	33 1	Rate in ZIP4 s  A	P (5  CUC AGU ACU U AAG UCC ACG A CCA ACA UCA C UCA GCC AGU G	MT1 RNA  "3' 3')  CG UGG ACU UTT AG UAC UGA GTT
MT1, DMT1, ZIP4, and Zn  F 2 MT1 T1 $(P < 0.05)$ , IP4  Table 2 P	RNA $(P < 0.05)$ RNA $IP4 RNA-66$ $IP4 RNA-71$	33 1	Rate in ZIP4 s  A	P (5  CUC AGU ACU U AAG UCC ACG A CCA ACA UCA C UCA GCC AGU G GUG CAC AAC U	(G (P < 0.05) MT1 RNA  G' . 3') CG UGG ACU UTT AG UAC UGA GTT AC UGG CUG ATT UG AUG UUG GTT
MT1, DMT1, ZIP4, and Zn $ \begin{array}{ccccccccccccccccccccccccccccccccccc$	RNA $(P < 0.05)$ RNA $IP4 RNA-66$ $IP4 RNA-71$	1 1 1 1883	Rate in ZIP4 s  A	P (5  CUC AGU ACU U  AAG UCC ACG A  CCA ACA UCA C  UCA GCC AGU G  GUG CAC AAC U  CAU CAG CGA A	(P < 0.05)  MT1 RNA  G' . 3')  CG UGG ACU UTT  AG UAC UGA GTT  AC UGG CUG ATT  UG AUG UUG GTT  UC GCU GAU GTT

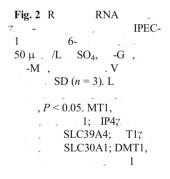
328 Н

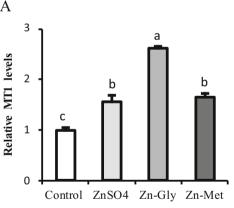


, DMT1 RNA IP4 RNA SO4 28.95 % (P < 0.05). S , , , IP4 26.44 % (P < 0.05),IP4- RNA -G -M 73.33 % (P < 0.05)

200

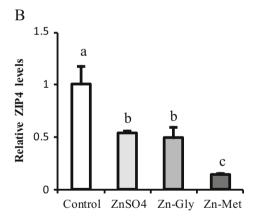
250

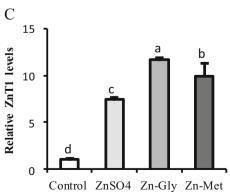


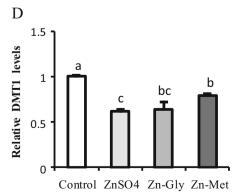


100

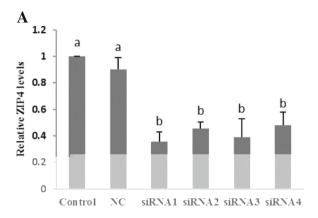
150 Zinc concentration ( µM) 24

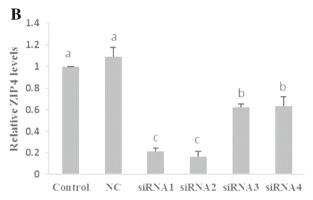


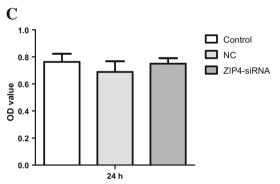












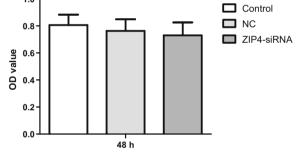


 Fig. 3
 IP4
 RNA
 IP4
 RNA

 IP4
 RNA
 IP6C-1
 .

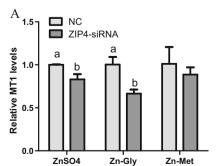
 R
 IP4
 24
 (a)
 48
 (b)
 IP4

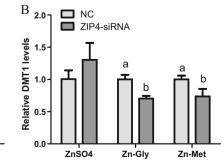
RNA1 ... SD (n = 3). L (c) 48 (d). V P < 0.05

