



● INVITED REVIEW

Phosphatidylserine improves axonal transport by inhibition of HDAC and has potential in treatment of neurodegenerative diseases

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Abstract

Familial dysautonomia (FD) is a rare children neurodegenerative disease caused due to a point mutation in the *IKBKAP* gene that results in decreased IKK complex-associated protein (IKAP) protein production. The disease affects mostly the dorsal root ganglion (DRG) and the sympathetic ganglion. Recently, we found that the molecular mechanisms underlying neurodegeneration in FD patients are defects in axonal transport of nerve growth factors and microtubule stability in the DRG. Neurons are highly polarized cells with very long axons. In order to survive and maintain proper function, neurons depend on transport of proteins and other cellular components from the neuronal body along the axons. We further demonstrated that IKAP is necessary for axon maintenance and showed that phosphatidylserine acts as an HDAC6 inhibitor to rescue neuronal function in FD cells. In this review, we will highlight our latest research findings.

Key Words: axonal transport; neurodegeneration; microtubule; familial dysautonomia; phosphatidylserine; HDAC6

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Introduction

Familial dysautonomia (FD) is a rare autosomal recessive congenital neurodegenerative neuropathy, which occurs almost exclusively in children of the Ashkenazi Jewish population with remarkably high carrier frequencies of 1 in 32 overall and of 1 in 18 in those of Polish descent (Lehavi et al., 2003). Individuals with FD suffer from a variety of symptoms including vomiting crises, pneumonia, ataxia, difficulty swallowing, gastrointestinal and cardiovascular dysfunction, and short life spans (Riley et al., 1949; Mahloudji et al., 1970; Axelrod et al., 2002; Wan et al., 2011; Palma et al., 2014). FD patients exhibit abnormal development and progressive depletion of unmyelinated sensory and autonomic neurons (Fogelson et al., 1967; Pearson and Pytel, 1978a, b; Pearson et al., 1978; Axelrod et al., 1995) and reductions in sizes and numbers of dorsal root ganglion (DRG) and sympathetic ganglion (SG) neurons (Pearson et al., 1975, 1978; Abashidze et al., 2014; Jackson et al., 2014). The genetic cause of FD is a point mutation in the *IKBKAP* gene, which encodes the IκB kinase com-

plex-associated protein (IKAP). The mutation alters the splicing pattern of the *IKBKAP* gene in a tissue-specific manner, leading to lower than normal levels of IKAP in the nervous systems. The exact role of IKAP in neurons and why neurons lacking IKAP degenerate are not entirely understood.

As neurons are highly polarized cells with very long axons that can be more than a meter long in adult humans, these cells are uniquely dependent on efficient intracellular transport to maintain spatiotemporal signaling, structural integrity, and function. Proper microtubule polymerization and stabilization is also essential to regulate axonal transport. Microtubules are formed from the dynamic polymerization of αβ-tubulin dimers required for the normal outgrowth of the axon and growth cone. Impairment of axonal transport appears to be one of the major pathogenic mechanisms that result in neurodegeneration in patients with diseases such as amyotrophic lateral sclerosis (ALS), Huntington's, Alzheimer's, Parkinson's, and Charcot-Marie-Tooth diseases (Perlson et al., 2010; Hinckelmann et al., 2013; Millecamps and Julien,

