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Research report

β-amyloid increases neurocan expression through regulating Sox9 in astrocytes: A potential relationship between Sox9 and chondroitin sulfate proteoglycans in Alzheimer's disease

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ABSTRACT

Objective: This study aimed to investigate whether β-amyloid (Aβ) was able to enhance neurocan expression in a Sox9 dependent manner in astrocytes.
Methods and materials: Astrocytes were incubated with Aβ at different concentrations, the expression of Sox9 and neurocan was detected by Western blot assay. Meanwhile, the viability and proliferation of astrocytes were assessed by MTT assay. Then, the Sox9 expression was silenced, and the expression of Sox9 and neurocan was examined.
Results: After incubation with Aβ, the viability of astrocytes was increased regardless silencing of Sox9 (all $P < 0.05$). The proliferation of astrocytes was also gradually increased with the increase in the time of Aβ incubation (all $P < 0.05$). With the increase in Aβ concentration, the expression of Sox9 and neurocan was also increased (all $P < 0.05$). However, after silencing of Sox9 expression, the neurocan expression was significantly reduced as compared to control group and scra-siRNA group (all $P < 0.05$).
Conclusion: Our study shows the viability and proliferation of astrocytes are significantly increased by Aβ in a dose dependent manner. Moreover, Aβ may effectively up-regulate the neurocan expression via regulating Sox9.

1. Introduction

Alzheimer's disease (AD) is the most common and devastating clinical neurodegenerative disorder with approximately 36 million patients worldwide and has caused a great burden due to its high disability and mortality (Ginter et al., 2015). Despite all scientific approaches, medications currently used only aim to relieve symptoms and provide transient benefit in a small group of patients. The main pathological characteristics of AD is the progressive loss of cognition and memory in the presence of cerebral atrophy, senile plaques (SP), neurofibrillary tangles (NFT) and neuronal cell necrosis, which has been proven to be closely associated with the overproduction of β-amyloid (Aβ), particularly its longer form, Aβ42 (Jarrett et al., 1993; Sato and Morishita, 2015). Aβ generally results from the abnormal proteolytic cleavage of amyloid-precursor protein (APP). Abnormal accumulation and aggregation of Aβ may result in calcium influx, synaptic dysfunctions and cell death, which cannot be timely repaired by the nervous system, and are regarded as the main cause of cognitive deterioration in AD (Palop and Mucke, 2010). This relatively low self-repair ability is mainly attributed to the existence of neurite growth inhibitors produced during the active glial reactions driven by the alterations of glial cells dysfunction following injury in the nervous system (Fitch et al., 1999).

Astrocytes are the most abundant type of glial cells in the central nervous system. These cells belong to a highly heterogeneous population with variable proportions and different functions (Doens and Fernandez, 2014; Xiao et al., 2014). A growing body of evidence has shown that astrocytes can biochemically support the endothelial cells that form the blood-brain barrier (BBB) (Figley and Stroman, 2011), provide trophic and metabolic nutrients to the nervous tissue (Perea et al., 2009; Sofroniew, 2015), and control the homeostasis by maintaining pH and ion balance (Fiacco et al., 2009; Obara et al., 2008). In addition, previous studies have pointed out that the reactive astrocytes can colocalize with SP and NFT, playing a role in the repair and scarring of the nervous system though providing a barrier between healthy and injured tissues (Armstrong, 2009). However, the role of astrocytes in the pathogenesis of AD is complicated and controversial. Several studies (Funato et al., 1998; Wyss-Coray et al., 2003) have established the notion that astrocytes can phagocytose

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Aβ via various mechanisms such as lysosomal degradation and formation of the complex with Aβ by secreting the receptor for advanced glycation end product (RAGE). In other studies on AD, astrocytes seem to be less responsive to Aβ accumulation and thus fail to uptake Aβ from the extracellular space (Mulder et al., 2012). Moreover, over-expression of APP may possibly facilitate astrocytes to synthesize Aβ (Zhao et al., 2011).

Of note, exposure to Aβ may alter the morphology of astrocytes with a marked increase in the expression of chondroitin sulfate proteoglycans (CSPGs), which is a marker of astrocyte activation (Iaci et al., 2007). CSPGs are a family of large extracellular matrix molecules consisting of a protein core and a chondroitin sulfate side chain, which are represented by several types including neurocan, brevican, aggrecan, phosphacan/DSD-1, NG2, etc (Grumet et al., 1993; Paulsson et al., 1987; Yamada et al., 1994) They are expressed throughout the nervous system and involved in the neural development, brain plasticity, and post-injury response (Siebert and Osterhout, 2011). Importantly, CSPGs were demonstrated to play an inhibitory role on neurogenesis and contribute to post-injury glial scar formation by acting as a barrier to prevent axon extension and regrowth into the injured site (Monnier et al., 2003). DeWitt et al. also found that CSPGs was abundant in the NFT and SP, which indicates that CSPGs are responsible for the regression of neurites around SP as an alternative to Aβ in AD (DeWitt et al., 1993). Therefore, the regulation on signal transduction pathways involved in the CSPGs expression may potentially contribute to the inhibition of AD progression. Neurocan is a brain-specific proteoglycan produced largely by astrocytes, which is involved in some nervous system diseases like bipolar disorder, schizophrenia, and attention-deficit hyperactivity disorder (Schimmelmann et al., 2013; Schultz et al., 2014). However, few studies have been conducted to investigate the roles of neurocan in AD.

