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Copper egress is induced by PMA in human THP-1 monocytic cell line

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Abstract Copper egress is an essential regulator of the kinetics of cellular copper and is primarily regulated by ATP7A, a copper-transporting P-type ATPase. However, little is known under which physiological condition copper egress is induced and its molecular consequence. In current manuscript, using THP-1 cells, a human monocytic cell line, we found that ATP7A expression was increased in cells exposed to phorbol-12-myristate-13-acetate (PMA), a potent inducer of neovascularization and cancer. Inductively coupled plasma mass spectrometry revealed that PMA also induced copper egress. Inhibition of ATP7A expression using small interfering RNA abrogated PMA induced copper egress. PMA treatment in THP-1 cells resulted in increased expression of matrix metalloproteinase (MMP) 9 and vascular endothelial growth factor receptor 1 (VEGFR1), whereas inhibition of ATP7A resulted in suppression of PMA-induced expression of VEGFR1, but not MMP9. Finally, addition of exogenous copper into the conditioned medium did not change VEGFR1 expression in THP-1 cells. Collectively, we demonstrate that PMA induces copper egress in THP-1 cells, which is regulated by ATP7A, and ATP7A regulates VEGFR1 expression. Considering the involvement of copper in neovascularization, our current finding provides the potential evidence to interpret the molecular mechanism.

Keywords Copper · ATP7A

Abbreviations

ICP-MS	Inductively coupled plasma mass spectrometry
MMP	Matrix metalloproteinase
PMA	Phorbol-12-myristate-13-acetate
VEGF	Vascular endothelial growth factor
VEGFR1	VEGF receptor 1

Introduction

Copper egress is defined as a spatial relocalization of cellular copper trafficking from cellular compartments, across the cell membrane, and release to the extracellular space. This cellular event is an essential regulator of the kinetics of cellular copper and is proposed to be a critical element of the copper

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signaling pathway in cells. In addition, such a copper relocalization is primarily regulated by ATP7A, a copper-transporting P-type ATPase [see Reviews (Schaefer and Gitlin 1999; La Fontaine and Mercer 2007; Lutsenko et al. 2007)]. Recently, we reported that ATP7A is highly expressed in the human vasculature and directly or indirectly modulates vascular function (Qin et al. 2006, 2008). However, little is known under which physiological condition copper egress is induced and its molecular consequence.

Vascular endothelial growth factor receptor 1 (VEGFR1) is a member of the VEGFR family, and it binds VEGF, placental growth factor, and VEGF-B. This receptor is expressed not only in endothelial cells but also in monocytes/macrophages (Clauss et al. 1996; Sawano et al. 2001), where it promotes monocyte/macrophage recruitment (Clauss et al. 1996; Hiratsuka et al. 1998), inflammation, cancer metastasis, and atherosclerosis (Cursiefen et al. 2004; Murakami et al. 2008). Activation of VEGFR1 also results in epithelial to mesenchymal transition, which is linked to increased invasion and migration of tumor cells (Fan et al. 2005; Wey et al. 2005; Yang et al. 2006). Targeting VEGFR1 may constitute a promising therapeutic approach since animal studies have proven its function in angiogenesis and collateral growth in ischemic heart and limb (Luttun et al. 2002). Thus, it is important to identify the upstream target that regulates VEGFR1 expression.

In the current study, we reported that ATP7A expression was increased in a human monocytic cell line after exposure to phorbol-12-myristate-13-acetate (PMA), a potent promoter of neovascularization and cancer (Montesano and Orci 1985). Next, we investigated whether the enhanced expression of ATP7A is associated with increased copper egress. Our data also indicated that VEGFR1 expression is regulated by ATP7A mediated copper egress. Because an association between copper egress and neovascularization in endothelial cells has recently been identified (Finney et al. 2007), here we provide additional evidence to the vascular role of copper egress.

Materials and methods

Cell culture

The human THP-1 cell line was obtained from the American Type Culture Collection (Rockville, MD) and maintained in culture using RPMI 1640 medium (Hyclone, Logan, UT) in the presence of 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 μg/ml). Cultures were maintained in 5% CO_2 tissue culture incubators maintained at 37°C until ready for use.

