Available online at www.sciencedirect.com
ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Semaphorin3A-induced axonal transport mediated through phosphorylation of Axin-1 by GSK3β

*Tomonobu Hida, Fumio Nakamura, Hiroshi Usui, Kan Takeuchi, Naoya Yamashita, Yoshio Goshima**

Departments of Molecular Pharmacology, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan

ARTICLE INFO

Article history:
Accepted 11 December 2014
Available online 18 December 2014

Keywords:
Axonal transport
GSK3β
Axin-1
Semaphorin3a
Axon guidance
Dorsal root ganglia

ABSTRACT

The establishment of neuronal polarity is necessary for proper neuronal wiring. Semaphorin3A (Sema3A), originally identified as a repulsive axon guidance molecule, exerts a wide variety of biological functions through signaling pathways including sequential phosphorylation of collapsin response mediator protein by cyclin-dependent kinase-5 (Cdk5) and glycogen synthase kinase-3β (GSK3β). Sema3A acts on its receptor neuropilin-1 to regulate axonal transport. To delineate mechanism by which Sema3A induces axonal transport, we investigate whether GSK3β is involved in mediating Sema3A-induced axonal transport. 4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione, an inhibitor of GSK3β, suppressed Sema3A-induced antero- and retrograde axonal transport. Introduction of either GSK3β mutants, GSK3β-L128A or K85M, suppressed Sema3A-induced axonal transport. On the other hand, introduction of GSK3β-R96A did not affect the Sema3A effect, suggesting that unprimed substrates are primarily involved in Sema3A-induced axonal transport. Overexpression of a partial fragment of frequently rearranged in advanced T-cell lymphomas 1 (FRATtide), which interferes the interaction between GSK3β and Axis inhibitor-1 (Axin-1), also suppressed Sema3A-induced transport. siRNA knockdown of Axin-1, an unprimed substrate of GSK3β, suppressed Sema3A-induced antero- and retrograde axonal transport. These results indicate that GSK3β and Axin-1 are involved in Sema3A-induced bidirectional axonal transport. This finding should provide a clue for understanding of mechanisms of a wide variety of biological activities of Sema3A.

1. Introduction

Developing neurons extend axon toward target cells as being guided by a variety of guidance cues that are expressed in the developing nerve system, and form synaptic connections with specific target cells (Tessier-Lavigne and Goodman, 1996). Axonal transport plays crucial roles during this process: a variety of proteins, organelles and RNAs which are required for axon

---

*Correspondence to: *Departments of Molecular Pharmacology and Neurobiology, Yokohama City University Graduate School of Medicine, #3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan.* Fax: +81 45 785 3645.

E-mail address: goshima@med.yokohama-cu.ac.jp (Y. Goshima).

http://dx.doi.org/10.1016/j.brainres.2014.12.028
0006-8993/

outgrowth and synapse formation are synthesized in cell body and are transported to target region in axon (anterograde axonal transport). While neurotrophic factors received in axonal growth cone are transported to cell body together with its receptors and transmit signals that support neuronal survival and circuit formation (retrograde axonal transport) (Ascano et al., 2012). Axonal transport is mediated by kinesin and dynein motor protein along microtubule, and is essential for viability and function of neurons (Hirokawa et al., 2010). Although molecular mechanism of axonal transport has been studied extensively, the regulatory mechanisms by which external stimuli regulate axonal transport remain largely unknown.

Sema3A, a secreted type of class 3 semaphorin, has originally been identified as a repulsive axon guidance molecule (Raper, 2000; Tran et al., 2009). The receptors for Sema3A constitute a complex of neuropilin-1 (NRP-1) and plexinAs, which are ligand-binding and signal-transducing subunit of class 3 semaphorin receptor complexes, respectively (Zhou et al., 2008). Sema3A is not only involved in regulating growth cone functions, but also in dendritic patterning, synapse maturation and neural cell polarity during neuronal development (Nakamura et al., 2009; Sasaki et al., 2002; Tran et al., 2009; Yamashita et al., 2007, 2014). Sema3A induces acceleration of fast axonal transport in dorsal root ganglion (DRG) and hippocampus neurons (Goshima et al., 1997; Li et al., 2004; Yamane et al., 2012; Yamashita et al., 2014). We recently found a molecular link between axonal transport and dendritic localization of GluA2 (Yamashita et al., 2014).

