Cystatin C Induces Insulin Resistance in Hippocampal Neurons and Promotes Cognitive Dysfunction in Rodents

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Dear Editor,

Cognitive impairment is a hallmark of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease. Growing evidence has demonstrated that cognitive impairment is closely associated with insulin resistance. For instance, the risk of AD is increased in diabetic patients [1]. Moreover, cognitively impaired persons exhibit high levels of fasting glucose and fasting insulin, elevated Homeostasis Model Assessment, an impaired insulin response to glucose, and increased frequency of diabetes. In accord with this, intranasal insulin administration greatly improves the delayed memory and caregiver-rated functional ability of patients with amnesic mild cognitive impairment or AD [2].

Cystatin C (CysC) is a ubiquitously-expressed protein and an inhibitor of cysteine protease. It has been demonstrated that plasma CysC is a positive indicator of central adiposity and insulin resistance [3]. Recently, a clinical survey indicated that a higher titer of plasma CysC impairs

insulin sensitivity in patients with type 1 diabetes [4]. This evidence implies that CysC serves as an inducer of insulin resistance. Indeed, we previously showed that CysC impairs the insulin signal pathway in hepatocytes by inducing endoplasmic reticulum (ER) stress [5]. Given that CysC is a causative factor for insulin resistance, it is reasonable to predict that elevated CysC levels lead to cognitive impairment by promoting insulin resistance.

To investigate whether CysC affects the actions of insulin, we treated rat primary hippocampal neurons with recombinant CysC at different dosages. As shown in Fig. S1A, phosphorylated Akt (p-Akt) and GSK3 β (p-GSK3 β) were dramatically increased by insulin stimulation in these neurons. However, the increases were decreased by CysC in a dose-dependent manner (Fig. S1A, B). To verify these findings, we also measured insulin-stimulated glucose uptake in primary hippocampal neurons. As expected, we found that the application of insulin greatly stimulated glucose uptake and this stimulation was largely blunted in the presence of CysC (Fig. S1C). These data showed that insulin signal transduction is blocked by CysC in primary hippocampal neurons.

Next, we explored the mechanism underlying the CysC-mediated blockade of insulin signal transduction in primary hippocampal neurons. It has been shown that CysC attenuates insulin signal transduction in hepatocytes by inducing ER stress [5]. Therefore, we measured the ER stress-related genes *Grp78*, *Chop*, and *Erdj4* and the results showed that CysC had no effect on their expression (data not shown). Suppressor of cytokine signaling 1/3 (SOCS1/3) are negative regulators of insulin signal transduction [6]. Thus, we measured SOCS1/3 expression and found that the mRNA level of *SOCS1* was significantly up-regulated by CysC, while *SOCS3* did not change (Fig. S2A). The protein level of SOCS1 was also enhanced by CysC (Fig. S2B, C).

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To investigate whether the CysC-mediated suppression of insulin signal transduction occurs *via* the stimulation of *SOCS1*, we down-regulated *SOCS1* expression by means of siRNA-mediated gene silencing. As shown in Fig. S2D, the mRNA level of *SOCS1* was decreased by siRNAs against *SOCS1*, especially by siRNA-#2 and siRNA-#3. To confirm the knockdown efficiency, we measured the protein levels of *SOCS1* and the results confirmed that *SOCS1* was reduced by the siRNAs (Fig. S2E). Of these, siRNA-#3 showed the best knockdown efficiency and thus it was used in subsequent experiments. As shown in Fig. S2F, in nonspecific control (NC) siRNA-transfected neurons, CysC greatly inhibited the insulin-stimulated p-Akt and p-GSK3 β . In the siRNA-#3-transfected neurons, however, the presence of CysC had no such effects (Fig. S2F). The above data clearly showed that, by stimulating *SOCS1*, CysC impairs insulin signal transduction in primary hippocampal neurons.

The above data showed that CysC induces insulin resistance in primary hippocampal neurons *via* stimulation of *SOCS1*. It has been well documented that insulin resistance in hippocampal neurons is closely correlated with cognitive dysfunction. Hence, we predicted that CysC would induce cognitive dysfunction. To test this hypothesis, we administered CysC to mice *via* the intranasal route and evaluated the potential roles of CysC on insulin signal transduction and cognitive function. First, we determined whether *SOCS1* and insulin signal transduction were affected by CysC. As shown in Fig. 1A, the mRNA level

of *SOCS1* was markedly up-regulated in the CysC-treated animals, while *SOCS3* expression was not altered. The protein level of *SOCS1* was also increased (Fig. 1B, C). The p-Akt was greatly reduced in the presence of CysC, while p-GSK3 β decreased slightly (Fig. 1B, C). Next, we measured the spatial and learning memory using the Morris water maze. As expected, the learning ability was decreased by CysC as evidenced by the increased escape latency (Fig. 1D). Moreover, spatial memory was also tested on day 7 when the platform was removed. The results showed that CysC decreased the crossing times and entry times, indicating that spatial memory is impaired by CysC (Fig. 1D). Moreover, the administration of CysC decreased the travel distance and swimming speed (Fig. 1E). These data indicated that central administration of CysC impairs cognitive function in mice, and elevated CysC concentrations in the brain might be a risk factor for cognitive impairment.