Western blotting

Cells were incubated for 30 min at 4°C in 1% Triton X-100 lysis buffer (50 mmol/l HEPES, 50 mmol/l NaCl, 5 mmol/l EDTA) with a tablet of protease inhibitors (Roche, Indianapolis, IN). Samples were then centrifuged at 18,000*g* for 10 min, and proteins in the supernatant were separated using SDS–PAGE, transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA), blocked, and incubated overnight at 4°C with the primary antibodies. After incubation with HRP-conjugated secondary antibodies, proteins were detected by chemiluminescence (Bio-Rad). ATP7A monoclonal antibodies were used (Qin et al. 2006). Equal gel loading was determined by Ponceau S staining of nitrocellulose membrane following transfer, and/or by blotting for α-tubuline antibody (Sigma).

Small interfering RNA inhibition of ATP7A

Approximately 5×10^5 cells were plated in 35-mm well of tissue culture-treated plates in presence of 100 nM of PMA (Sigma, St Louis, MO) for 24 h at 37°C in 5% CO_2 in normal culture media. The transfection of small interfering RNA (siRNA) oligonucleotides was carried out using Oligofectamine Transfection Reagent (Invitrogen, Carlsbad, CA). The ATP7A siRNA sequences were obtained from Ambion (Austin, TX) siRNA library (ID number 120175) and targeted exon 11 (sense, GCAACUAUUGUAA CUCUUGtt; antisense, CAAGAGUUACAAUAGUU GCtt). Scrambled siRNA sequences were used as negative controls. All siRNAs were obtained in lyophilized, annealed form, resuspended in double-distilled H_2O to a stock concentration of 20 μM, and stored at −20°C in 100 μl aliquots.

Sample preparation for total copper determination

To measure intracellular copper content, cells were first collected, pelleted and washed twice with PBS.

After incubation for 30 min at 4°C in 1% Triton X-100 lysis buffer (50 mmol/l HEPES, 50 mmol/l NaCl, 5 mmol/l EDTA) with a tablet of protease inhibitors (Roche, Indianapolis, IN), samples were centrifuged at 18,000*g* for 10 min in order to separate the supernatant and pellet. Subsequently, 10% of the supernatant was used for protein concentration determination by the Bradford assay (Bio-Rad). The remainder of the supernatant was diluted to 1.5 ml with 18 MΩ/cm doubly deionized water processed by Sybron/Barnstead (Boston, MA) containing 5% v/v nitric acid (HNO_3) (Pharmco Products Inc., Brookfield, CT). The copper elemental standard used for quantification was acquired from SpexCertiPrep (Metuchen, NJ). Calibration standards of 1.0–500 μg/l were prepared through dilution from a stock solution with 5% v/v HNO_3.

Inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) used for specific copper detection was an Agilent 7500ce by Agilent Technologies (Santa Clara, CA). The instrument was equipped with a microconcentric nebulizer made by Glass Expansion (Pocasset, MA), a Scott double channel spray chamber (2°C), a shielded torch with a sampling depth of 7 mm, nickel sampling and skimmer cones, a CE lens stack, an octopole collision/reaction cell with hydrogen gas (Matheson Gas Products, Parisppany, NJ) pressurization (purity of 99.999%), a quadrupole mass analyzer with a dwell time of 100 ms per isotope and an electron multiplier for detection. Instrumental parameters were as follows: forward power, 1,500 W; plasma gas flow rate, 15.0 l/min; auxiliary gas flow rate, 1.0 l/min; carrier gas flow rate, 0.99 l/min; makeup gas flow rate, 0.14 l/min; monitored isotope, ^{63}Cu. Samples were read in 5 times and averaged for reproducibility. The sample values were normalized to the total protein content. The pellets were not further analyzed in subsequent experiments considering the contained quantity of copper was less than 5% in our condition.

RNA preparation

TRI Reagent (Sigma) was used in isolation of total RNA following the manufacturer's instruction with minor modifications. After the ethanol precipitation step in the TRI Reagent extraction procedure, an additional cleanup was performed using RNeasy Mini kit (QIAGEN, Valencia, CA) to improve the purity of total RNA. In some cases, the quality of RNA was assessed using standard techniques, including examination of the absorption spectrum to measure the 260–280-nm optical density absorbance ratio, and detection of distinct 28S and 18S rRNA bands on ethidium bromide-stained agarose gels.

Reverse transcriptase-polymerase chain reaction

cDNA was synthesized using Retroscript First-Strand Synthesis Kit (Ambion) following the manufacturer's instructions. Reverse transcriptase-polymerase chain reaction (RT-PCR) primers for the target genes were designed using Primer 3 (Whitehead Institute for Biomedical Research) as shown in Table 1. PCR was performed in the Mx3000P™ PCR system (Stratagene, La Jolla, CA) under the following conditions: denaturation at 94°C for 1 min, annealing at 55°C for 30 s, and extension at 72°C for 1 min. An equal amount of aliquots from 25 or 30 thermocycles was electrophoresed in 1.5% agarose gel and quantified by densitometry analysis (Kodak Digital 1D Science).

