Neurobiology of Aging 36 (2015) 1310–1315

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

The role of copper and the copper-related protein CUTA in mediating APP processing and Aβ generation

Ping Hou [a,b], Guiying Liu [a], Yingjun Zhao [c], Zhun Shi [a], Qiuyang Zheng [a], Guojun Bu [a], Huaxi Xu [a,c], Yun-wu Zhang [a,*]

[a] Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, College of Medicine, Xiamen University, Xiamen, China
[b] School of Pharmaceutical Sciences, Xiamen University, Xiamen, China
[c] Degenerative Disease Research Program, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA

ARTICLE INFO

Article history:
Received 17 May 2014
Received in revised form 11 November 2014
Accepted 4 December 2014
Available online 11 December 2014

Keywords:
β-amyloid
β-amyloid precursor protein
Alzheimer's disease
Copper
CutA divalent cation tolerance homolog of *E. coli*

ABSTRACT

One major pathologic hallmark and trigger of Alzheimer's disease (AD) is overproduction and accumulation of β-amyloid (Aβ) species in the brain. Aβ is derived from β-amyloid precursor protein (APP) through sequential cleavages by β- and γ-secretases. Abnormal copper homeostasis also contributes to AD pathogenesis. Recently, we find that a copper-related protein, CutA divalent cation tolerance homolog of *Escherichia coli* (CUTA), interacts with the β-secretase β-site APP cleaving enzyme 1 (BACE1) and inhibits APP β-processing and Aβ generation. Herein, we further found that overexpression of CUTA increases intracellular copper level, whereas copper treatments promote CUTA expression. We also confirmed that copper treatments promote APP expression and Aβ secretion. In addition, copper treatments promoted the increase of Aβ secretion induced by CUTA downregulation but had no effect on CUTA-β-site APP cleaving enzyme 1 interaction. On the other hand, CUTA overexpression ameliorated copper-induced Aβ secretion but had no effect on APP expression. Moreover, we found that Aβ treatments can reduce both CUTA and copper levels in mouse primary neurons. Consistently, both CUTA and copper levels were decreased in the hippocampus of APP/PS1 AD mouse brain. Together, our results reveal a reciprocal modulation of copper and CUTA and suggest that both regulate Aβ generation through different mechanisms, although Aβ mutually affects copper and CUTA levels.

1. Introduction

A major pathologic hallmark of Alzheimer's disease (AD) in the brain is the formation of senile plaques whose major components are heterogenous β-amyloid (Aβ, mostly Aβ40 and Aβ42) peptides. Multiple lines of evidence demonstrate that Aβ peptides are neurotoxic and can trigger a cascade of neurodegenerative steps including the formation of neurofibrillary tangles, synaptic deficits, and neuronal loss, indicating that Aβ plays a pivotal role in the pathogenesis of AD (Eimer and Vassar, 2013; Hardy and Higgins, 1992; Hardy and Selkoe, 2002). Aβ is generated from β-amyloid precursor protein (APP) through sequential cleavages first by β-secretase and then by γ-secretase. Excessive Aβ can trigger a cascade of neurodegenerative steps, including the formation of senile plaques and intra-neuronal fibrillary tangles and neuronal loss in susceptible brain regions. Alternatively, APP can be cleaved by α-secretase within the Aβ domain. α-cleavage precludes Aβ generation and releases a large extracellular domain of APP known as soluble APPα (sAPPα) instead (Zhang et al., 2011; Zheng and Koo, 2011).

Environmental factors such as heavy metals also play important roles in the pathogenesis of AD, either as triggers or as modulators of disease progression (Bush, 2003). Copper is indispensable in the human central nervous system and may function as a cofactor for multiple enzymes, activate neuropeptides and hormones, protect against reactive oxygen species, and so forth (Lutsenko et al., 2010). Abnormal homeostasis of copper has been shown to be involved in AD. However, the contribution of copper to disease pathology and development is far from being elucidated. Copper is enriched in amyloid plaques of AD patients compared with age-matched subjects (Bush, 2003). Copper can interact with APP and one of the 2

* Corresponding author at: Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, College of Medicine, Xiamen University, Xiamen 361102, China. Tel./fax: +86 592 2188528.
E-mail address: yunzhang@xmu.edu.cn (Y.-w. Zhang).

0197-4580/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.12.005

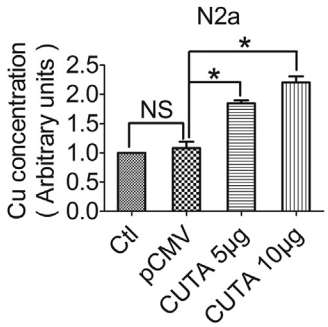

Fig. 1. Overexpression of CUTA increases intracellular copper level. N2a cells were transfected with indicated amounts of CUTA plasmid or pCMV vector for 24 hours. Untransfected cells were also used as control (Ctl). Cellular Cu levels were quantified by ICP-MS. N = 3, $^*p < 0.05$. Abbreviations: CUTA, CutA divalent cation tolerance homolog of *Escherichia coli*; ICP-MS, inductively coupled plasma mass spectrometry; NS, not significant.