GSK3β is a multifunctional proline-directed serine/threonine kinase, and plays crucial roles in a wide range of cellular processes including neuronal polarization, neurogenesis, neural migration, axon growth and guidance during neuronal development (Hur and Zhou, 2010). Over 40 GSK3β substrates have been identified and classified into two categories: primed substrates and unprimed substrates (Sutherland, 2011). For instance, collapsin response mediator protein-2 (CRMP2) and β-catenin, a primed substrate, requires the priming phosphorylation by another kinase at a serine/threonine residue located four residues C-terminal to the side of GSK3β phosphorylation sites (Hagen and Vidal-Puig, 2002; Uchida et al., 2005). Unprimed substrates, such as Axin-1 and presenilin-1, are also GSK3β substrates but do not require the priming phosphorylation (Ikeda et al., 1998; Twomey and McCarthy, 2006). We have previously reported that phosphorylation of Axin-1 by GSK3β contributes to intracellular signaling of Sema3A to induce growth cone collapse (Hida et al., 2012). In this study, we show that the phosphorylation of Axin-1 by GSK3β is also involved in intracellular signaling of Sema3A that induce acceleration of axonal transport. We here propose a novel role of Axin-1 in Sema3A-induced bidirectional axonal transport that may be involved in axon guidance, localization of glutamatergic receptor and dendritic development (Sasaki et al., 2002; Yamashita et al., 2014).

2. Results

2.1. *An inhibitor of GSK3β suppresses Sema3A-induced facilitation of axonal transport in chick DRG neurons*

We have previously reported that sequential phosphorylation of CRMP2 by Cdk5/GSK3β and phosphorylation of Axin-1 by GSK3β mediates growth cone collapse response to Sema3A (Uchida et al., 2005; Hida et al., 2012). In terms of different sensitivity against several reagents of Sema3A-induced growth cone collapse and axonal transport (Goshima et al., 1997; Yamane et al., 2012), distinct signaling pathways may be involved in Sema3A-induced growth cone collapse and axonal transport. For example, tetrodotoxin, a Na^+ channel blocker, suppresses Sema3A-induced axonal transport, but not growth cone collapse (Goshima et al., 1997; Yamane et al., 2012; Yamashita et al., 2014). To test whether GSK3β was also involved in Sema3A-induced facilitation of axonal transport, we examined the effect of a GSK3β inhibitor, 4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (TDZD-8) on axonal transport in chick DRG neurons (Fig. 1). TDZD-8 (15–150 nM) concentration-dependently decreased the basal numbers of moving vesicles in antero- and retrograde axonal transport (Table 1). These findings suggest that GSK3β (Guzman et al., 2007; Martinez et al., 2011) was involved in the mechanism regulating the basal axonal transport. We next examined the effect of TDZD-8 on the Sema3A-induced facilitation of axonal transport (Fig. 2). In control DRG neurons, Sema3A at 5 nM accelerated antero- and retrograde axonal transport within 1–3 min after application with the peak effect being seen at 4–6 min. The enhancement returned to basal levels 15 min after Sema3A application (Fig. 2A and B). In DRG neurons pretreated with TDZD-8 (15 nM), Sema3A did not accelerate the antero- and retrograde axonal transport (Fig. 2A and B).

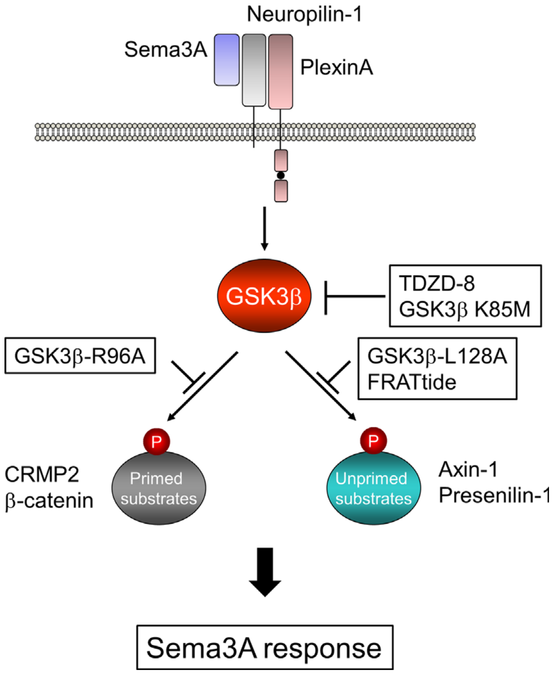

Fig. 1 – The targets of pharmacological reagent, glycogen synthase kinase-3β (GSK3β) mutants and a partial fragment of frequently rearranged in advanced T-cell lymphomas 1 (FRATtide) used in this study. 4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (TDZD-8) is a GSK3β specific inhibitor; GSK3β K85M mutant lacks the ability to phosphorylate primed substrates and unprimed substrates; GSK3β R96A mutant lacks the ability to phosphorylate primed substrates; GSK3β L128A mutant lacks the ability to phosphorylate unprimed substrates; FRATtide inhibits phosphorylation of Axin-1 by GSK3β. Other details are described in the text.