Statistical analyses

Data are presented as mean ± SEM. Data were compared between groups of cells by Student's *t*-test when one comparison was performed or by ANOVA for multiple comparisons. When significance was indicated by ANOVA, the Tukey–Kramer post hoc test was used to specify between group differences. Values of $P < 0.05$ were considered statistically significant.

Table 1 PCR primer sequences

Name	Sequence	
VEGFR1	Forward	GGCTCTGTGGAAAGTTCAGC
	Reverse	GCTCACACTGCTCATCCAAA
MMP9	Forward	TTGACAGCGACAAGAAGTGG
	Reverse	GCCATTCACGTCGTCCTTAT
GAPDH	Forward	AACACAGTCCATGCCATCAC
	Reverse	TCCACCACCCTGTTGCTGTA

Results

PMA induces the expression of ATP7A

To determine whether the abundance of ATP7A is regulated in THP-1 cells after exposed to PMA, we measured the ATP7A protein levels using Western blot. As shown in Fig. 1a, PMA (10 nM) induced the protein level of ATP7A, with a peak occurring 48 h after PMA addition (8- ± 0.5-fold increase), which persisted at 72 h. In addition, PMA-induced ATP7A expression was dose-dependent, with a maximal effect occurring at 10 nM (Fig. 1b).

PMA induces copper egress

Because ATP7A is a key regulator of copper egress, we determined whether copper egress is enhanced in THP-1 cells after exposure to PMA using ICP-MS. We observed that the cellular copper concentration was significantly decreased (approximately, two-folds) in THP-1 cells after incubation with PMA (10 nM) for 48 h (Fig. 2, left panel). This decrease in cellular copper by PMA was further confirmed when THP-1 cells were co-treated with $CuSO_4$ (10 μg/ml) for 48 h (Fig. 2, right panel). The concentration of $CuSO_4$ employed here has been applied in copper overload experiments in cells (Gu et al. 2001). As expected, the cellular copper concentration was significantly increased in THP-1 cells treated with $CuSO_4$. These results indicated that PMA induces copper egress in human THP-1 cells.

ATP7A-specific siRNA oligonucleotides down-regulate the expression of ATP7A

To begin to investigate the function of ATP7A in THP-1 cells, a siRNA technique was employed to