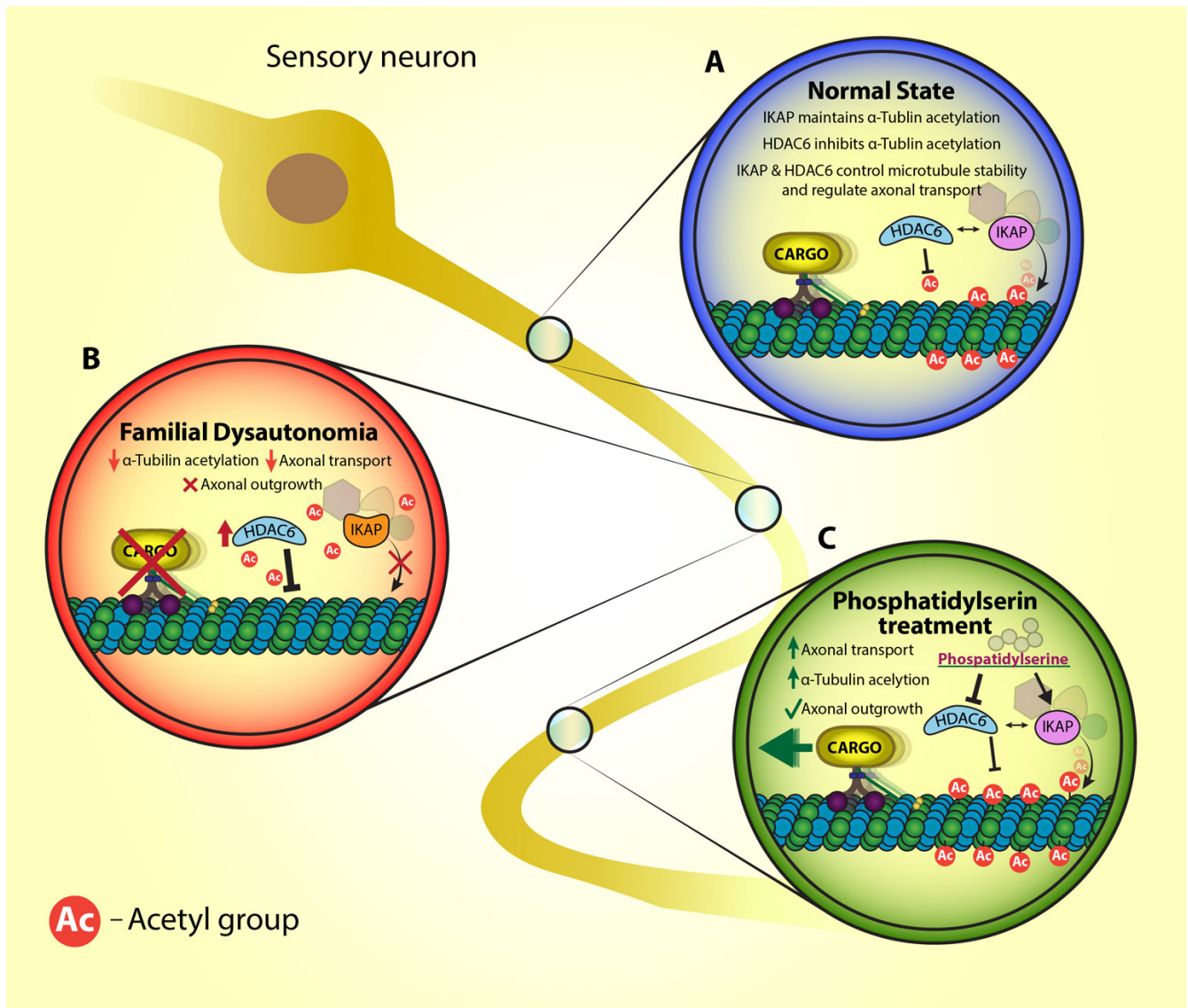


Figure 1 A suggested model for the impaired axonal transport in familial dysautonomia (FD) and the effect of phosphatidyserine. A scheme representation of IKK complex-associated protein (IKAP) involvement in axonal transport in sensory neuron at (A) normal state, (B) Familial dysautonomia patients and (C) after phosphatidyserine treatment. (A) In normal state IKAP is part of a complex that acetylates α -tubulin and histone deacetylase 6 (HDAC6) is the major α -tubulin deacetylates. Acetylated α -tubulin is essential for dynein movement and polymerization of microtubules. (B) In FD, lower levels of IKAP are indicated and influence HDAC6 levels. As a result of the imbalance interplay in IKAP and HDAC6 levels, microtubule stabilization and axonal transport affected. (C) Phosphatidyserine elevates IKAP levels and downregulates HDAC6 levels and thus facilitates axonal transport and microtubule stabilization.

2013). Therefore, we speculated that axonal transport and microtubule stability are defective in FD patients.