CysC is abundant in the brain and is expressed by astrocytes, microglial cells and neurons in brains of different species [7, 8]. The CysC level in the cerebrospinal fluid is five times that in plasma [9], indicating that CysC plays an important role in the brain. The precise biological functions of CysC in the brain are still a matter of debate. In the present study, we found that application of CysC attenuated insulin signal transduction in hippocampal neurons. This is in line with our previous finding that CysC impairs insulin signal transduction in hepatocytes [5]. These data imply that CysC is a detrimental factor for

Fig. 1 Cystatin C impairs insulin signal transduction in the hippocampus and promotes cognitive dysfunction. **A** The relative mRNA levels of *SOCS1* and *SOCS3*. 18S rRNA was used as a house-keeping gene. **B** Western blots of the protein levels using the indicated antibodies. **C** Quantification of immunoblots as shown in **B**. **D** Morris

water maze analysis showing impaired spatial and learning memory in CysC-treated mice. **E** CysC administration affects swimming behavior. Values are presented as mean $\pm$ SEM from two independent experiments ($n = 10$; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus vehicle-treated mice).

health. Indeed, clinical survey data have suggested that CysC is a strong indicator for the risk of death and cardiovascular disorders in elderly persons [10]. However, another study has shown that the neuroprotective role of CysC is dependent upon cysteine protease inhibition, evocation of autophagy, and initiation of proliferation in chronic and acute neurodegeneration [11]. This discrepancy may be due to the differences in cell type and/or the concentration of exogenous CysC applied.

Impaired insulin signal transduction is linked to an increased risk of both AD and cognitive decline [1, 12]. Therefore, insulin resistance is a promising causative factor for cognitive impairment. Consistently, our data showed that the intranasal administration of CysC greatly impairs spatial learning and memory in mice. Moreover, we also found that the application of CysC slows motor activity in mice. Epidemiologic evidence has also shown that CysC levels are positively correlated with physical disabilities in the elderly [13]. The underlying mechanisms remain unclear. The substantia nigra is likely to be the target of CysC, as this is responsible for movement.

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Compliance with Ethical Standards

Conflict of interest All authors claim that there are no conflicts of interest.

References

1. Cheng D, Noble J, Tang MX, Schupf N, Mayeux R, Luchsinger JA. Type 2 diabetes and late-onset Alzheimer’s disease. *Dement Geriatr Cogn Disord* 2011, 31: 424–430.

2. Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, *et al.* Intranasal insulin therapy for Alzheimer disease and amnesic mild cognitive impairment: a pilot clinical trial. *Arch Neurol* 2012, 69: 29–38.
3. Shankar A, Teppala S. Relationship between body mass index and high cystatin levels among US adults. *J Clin Hypertens (Greenwich)* 2011, 13: 925–930.
4. Uruska A, Araszkiewicz A, Zozulinska-Ziolkiewicz D, Wegner M, Grzelka A, Wierusz-Wysocka B. Does serum cystatin C level reflect insulin resistance in patients with type 1 diabetes? *Clin Biochem* 2014, 47: 1235–1238.
5. Ji X, Yao L, Wang M, Liu X, Peng S, Li K, *et al.* Cystatin C attenuates insulin signaling transduction by promoting endoplasmic reticulum stress in hepatocytes. *FEBS Lett* 2015, 589: 3938–3944.
6. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, *et al.* SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor- α in the adipose tissue of obese mice. *J Biol Chem* 2001, 276: 47944–47949.
7. Yasuhara O, Hanai K, Ohkubo I, Sasaki M, McGeer PL, Kimura H. Expression of cystatin C in rat, monkey and human brains. *Brain Res* 1993, 628: 85–92.
8. Hakansson K, Huh C, Grubb A, Karlsson S, Abrahamson M. Mouse and rat cystatin C: *Escherichia coli* production, characterization and tissue distribution. *Comp Biochem Physiol B Biochem Mol Biol* 1996, 114: 303–311.
9. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol* 1992, 38 Suppl 1: S20–27.
10. Shlipak MG, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, *et al.* Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med* 2005, 352: 2049–2060.
11. Gauthier S, Kaur G, Mi W, Tizon B, Levy E. Protective mechanisms by cystatin C in neurodegenerative diseases. *Front Biosci (Schol Ed)* 2011, 3: 541–554.
12. Ueki K, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 2004, 24: 5434–5446.
13. Sarnak MJ, Katz R, Fried LF, Siscovick D, Kestenbaum B, Seliger S, *et al.* Cystatin C and aging success. *Arch Intern Med* 2008, 168: 147–153.