Protein misfolding and clearance in demyelinating peripheral neuropathies: therapeutic implications

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Peripheral neuropathies such as Charcot-Marie-Tooth disease (CMT) are a group of neurological disorders that affect the peripheral nervous system. Although demyelinating CMT is the most prevalent hereditary peripheral neuropathy, there are currently no effective treatments for patients suffering from this disease. Recent studies by our group and others have provided a link between protein misfolding and demyelinating CMT and indicate that impairment of the proteasome and aggresome-autophagy pathways may contribute to CMT pathogenesis. These studies suggest that targeting protein quality control systems involved in cytoprotection against CMT-associated misfolded proteins could have therapeutic benefits for treating demyelinating CMT.

Peripheral neuropathies are a group of disorders that affect the peripheral nervous system, with demyelinating Charcot-Marie-Tooth disease (CMT) being the most common inherited peripheral neuropathy.1 Demyelinating CMT is characterized by motor weakness, sensory loss and muscle wasting, all of which drastically decrease the quality of life.2 There are currently no effective treatments for demyelinating CMT. Recent studies by our laboratory and others have provided important insights into the molecular and cellular pathways involved in demyelinating CMT, which may help develop better treatments for this disease.

Protein Misfolding is a Common Feature in Demyelinating CMT

Our recent study revealed that mutations causing demyelinating CMT type 1C (CMT1C) are clustered within or around the transmembrane domain of the C-tail anchored protein SIMPLE and disrupt its insertion into the membrane, leading to accumulation of misfolded SIMPLE in the cytosol.3 Similarly, mutations causing the overexpression or single amino acid substitutions of the type III tetra-spanning1 membrane protein peripheral myelin protein 22 (PMP22) and type II single-spanning2 membrane protein myelin protein zero (MPZ) are linked to the major subtypes of demyelinating CMT, type 1A (CMT1A) and type 1B (CMT1B), respectively, which lead to the misfolding of PMP22 and MPZ.3-8 How misfolding of these membrane proteins with different topologies contributes to demyelinating neuropathy remain unknown. We found that misfolded SIMPLE forms abnormal cytosolic aggregates and mediates erroneous interactions with cellular proteins,3 which are pathogenic mechanisms characteristic of many protein-misfolding diseases.4,9,10 These findings suggest that demyelinating CMT may be a protein-misfolding disease of Schwann cells. The Proteasome and Aggresome-Autophagy Pathways Protect Schwann Cells against Toxic Build-up of Misfolded Proteins

Misfolded myelin proteins, such as PMP22 and MPZ (Fig. 1 and step 1a), may be refolded by molecular chaperones at the endoplasmic reticulum (ER).11,12 When refolding is not possible, these misfolded proteins are ret-
Our study found that misfolded SIMPLE proteins translocated from the early endosome to the cytosol (Fig. 1 and step 3) and cleared by the proteasome (Fig. 1 and step 4) in a process known as ER-associated degradation (ERAD).\(^1^4\) Our study found that misfolded SIMPLE or also degrades other C-tail anchored membrane proteins will need to be addressed by future studies. In addition, the E3 ligases targeting misfolded proteins for ERAD-dependent and -independent clearance of misfolded proteins in Schwann cells remain to be identified.

The proteasome system only degrades monomeric misfolded proteins but not aggregated proteins. When proteasome is impaired or overwhelmed, misfolded proteins accumulate and form soluble oligomers (Fig. 1 and step 5) that inhibit proteasome function (Fig. 1 and step 6) and may impair Schwann cell functions including myelination\(^1^3,^1^4\) (Fig. 1 and step 7). The aggresome-autophagy pathway has emerged as another crucial protein quality control system in Schwann cells that degrades misfolded and aggregated proteins.\(^1^4-^1^6\) Reports by our group and others indicate that Schwann cells handle misfolded PMP22 and SIMPLE by sequestering them into perinuclear aggresomes (Fig. 1 and step 8). Although the signaling events that target misfolded PMP22 and SIMPLE into aggresomes have not been identified, our previous finding\(^2\) of parkin-mediated K63 linked poly-ubiquitination in targeting misfolded protein to aggresomes via interactions with the dynein adaptor protein HDAC6 suggest that as yet-to-be identified E3 ligases and adaptor proteins could be responsible for targeting misfolded PMP22 and SIMPLE to aggresomes. Moreover, we\(^3\) and others\(^1^8\) showed that PMP22- and SIMPLE-positive aggresomes are tightly surrounded by autophagosome markers (Fig. 1 and step 9) and degraded by autophagy (Fig. 1 and step 10).