Transport along DRG Axons is Impaired in Neurons that Lack IKAP

Several FD models indicate that there are alterations in microtubule acetylation (Gardiner et al., 2007; Creppe et al., 2009), a process that regulates axonal transport, in cells deficient in IKAP. Tubulin acetylation is a reversible post-translational modification that occurs at lysine 40 in the N-terminal region of α -tubulin. Acetylated tubulin levels impact the degree of protein trafficking along micro-

tubules (Reed et al., 2006; Li et al., 2011; Jakovcevski and Akbarian, 2012) and stability of the microtubule backbone (Rosenbaum, 2000; Westermann and Weber, 2003), and defects in tubulin acetylation are associated with Alzheimer's, Huntington's, and ALS diseases (Hempfen and Brion, 1996; Dompierre et al., 2007).

Histone deacetylase 6 (HDAC6) is a key regulator of axonal α -tubulin acetylation (Hubbert et al., 2002). HDAC6 expression increases following neuronal injury (Rivieccio et al., 2009), and treatment with an HDAC inhibitor promotes neuronal outgrowth (Gaub et al., 2010). Also inhibition of HDAC6 expression or use of

the deacetylase inhibitor trichostatin A (TSA) elevates axonal transport rates by enhancing acetylated α -tubulin levels (Dompierre et al., 2007; d'Ydewalle et al., 2011; Godena et al., 2014). Thus, HDAC inhibitors have been evaluated pre-clinically and clinically for treatment of neurodegenerative diseases and neuropathologic events; however, side effects and toxicity limit their utility (Dietz and Casaccia, 2010).

Recently, we developed a novel conditional knockout (CKO) mouse model of FD using Cre-loxP system to extract *IKBKAP* exon 20 in the nervous system and DRGs; these mice demonstrated downregulate of IKAP levels in the DRGs and have many FD symptoms. Using this model we identified the underlying causes of degeneration in FD. We demonstrated using live imaging assays in microfluidic chambers that there is a significant decrease of nerve growth factor (NGF) retrograde transport along DRG axons derived from the FD mice compared to controls. We further found using several models, including FD patient cells, that this axonal transport inhibition is accompanied by lower levels of acetylated α -tubulin and by an increase in HDAC6 levels. These findings demonstrate the urgent need to find an effective, non-toxic HDAC6 inhibitor that can be used to treat FD patients.

Phosphatidylserine Enhances NGF Axonal Transport by Inhibiting HDAC6 Levels

Phosphatidylserine (PS), a food supplement with no reported side effects, was previously shown to have potential as an FD therapy (Keren et al., 2010; Bochner et al., 2013; Salani et al., 2013; Donyo et al., 2016). PS promotes cell survival (Maragno et al., 2015), reduces pro-inflammatory signals (Monastra and Bruni, 1992), activates the MAP/ERK kinase pathway (Donyo et al., 2016), and inactivates JNK and p38 signaling after lipopolysaccharide treatment (Nolan et al., 2004). In FD mouse models, PS increases NGF axonal transport, downregulates HDAC6 levels, and elevates acetylated α -tubulin levels (Naftelberg et al., 2016). When treated with PS, cultured DRG neurons deficient in IKAP have more NGF tracks per axon and transport occurs at higher velocity than in untreated neurons (Naftelberg et al., 2016). Interestingly, phosphatidylserine also significantly improves axonal transport in normal healthy DRGs compared to vehicle-treated controls, which indicates that the beneficial effects of PS are not limited to FD. The effect of PS in other neuropathological disease models and neuron types should be tested in the near future. Our analysis suggests that PS inhibits HDAC, elevating acetylated α -tubulin levels and impacting dynamics, stability, and growth of axons (Naftelberg et al., 2016).

Conclusions

In our work to elucidate the neurodegenerative pathway in familial dysautonomia disease, we demonstrated that the mechanism involves an increase in HDAC6 expression, which results in aberrant NGF axonal transport and decreased DRG neuron survival with attenuated outgrowth axons (**Figure 1**). We discovered that PS is a safe, potent regenerative therapy that acts as a HDAC inhibitor. Pharmacological inhibition of HDAC6 activity by PS treatment will likely enhance neuronal survival in FD patients and could be effective for treatment of patients with other neurodegenerative disorders that have molecular features similar to FD such as elevated HDAC6 levels, reduced acetylated tubulin levels, and alterations in axonal transport (**Figure 1**).

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