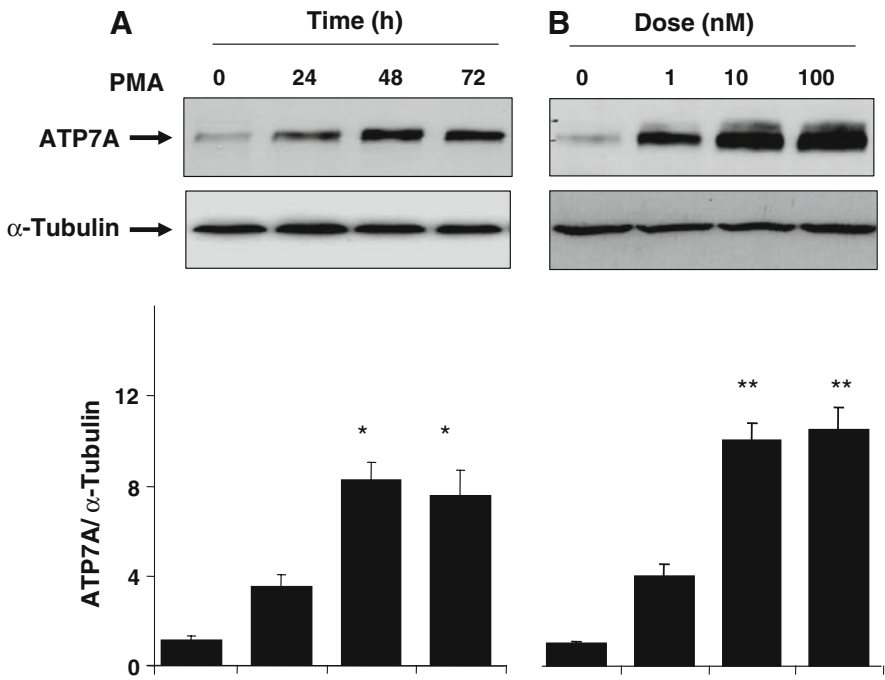

Fig. 1 Effect of PMA on the expression of ATP7A. After incubation of THP-1 cells (1×10^6 cells/ml) with PMA, cell lysates were prepared and the protein expressions of ATP7A were then assessed as described in the "Materials and methods". **a** Time course for ATP7A protein level in THP-1 cells stimulated by PMA. Cells were treated with 10 nM PMA for the indicated times. **b** Dose response of ATP7A protein level in human THP-1 cells stimulated by PMA. Cells were treated with various concentrations of PMA (0–100 nM) for 48 h. In **a**, **b**, *top panels* are representative immunoblots of PMA-induced expression of ATP7A and loading control α-Tubulin. *Bottom panels* represent average data quantified by densitometry of immunoblots, expressed as -fold increase in protein, in which the protein observed in cells at time 0 (**a**) or in unstimulated cells (**b**) was defined as 1.0 (control). In these experiments, alpha-tubulin served as a loading control. Values are the mean ± SE for three independent experiments. *$P < 0.05$ versus control. **$P < 0.01$ versus control

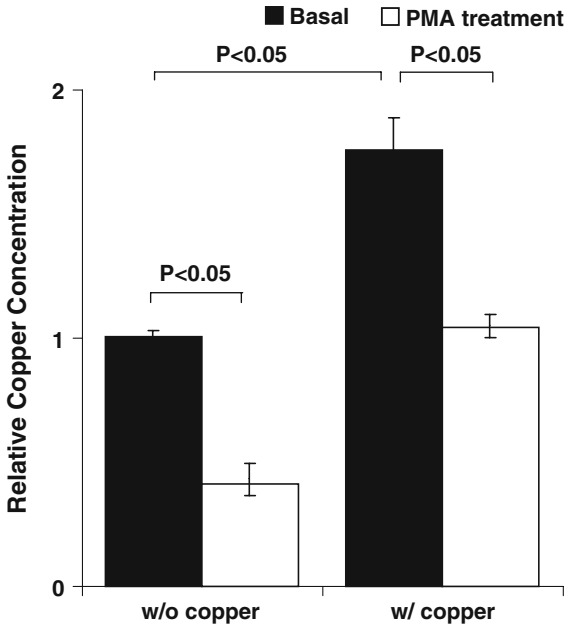

Fig. 2 Effect of PMA on cellular copper concentration. *Left panel* Human THP-1 cells (1×10^6 cells/ml) were incubated in the absence (*filled bar*) or the presence (*empty bar*) with 10 nM PMA for 48 h; *Right panel* Human THP-1 cells (1×10^6 cells/ml) were incubated with 10 μg/ml $CuSO_4$ in the absence (*filled bar*) or the presence (*empty bar*) with 10 nM PMA for 48 h. After that, cell lysates were prepared and the supernatants were subjected to copper measurement as described in the "Materials and methods". The sample values were normalized to the total protein content. The data presented as -fold increase in copper concentration, in which the copper concentration observed in cells in the absence of PMA and copper was defined as 1.0. Values are the mean ± SE for three independent experiments and each experiment are repeated 5 times

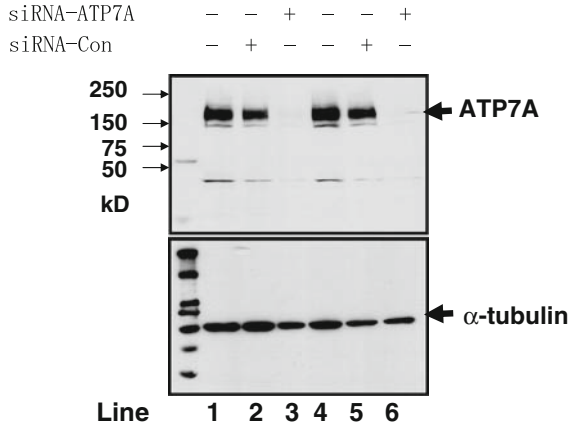

Fig. 3 Effect of siRNA on ATP7A protein expression. Following 100 nM of PMA treatment, human THP-1 cells (5×10^5 cells/ml) were incubated with either 5 nM of ATP7A-specific (*lanes 3* and *6*) or control (*lanes 2* and *5*) siRNA oligonucleotides for 24 h. Control cells (*lanes 1* and *4*) were exposed to transfectant without siRNA oligonucleotides. After incubation, cell lysates were prepared and the protein expressions of ATP7A was then assessed as described in the "Materials and methods". In these experiments, α-tubulin served as a loading control. Experimental results represent data obtained from one of five similar experiments

knockdown ATP7A expression. In order to make it easier to demonstrate the effect of ATP7A, cells were first exposed to PMA to increase ATP7A expression (Fig. 1) prior to the treatment with siRNA oligonucleotides. As shown in Fig. 3, after treatment with ATP7A-specific siRNA oligonucleotides for 24 h, ATP7A protein was downregulated up to 90% compared with that of scrambled oligonucleotides and non-oligonucleotides treated cells.