The sex-determining region Y (Sry)-box-containing (Sox) factors are a structurally-related family of transcription factors with a DNA-binding HMG domain, and have been found to be involved in a great number of developmental processes such as sex determination, skeleton formation, hemacytopoiesis and neuronal growth (Gubbay et al., 1990; Pevny and Lovell-Badge, 1997; Wegner, 1999). Sox9 as an important factor in the developmental processes of heart, kidney and brain has been well investigated recently (Bowles et al., 2000). Cheung and Briscoe (2003) pointed out that Sox9 could suppress the normal differentiation of neural progenitor cells and induce the neural crest differentiation of neural cells. Furthermore, Sox9 knock-down in primary astrocytes causes a decrease in CSPG expression, and conditional Sox9 ablation reduces CSPGs expression to improve the motor function following spinal cord injury (Gris et al., 2007; McKillop et al., 2013). However, in the central nervous system diseases such as AD, the effect of Sox9 on CSPGs expression still remains unclear. Although the direct neurotoxicity of Aβ in AD is well documented, the influence of Aβ on Sox9 expression also requires further investigation. Thus, in the present study, the brain-specific proteoglycan neurocan was used to determine whether Aβ affects the CSPGs expression in a Sox9 dependent manner in astrocytes.

2. Results

2.1. RNA interference significantly reduced Sox9 expression in astrocytes

The silencing efficiency of Sox9-siRNA was determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and Western blot assay. Results showed that the Sox9 expression at mRNA and protein levels was reduced by more than 80% following incubation with 60-nM Sox9-siRNA for 48 h as compared to control and scra-siRNA groups (Figs. 1 and 2). These indicate that Sox9 siRNA significantly inhibits the Sox9 expression at both mRNA and protein levels.

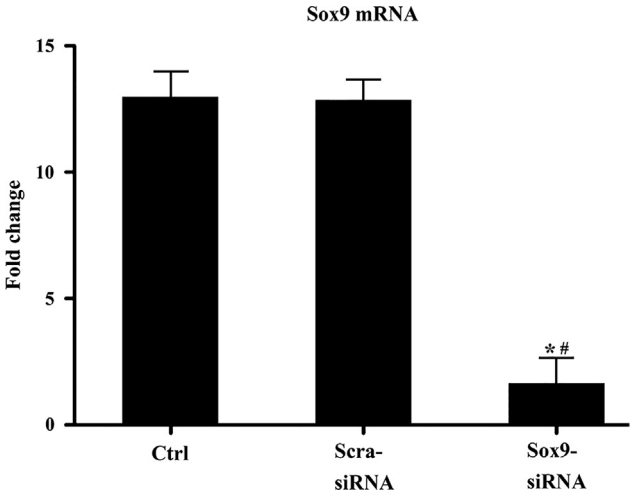

Fig. 1. The silencing efficiency of siRNA determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay. The Sox9 mRNA expression was significantly reduced following RNA interference compared with control group and scra-siRNA group, which indicates a successful knock down of Sox9 expression. RT-qPCR assay was carried out in triplicate using three repeated wells per condition, and experiments were performed at least three times on separate plates. *$P < 0.05$ vs. control group; #$P < 0.05$ vs. scra-siRNA group.

2.2. Aβ increased viability and proliferation of astrocytes at different time intervals

MTT assay showed that the cell viability was significantly increased in Aβ (0.70 ± 0.05 OD), scra-siRNA (0.73 ± 0.08 OD) and Sox9-siRNA (0.71 ± 0.07 OD) groups, as compared to control group (0.21 ± 0.06 OD; Fig. 3A; all $P < 0.05$) when the astrocytes were incubated 40 μM Aβ1-42 for 24 h. Moreover, when compared with control group, the cell proliferation of astrocytes was gradually increased with the increase in the time of Aβ1-42 incubation (Fig. 3B, all $P < 0.05$).

2.3. Aβ at different concentrations simultaneously up-regulated the expression of both Sox9 and neurocan

The expression of Sox9 and CSPGs was detected in astrocytes incubated with Aβ1-42 at different concentrations (10, 20, 30 and 40 μM). Western blot assay indicated that the Sox9 expression was significantly up-regulated with the increase in Aβ1-42 concentration (all $P < 0.05$ vs. 0 μM group; Fig. 4 A and B). Similarly, the neurocan expression was also significantly enhanced by Aβ1-42 in a concentration dependent manner as compared to 0 μM group (all $P < 0.05$; Fig. 4 C and D).

2.4. Aβ possibly regulated neurocan expression by targeting Sox9 in astrocytes

To investigate the potential effect of Sox9 on the neurocan expression, the expression of Sox9 and neurocan was compared among four groups. Results showed Aβ could significantly increase the expression of Sox9 and neurocan as compared to control group (both $P < 0.05$; Fig. 5). After silencing of Sox9 expression by siRNA, the neurocan expression was also significantly reduced ($P < 0.05$ vs. Aβ group; Fig. 5 C and D). These indicate that the alteration in neurocan expression is dependent on Sox9, suggesting that Aβ induced enhancement of neurocan expression is mediated by Sox9.