Autophagy 7:2, 238-239; February 2011; © 2011 Landes Bioscience

Autophagy aids membrane expansion by neuropathic Schwann cells

Sunitha Rangaraju and Lucia Notterpek*
Department of Neuroscience; College of Medicine; McKnight Brain Institute; University of Florida; Gainesville, FL USA

Demyelinating peripheral neuropathies associated with abnormal expression of peripheral myelin protein 22 (PMP22) involve the formation of cytosolic protein aggregates within Schwann cells. Towards developing a therapy for these progressive neurodegenerative diseases, we assessed whether pharmacological activation of autophagy by rapamycin (RM) could prevent protein aggregation and enhance Schwann cell myelination. Indeed, we found that glial cells from neuropathic mice activate autophagy in response to RM and produce abundant myelin internodes. Lentivirus-mediated shRNA shutdown of Atg12 abrogates the improvements in myelin production, demonstrating that autophagy is critical for the observed benefits.

Key words: myelination, protein aggregation, neuropathy, neurodegeneration, Schwann cell

Submitted: 11/21/10

Revised: 11/23/10

Accepted: 11/24/10

Previously published online: www.landesbioscience.com/journals/autophagy/article/14278

DOI: 10.4161/auto.7.2.14278

*Correspondence to: Lucia Notterpek; Email: notterp@mbi.ufl.edu

Punctum to: Rangaraju S, Verrier JD, Madorsky I, Nicks J, Dunn WA Jr, Notterpek L. Rapamycin activates autophagy and improves myelination in explant cultures from neuropathic mice. J Neurosci 2010; 30:11388–97; PMID: 20739560; DOI: 10.1523/JNEUROSCI.1356-10.2010.

Myelin, a specialized lipid-rich, multilayered membrane, is deposited along axons by Schwann cells in the peripheral nervous system. PMP22, a key component of Schwann cell myelin is prone to misfolding and the majority (~80%) of the newly synthesized protein is targeted for degradation by the proteasome. In neuropathic Schwann cells, when the *PMP22* gene is mutated or overexpressed, the quality control mechanisms responsible for the processing of PMP22 are overwhelmed, leading to the formation of cytosolic protein aggregates. These protein aggregates in turn accumulate chaperones and other essential glial molecules and likely contribute to the inability of the cells to maintain their differentiated, myelinated phenotype.

Compounds that enhance the activity of intracellular quality control mechanisms offer potential treatment opportunities for protein misfolding disorders, including PMP22-linked neuropathies. Autophagy is a particularly appealing therapeutic target mechanism for progressive neurodegenerative diseases, as activation of this pathway in aged mice extends life span without any apparent negative side effects. Reports in the literature also show that enhancement of autophagy by small molecules is able to decrease the aggregation of several disease-linked neuronal proteins; however, similar experiments in myelinating glial cells have not been carried out. As a proof-of-principle intervention to activate chaperones and autophagy in neuropathic mice, we previously tested the intermittent fasting regimen, which led to an improvement in locomotor performance and myelination of peripheral nerves. Since such drastic dietary restriction is not suitable for humans, in our recent study we asked whether RM could be used for activating autophagy in actively myelinating Schwann cells.

Using explant cultures of dorsal root ganglion neurons, we found that Schwann cells isolated from normal mice maintain their myelinating phenotype when autophagy is enhanced by RM exposure. These data show that myelinating glial cells are amenable to pharmacological stimulation of autophagy, and RM is not toxic to these cells. Cultures from neuropathic mice also reveal a decrease in p62 and in polyubiquitinated proteins in response to RM, supporting the idea that undegraded proteasome substrates are removed by autophagy. Along with the activation of autophagy, the processing and trafficking of PMP22, as judged from its carbohydrate moiety, also improves and is associated with an increase in the abundance and length of myelin internodes.

Significantly, activation of autophagy by RM improves myelination in cells from two genetically distinct neuropathic models, including a PMP22 overexpressor and a point mutation genotype. Because inhibition of Atg12 by lentiviral gene suppression abolishes the improvements in both models, we conclude that intact autophagy is critical for the observed effect.

How might the activation of autophagy benefit myelination by neuropathic Schwann cells? Restoration of protein homeostasis, including the degradation of misfolded PMP22, likely provides a more conducive environment for glial membrane expansion, a process that is known to demand extensive de novo protein synthesis, and correct protein folding and trafficking. Since myelin proteins interact, improvement in the processing of PMP22 also likely benefits the trafficking of other glial molecules. Compared to the neuropathic models, the reason for enhanced myelin production by normal Schwann cells in response to RM treatment is rather puzzling. While the increase in normal cultures is less pronounced than in affected samples, the finding is reproducible between numerous independent experiments. As myelination demands de novo membrane synthesis, the activation of autophagy within glial cells may improve myelin expansion by affecting the endocytic pathway. It is feasible that during myelinogenesis, patches of the Schwann cell membrane are recycled via the endocytic pathway in order to establish the distinct compact and uncompact subdomains of myelin. The more pronounced increase in the lengths rather than in the number of myelin internodes in normal cultures supports the notion that RM affects membrane expansion rather than the differentiation of Schwann cells.

As our dorsal root ganglion myelinating explant cultures contain both neurons and glia, we also investigated the autophagic response of the two cell types independently. Compared to Schwann cells, peripheral sensory neurons show muted activation of autophagy in response to low concentration (25 nM) of RM. Still, it is possible that besides activating autophagy, RM may have affected signaling pathways involving Akt, which was reported to modulate neuregulin-mediated Schwann cell survival. Therefore, while the rationale for our work with RM as a therapeutic agent for PMP22-associated neuropathies stems from our goal to prevent protein aggregation, based on the current study we cannot rule out the possibility that RM benefits neuropathic Schwann cells through other mechanisms, as well. The observed improvements in samples from normal mice support this hypothesis.

Based on our positive results, we propose that RM is a suitable compound for further testing in neurological disorders, particularly those involving peripheral nerves. Schwann cells respond to low concentration of RM (25 nM) and the peripheral nervous system is outside of the blood brain barrier, which will permit better access to the target tissue. Therefore, it is possible that peripheral nerves will react to this compound more robustly and at lower dosages than the central nervous system. In summary, our results provide further support for the involvement of autophagy as a protective pathway in protein aggregation disorders such as PMP22-associated neuropathies. Furthermore, we also show that enhancement of this pathway is permissive and likely beneficial for membrane expansion by glial cells.