Inhibition of ATP7A recovers the cellular copper concentration after exposed to PMA

To determine whether the copper egress we observed is mediated by ATP7A, we measured cellular copper concentration in human cells incubated with ATP7A or control siRNA oligonucleotides for 24 h. Cellular copper measured by ICP-MS was significantly higher in THP-1 cells incubated with ATP7A oligonucleotides versus control oligonucleotides (Fig. 4, left panel). The increase of cellular copper by ATP7A siRNA oligonucleotides was further confirmed when THP-1 cells were treated as well with $CuSO_4$ (10 μg/ml) to enhance copper levels in untreated cells (Fig. 4, right panel). These experiments indicate that ATP7A mediates copper egress induced by PMA in human THP-1 cells.

VEGFR1 expression is decreased after suppression of ATP7A

In order to investigate the molecular consequence of inhibition of ATP7A in human THP-1 cells, we performed cDNA microarray analysis to compare global gene expression responses in control versus ATP7A-knockdown THP-1 cells (Affymetrix human genome U133 plus). We found that VEGFR1 expression was among the most affected in the group of significantly downregulated genes. Moreover, we performed RT-PCR to determine the expression of VEGFR1 and MMP9. First, consistent with previous reports (Akuzawaet al. 2000; Worley et al. 2003;

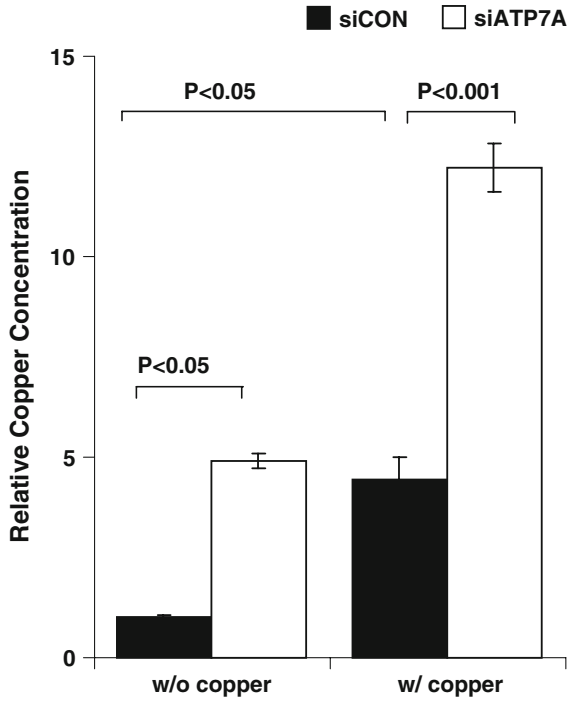

Fig. 4 Effect of suppression of ATP7A on cellular copper concentration. After incubation of ATP7A or control siRNA oligonucleotides for 24 h, human THP-1 cells were incubated for another 24 h in the absence (*left panel*) or the presence (*right panel*) of 10 μg/ml $CuSO_4$. After that, cell lysates were prepared and the supernatants were subjected to copper measurement, as described in the "Materials and methods". The sample values were normalized to the total protein content. The data presented as -fold increase in copper concentration, in which the copper concentration observed in cells treated with control siRNA oligonucleotides in the absence of copper was defined as 1.0. Values are the mean ± SE for three independent experiments and each experiment was repeated 5 times