copper-binding domains on APP is within the Aβ region (Stefansson et al., 2005). On binding to APP and Aβ, Cu(II) can be reduced to Cu(I), which may generate reactive oxygen species during this process (Multhaup et al., 1996) and contribute to the oxidative stress observed in AD brain (Eskici and Axelsen, 2012). However, although copper overload has been shown to promote APP expression (Armendariz et al., 2004; Borchardt et al., 1999) and copper depletion can downregulate APP expression (Bellingham et al., 2004), the effect of copper on Aβ is controversial: one study found that copper can reduce Aβ level (Borchardt et al., 1999), whereas another study showed that copper can enhance APP dimerization and promote Aβ production (Noda et al., 2013). Results from in vivo studies are also controversial: one study showed that copper level is decreased in APP23 transgenic mice and dietary copper can reduce Aβ production and stabilize brain superoxide dismutase 1 activity (Bayer et al., 2003). Copper level was also found to be increased in APP knockout mice (White et al., 1999). However, other studies found that copper exposure may cause Aβ plaques and learning deficits in a rabbit model of AD possibly through affecting Aβ clearance and exacerbate both amyloid and tau pathology in APP/PS1/tau triple transgenic AD mice by upregulating β-site APP cleaving enzyme 1 (BACE1), the essential β-secretase (Chami and Checler, 2012; Kitazawa et al., 2009).

We recently found that a copper-related protein, the mammalian CutA divalent cation tolerance homolog (*Escherichia coli*), CUTA, can modulate Aβ generation. Human CUTA has several variants that differ in their amino-terminal length and can be separated as heavy and light components. We demonstrated that the heavy component (but not the light component) of CUTA can interact with BACE1 and mediates its intracellular trafficking, therefore affecting β-processing of APP and Aβ production (Zhao et al., 2012a). CUTA can form trimers through a region of about 100 residues that is conserved from bacteria to vertebrates (Savchenko et al., 2004). In bacteria, CutA is involved in copper tolerance and some mutations in the *cutA* gene have been found to lead to copper sensitivity because of its increased uptake (Fong et al., 1995). Additional studies show that many CutA proteins have a high copper-binding capacity and that copper could induce reversible aggregation of the CutA protein (Arnesano et al., 2003; Tanaka et al., 2004). Therefore, in the present study, we further investigated the correlation between CUTA and copper and any potential interplay between the two during their modulating APP processing and Aβ generation.

2. Methods

2.1. Cell culture

Mouse neuroblastoma N2a cells, N2a cells stably expressing human APP695 (N2a-APP695), and HEK293T cells were cultured as previously described (Zhao et al., 2012b). Primary neurons derived from embryonic day 14.5–16.5 C57BL/6 wild type or APP/PS1 mouse embryos were maintained in neurobasal medium supplemented with B27 (Life Technologies). All animal procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Xiamen University.

2.2. Antibodies

The rabbit polyclonal antibody R-CUTA against CUTA (Zhao et al., 2012a) and the rabbit polyclonal antibody Ru369 against APP (Xu et al., 1997) were developed in our laboratories. The mouse monoclonal antibody 22C11 was from Millipore. Mouse anti-α-tubulin, rabbit anti-β-actin, and mouse anti-HA antibodies were from Sigma. Mouse anti-Myc, mouse anti-GAPDH, and mouse anti-PARP antibodies were from Santa Cruz Biotechnology.

2.3. Cell manipulation

For copper treatments, cells were incubated with various amounts of $CuSO_4$ for different time periods. In some experiments, cells were transiently transfected with pCMV, BACE1-HA, and CUTA plasmids, using Turbofect reagent (Fermentas). For RNA interference to downregulate CUTA expression, cells were transiently transfected with a scrambled control shRNA and a shRNA targeting CUTA (piLenti-siRNA 494-GFP: 5′-TCACAGAATCGGTTTCAAATTCTGGCACA-3′), using Lipofectamine2000 reagent (Invitrogen).

For Aβ treatments, primary neurons from wild-type C57BL/6 mice were insulted with 40 μM Aβ42 for 24 hours. Cell lysates were measured for CUTA and copper levels.

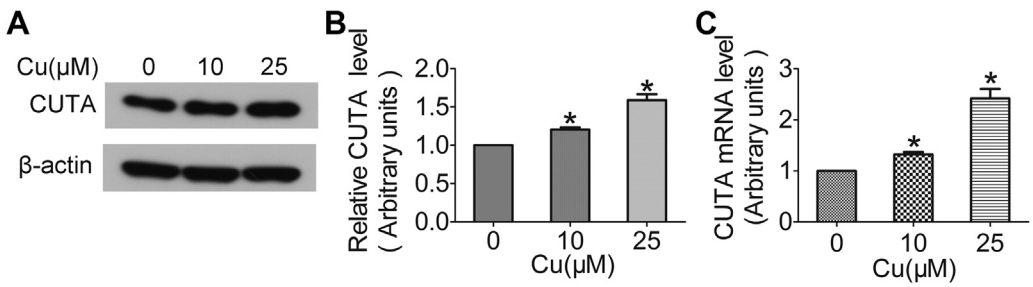

Fig. 2. Copper treatments induce CUTA expression. N2a cells were treated with indicated amounts of Cu for 24 hours. (A) Cell lysates were assayed for CUTA by Western blot. (B) CUTA levels were quantified by densitometry for comparison. (C) The mRNA level of CUTA was determined by quantitative real-time PCR for comparison. N = 3, $^*p < 0.05$. Abbreviations: CUTA, CutA divalent cation tolerance homolog of *Escherichia coli*; mRNA, messenger RNA; PCR, polymerase chain reaction.