 $SO_4$  -G -FNA -F

1.0 7

Fig. 4 D 7 MT1 RNA IPEC-1 IP4 RNA. V SD (n = 3). L , P < 0.05







330 H

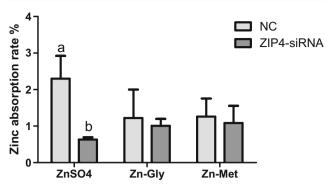


Fig. 5 D  $P^{2}$  IP4 RNA. V  $P^{2}$  SD  $P^{2}$  SD  $P^{2}$  SD  $P^{2}$  SD  $P^{2}$   $P^{2}$  SD  $P^{2}$ 

#### Discussion

7. 7. 7. 17. T 100 μ /L . T \_ -IPEC-J2 18. T -M  $SO_4$ . I SO<sub>4</sub>, 1 (MT1) <sub>-</sub> 7. 19, 20 . I MT1 5, 21, MT1 RNA 7 ', -G 7. 1. L  $200~\mu$  , /L  $SO_4$  0 IPEC-J2 C \_ -2 24 . M . (2007) \_ -MT RNA НСТ 25 . T 7\_ ', (MTF-1) MT 27. . (2013) T . (2012) Η ,

IP4. , MT. , T1, 7. MT 28, 29 . IP4 IP4 RNA RNA 30, 31 . I IP4 RNA 5, 31, 32. T1 . RNA \_\_\_\_\_\_ 33 . T 7. T1 RNA \_ (P < 0.05)IP4 RNA RNA T1 34 . DMT1, 35.T DMT1 (2008) IP4 RNA DMT1 22. I 36.T IP4, T1, DMT1 , MT1,. IP4 DMT1 7. IP4 RNA RNA RNA 7. 37 .I MT1 RNA (P < 0.05 SO RNA ; **7**. ; G



IP4 30 . H , DMT1 RNA , , , , , , , , , , -G -G , -M  $SO_4$ IP4- RNA IP4 ', ', ', **-**G IP4 ,. 7. 7. 7. -M

#### **Conclusions**

 $SO_4$ . M , G G G G

 Acknowledgments
 T
 N
 N
 N
 N

 S
 F
 C
 (G
 N
 31472102), K
 K
 S

 P
 "973" A
 N
 N
 S
 T
 T

#### References

- 1. K T, T T, H . . A, . (2015) T P R 95:749–784

- . J N 143: 1205-1210
- 6. K T, -I , S R, N M (2004) O ... C M L S 61:49–68
- 8. W KJ, L AJ, G MA, M PS (1994)
- . J A S 72:2681–2689
- TL, A GA, L GF, P. DS (1996)

- 10. W , T , JW, M WQ, F , J (2010) D 7. 7 . B. T É 7-133:325–334
- 11. M WQ, N H, F , J, W , , F , J (2011) E .B. T E R 142:546–
- 12. M, F , SL, , L DD, F , J (2015) P A N 99:457–464
- 13. L S, , , M , S, C , X, B DD (2002) O
- A-1V

  J B. C 277:31929–31937

  14. D S, C F, N N, P A, C
  C J, F A, S M (2004) D

  7 T-1, T-5 T-7

  7 : RT-PCR B P

- 379–386
- 18. L , U, E , R, S , F, ... (2013) E , 7. ...
- 19. H TC, K DW, S SV, (2015) A 7\_ '; 7\_ 7\_ '; . B. . . :10.1007/ 10534-015-9883-3
- 20. G V, S DA, S S, (2005) P 1 2 ... MCF-10 A ... T ... S 85:906–915
  21. Q , T ... D, F ... CP, C ... RA (2009) S ... L 450:206–210 L 450:206-210
- 22. S H, Q HH, G JS (2008) C
- . J N 131:46–52 24. L U, G EM, A JR, . (2015) D . J B. M T. 29:410-
- 417 25. M H, O , O , . . (2007) A7. (II)-

- . M A M 34:548–560

332 H

29.	T	S (2012) M
	, ;	.JH O 5:41
30.		J, V , KJ, D L RC, A GK (2012) A
	_	; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
	_	IP4 (S 39 4)
		. PL S G 8(6): 1002766
31.	D ,	-B J, K M, G J, A GK (2004) T
		<b>7</b> ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
		, <b>z</b> ,, , <b>z</b> ,
	IP4	IP5. J B. C 279:49082–49090
32.	W	BP, D -B J, K T, A GK (2007) N
	7. , -	naj kala <del>-</del> Granda da Karana da Kar
	_	S 39 4 , S 39 5 ,
	( 4	5). J B, C 388:1301–1312
33.		, K CP, H L (2007) I
	_	T1, 4, 5, 6, 7
		C 55:223–234