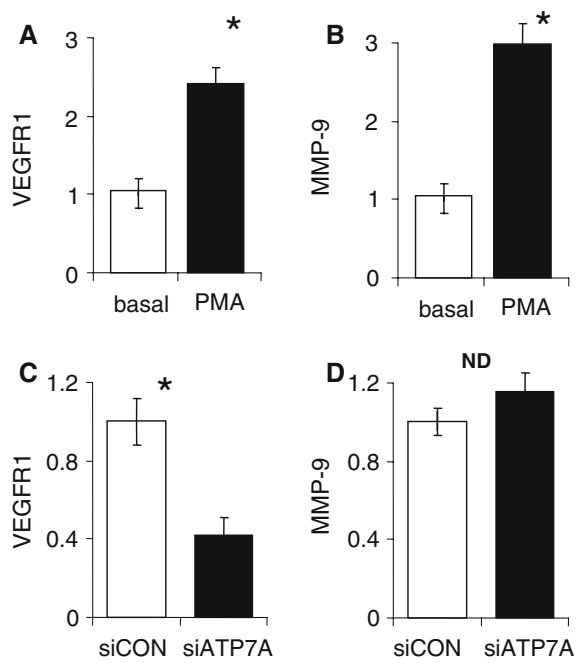

Fig. 5 **a**, **b** Effect of PMA on the mRNA levels of VEGFR1 and MMP9. Following treatment with 100 nM of PMA for 8 h, total RNA of THP-1 cells was isolated. **c** and **d** Effect of suppression of ATP7A on the mRNA levels of VEGFR1 and MMP9 in PMA treated THP-1 cells. Following 100 nM of PMA treatment, THP-1 cells were then treated with ATP7A specific and control siRNA oligonucleotides for 48 h. After that, total RNA was isolated. **a**–**d** After preparation of total RNA, human VEGFR1 and MMP9 and GAPDH expression was quantified by RT-PCR, as described in the "Materials and methods". The abundance of target mRNA was calculated in relation to the GAPDH mRNA in the same sample. The data are presented as -fold increase in mRNA level, in which the mRNA level observed in untreated cells was defined as 1.0. Each data point is the mean of triplicate measurements. *$P < 0.01$ versus untreated THP-1 cells or ATP7A-silenced THP-1 cells

Tuomisto et al. 2005), our results showed that PMA markedly induced the expression of VEGFR1 (Fig. 5a) and MMP9 (Fig. 5b). Whereas VEGFR1 expression was significantly reversed in ATP7A-silenced cells as compared with that in controls (Fig. 5c). In contrast, inhibition of ATP7A did not change the expression of MMP9 (Fig. 5d). These observations suggested that VEGFR1 but not MMP9 expression is regulated by ATP7A.

VEGFR1 expression is not changed after the addition of copper into the conditioned medium

Intracellular copper is increased after inhibition of ATP7A and the addition of copper into the conditioned medium also increases intracellular copper (Figs. 2, 4). It is unclear whether these two events have same effect on VEGFR1 expression. We, therefore, determined whether the addition of copper also inhibits the expression of VEGFR1 in THP-1 cells using RT-PCR. Our results showed that there was no marked change in VEGFR1 gene expression observed in THP-1 cells in the presence and the absence of copper (Fig. 6), suggesting that the molecular consequence of ATP7A mediated copper egress is, at least in part, independent of the effect of direct copper addition into the cell culture medium.

Discussion

We previously reported that ATP7A is highly expressed in the human vasculature, including aortic smooth muscle cells, aortic endothelial cells and umbilical vein endothelial cells (Qin et al. 2006, 2008). Moreover, we identified a novel vascular function of ATP7A: modulation of the expression and activity of a copper-accepting enzyme, extracellular superoxide dismutase (Qin et al. 2006, 2008). ATP7A contains several functional domains, including the NH_2-terminal metal binding domain, the transmembrane domain, the ATP-binding domain, and the COOH-terminal dileucine motifs. These functional domains facilitate this ATPase as a cellular pump to egress copper from cells. In the current study, we found that PMA induces the expression and function of this cellular copper pump and sheds a new light to the regulation of this copper transporter.

Recently, the cultured human fibroblasts over-expressing the ATP7A has been proposed as a novel approach to investigate the role of copper in gene regulation (Bellingham et al. 2004). As an extension of this approach, we demonstrated that inhibition of ATP7A in human THP-1 cells suppresses copper egress. Our study (Fig. 6) also indicates that this cellular copper egress system is different from the approach using exogenous copper treatment. It is worth noting that ATP7B is another important player to regulate copper egress. [see Review (Lutsenko et al. 2007)]. Our study focused on ATP7A is because that ATP7B is primarily expressed in the liver and the brain, whereas ATP7A appears expressed in most of tissues except liver.

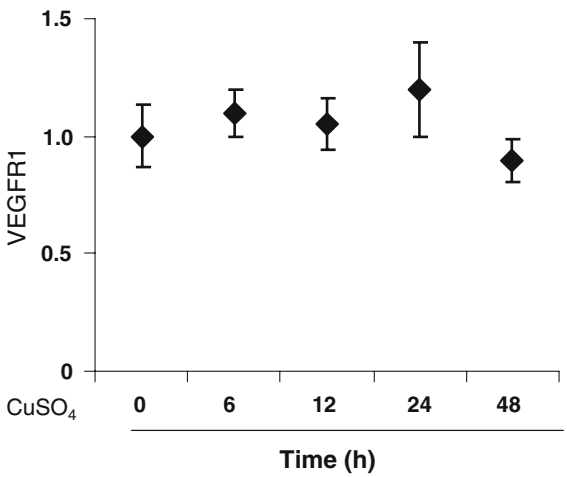

Fig. 6 Effect of exogenous copper on the expression of VEGFR1. THP-1 cells (5×10^5 cells/ml) were treated with 10 μg/ml $CuSO_4$ for the indicated times. After RT-PCR, the abundance of target mRNA was calculated in relation to the GAPDH mRNA in the same sample. The data are presented as -fold increase in mRNA level, in which the mRNA level observed in untreated cells was defined as 1.0. Each data point is the mean of triplicate measurements

Vascular endothelial growth factor receptor 1 is a tyrosine kinase receptor for VEGF discovered by de Vries et al. in 1992. This receptor has attracted significant attention since the discovery that the inhibition of VEGFR1 may provide a novel treatment for the inhibition of tumor angiogenesis and atherosclerotic plaque growth (Luttun et al. 2002). Importantly, the inhibition of VEGFR1 using anti-VEGFR1 antibody not only reduces the size of atherosclerotic plaque but also stabilizes atherosclerotic lesions, which might prevent plaque rupture and fatal thrombotic events (Luttun et al. 2002). Our current report and the study of Akuzawa et al. (2000) show that VEGFR1 expression is strongly up-regulated by PMA in human THP-1 cells, suggesting PMA promotion of neovascularization involves VEGFR1. Specifically, we find that after inhibition of ATP7A, VEGFR1 expression is also decreased, suggesting that ATP7A contributes to the regulation of VEGFR1 expression.

The present study shows that the treatment of siRNA for ATP7A can increase cellular copper content and inhibit VEGFR1 expression, but increased cellular copper content by copper treatment is not able to inhibit VEGFR1 expression. In spite of eventual similar effect to increase total cellular copper content (siRNA ATP7A vs. copper treatment), the mechanism related to this discrepancy between the two different treatments is unknown. Interestingly, in a recent study, although the gene expression of both amyloid-β precursor protein and prion protein is increased in ATP7A deficient cells, copper treatment only increases the expression of amyloid-β precursor protein, but not prion protein (Armendariz et al. 2004). Thus, in addition to the total cellular copper content, other factors may be also involved into the regulation of gene expression in these contexts. For example, cellular copper distribution and transport are different between the ATP7A inhibition and the copper treatment, and this may contribute to their differential effect on gene expression.

Finney et al. (2007) reported that copper egress is induced during endothelial tube formation in response to angiogenic factors such as VEGF. Although it is unknown whether this process is regulated by ATP7A, this finding provides new insight into the molecular mechanism in which copper regulates neovascularization. Indeed, previous studies have shown that neovascularization is sensitive to exogenous copper (Raju et al. 1982; Ziche et al. 1982), copper chelation inhibits neovascularization (Goodman et al. 2004). We now demonstrate that ATP7A is responsible, at least in part, for PMA induced copper egress in THP-1 cells. In addition, similar to the effect of VEGF, PMA is a potent inducer of neovascularization and cancer (Montesano and Orci 1985). Both VEGF and PMA can share common downstream pathways, such as protein kinase C (Taylor et al. 2006). Therefore, the findings from these two different models (endothelial cells vs. a monocytic cell line; VEGF vs. PMA) suggest the possibility of the copper egress as a novel endogenous positive mediator of neovascularization, which are subjects of further investigation.

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References

Akuzawa N, Kurabayashi M et al (2000) Zinc finger transcription factor Egr-1 activates Flt-1 gene expression in THP-1 cells on induction for macrophage differentiation. Arterioscler Thromb Vasc Biol 20(2):377–384

Armendariz AD, Gonzalez M et al (2004) Gene expression profiling in chronic copper overload reveals upregulation of Prnp and App. Physiol Genomics 20(1):45–54. doi:10.1152/physiolgenomics.00196.2003

Bellingham SA, Lahiri DK et al (2004) Copper depletion down-regulates expression of the Alzheimer's disease amyloid-beta precursor protein gene. J Biol Chem 279(19):20378–20386. doi:10.1074/jbc.M400805200

Clauss M, Weich H et al (1996) The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis. J Biol Chem 271(30):17629–17634. doi:10.1074/jbc.271.30.17629

Cursiefen C, Chen L et al (2004) VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest 113(7):1040–1050

de Vries C, Escobedo JA et al (1992) The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 255(5047):989–991. doi:10.1126/science.1312256

Fan F, Wey JS et al (2005) Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. Oncogene 24(16):2647–2653. doi:10.1038/sj.onc.1208246

Finney L, Mandava S et al (2007) X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis. Proc Natl Acad Sci USA 104(7):2247–2252. doi:10.1073/pnas.0607238104

Goodman VL, Brewer GJ et al (2004) Copper deficiency as an anti-cancer strategy. Endocr Relat Cancer 11(2):255–263. doi:10.1677/erc.0.0110255

Gu YH, Kodama H et al (2001) ATP7A gene mutations in 16 patients with Menkes disease and a patient with occipital horn syndrome. Am J Med Genet 99(3):217–222. doi:10.1002/1096-8628(2001)9999:9999<::AID-AJMG1167>3.0.CO;2-R

Hiratsuka S, Minowa O et al (1998) Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. Proc Natl Acad Sci USA 95(16):9349–9354. doi:10.1073/pnas.95.16.9349

La Fontaine S, Mercer JF (2007) Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. Arch Biochem Biophys 463(2):149–167. doi:10.1016/j.abb.2007.04.021

Lutsenko S, Barnes NL et al (2007) Function and regulation of human copper-transporting ATPases. Physiol Rev 87(3):1011–1046. doi:10.1152/physrev.00004.2006

Luttun A, Tjwa M et al (2002) Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat Med 8(8):831–840

Montesano R, Orci L (1985) Tumor-promoting phorbol esters induce angiogenesis in vitro. Cell 42(2):469–477. doi:10.1016/0092-8674(85)90104-7

Murakami M, Zheng Y et al (2008) VEGFR1 tyrosine kinase signaling promotes lymphangiogenesis as well as angiogenesis indirectly via macrophage recruitment. Arterioscler Thromb Vasc Biol 28(4):658–664. doi:10.1161/ATVBAHA.107.150433

Qin Z, Itoh S et al (2006) Essential role for the Menkes ATPase in activation of extracellular superoxide dismutase: implication for vascular oxidative stress. FASEB J 20(2):334–336

Qin Z, Gongora MC et al (2008) Role of Menkes ATPase in angiotensin II-induced hypertension: a key modulator for extracellular superoxide dismutase function. Hypertension 52(5):945–951

Raju KS, Alessandri G et al (1982) Ceruloplasmin, copper ions, and angiogenesis. J Natl Cancer Inst 69(5):1183–1188

Sawano A, Iwai S et al (2001) Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. Blood 97(3):785–791. doi:10.1182/blood.V97.3.785

Schaefer M, Gitlin JD (1999) Genetic disorders of membrane transport. IV. Wilson's disease and Menkes disease. Am J Physiol 276(2 Pt 1):G311–G314

Taylor CJ, Motamed K et al (2006) Protein kinase C and downstream signaling pathways in a three-dimensional model of phorbol ester-induced angiogenesis. Angiogenesis 9(2):39–51. doi:10.1007/s10456-006-9028-y

Tuomisto TT, Riekkinen MS et al (2005) Analysis of gene and protein expression during monocyte-macrophage differentiation and cholesterol loading–cDNA and protein array study. Atherosclerosis 180(2):283–291. doi:10.1016/j.atherosclerosis.2004.12.023

Wey JS, Fan F et al (2005) Vascular endothelial growth factor receptor-1 promotes migration and invasion in pancreatic carcinoma cell lines. Cancer 104(2):427–438. doi:10.1002/cncr.21145

Worley JR, Baugh MD et al (2003) Metalloproteinase expression in PMA-stimulated THP-1 cells. Effects of peroxisome proliferator-activated receptor-gamma (PPAR gamma) agonists and 9-cis-retinoic acid. J Biol Chem 278(51):51340–51346. doi:10.1074/jbc.M310865200

Yang AD, Camp ER et al (2006) Vascular endothelial growth factor receptor-1 activation mediates epithelial to mesenchymal transition in human pancreatic carcinoma cells. Cancer Res 66(1):46–51. doi:10.1158/0008-5472.CAN-05-3086

Ziche M, Jones J et al (1982) Role of prostaglandin E1 and copper in angiogenesis. J Natl Cancer Inst 69(2):475–482