**Impairment in the Proteasome and Aggresome-Autophagy Pathways Contributes to Peripheral Neuropathies**

The accumulation of misfolded PMP22 and MPZ at the ER\(^1^9,^2^0\) suggests that ERAD dysfunction and subsequent ER stress may be involved in causing demyelinating CMT. Our recent finding that

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**Figure 1.** Protein quality control systems are potential targets for mechanism-based treatments of demyelinating Charcot-Marie-Tooth disease (CMT). Genetic mutations and increased protein expression levels linked to demyelinating CMT induce misfolding of hydrophobic proteins such as peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), and SIMPLE in Schwann cells (step 1a and 1b). Chaperones at the endoplasmic reticulum (ER) and the cytosol refold misfolded PMP22 and MPZ proteins (step 2), but when refolding is not possible, these proteins are recognized and retrotranslocated to the cytosol by the p97/VCP-dependent ER-associated degradation (ERAD) machinery (step 3). In contrast, misfolded SIMPLE is translocated to the cytosol by an ERAD-independent mechanism (step 1b). These misfolded proteins are targeted to the 26S proteasome by K48-linked poly-ubiquitination for degradation (step 4). When the chaperone and proteasome systems are damaged or overwhelmed, misfolded proteins accumulate and aggregate into toxic oligomers (step 5) that could inhibit proteasome function (step 6) and impair myelination by Schwann cells, leading to demyelinating CMT (step 7). The aggresome-autophagy pathway is another protein quality control system in which misfolded and aggregated proteins are transported by the microtubule-dependent dynein motor complex to the aggresomes (step 8). Aggresomes not only sequester toxic misfolded and aggregated proteins but also recruit autophagic membrane for the formation of autophagosomes (step 9) and subsequent degradation by lysosomal hydrolases upon autophagosome-lysosome fusion (step 10). Therapeutic strategies and currently available agents that enhance the targeting of misfolded and aggregated proteins for turnover are indicated by the color green (upward arrows), and drugs exacerbating the CMT neuropathy phenotype are marked by the color red (downward arrows).
misfolded SIMPLE is not cleared by ERAD, indicating that CMT pathogenesis can be mediated by dysfunction in ERAD-independent pathways. Proteasome impairment induced by misfolded and aggregated proteins was observed in several mouse models of demyelinating CMT1A. Moreover, proteasome inhibition by the chemotherapeutic medication bortezomib causes demyelinating neuropathy and worsens the neuropathic symptoms of CMT patients. These findings suggest that proteasome dysfunction could contribute to demyelinating CMT pathogenesis.

Impairment of the aggresome-autophagy pathway by microtubule-disrupting drugs such as vincristine, cisplatin and docetaxel (Fig. 1 and step 8) block aggresome-targeting of misfolded PMP22 and exacerbate the neuropathy phenotype in CMT patients. Inhibition of microtubule polymerization observed in Chediak-Higashi syndrome, a genetic disease caused by LYST mutations, leads to severe demyelinating peripheral neuropathy as well. Meanwhile, the raise in luminal pH of lysosomes and disruption of autophagosome-lysosome fusion by chloroquine also induce peripheral neuropathy. These evidence suggests that dysfunction in the aggresome-autophagy pathway is another major contributing factor of demyelinating peripheral neuropathies.

### Conclusions

Recent evidence by our group and others has implicated protein misfolding and aggregation in the pathogenesis of demyelinating CMT. The findings that the proteasome and aggresome-autophagy pathways clear misfolded proteins in Schwann cells and their impairment in demyelinating neuro-pathies suggest that augmentation of these pathways may provide therapeutic benefits for treating demyelinating CMT. Future studies to identify Schwann cell-specific E3 ligases and adaptor proteins that target misfolded proteins for clearance will facilitate the development of new therapeutic strategies that are clinically viable in treating demyelinating CMT.

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### The Proteasome and Aggresome-Autophagy Pathways are Potential Therapeutic Targets in CMT

The studies described above suggest that the augmentation of the proteasome and aggresome-autophagy pathways in Schwann cells could help protect against demyelination by aggregating proteins. Proteasome activation by oleuropein or enhanced targeting to proteasome by an USP14 inhibitor both demonstrated cytoprotective effects. In addition, the chemical compounds B2 and B5 identified from a recent drug screen are inducers of aggresome formation that reduce the cytotoxicity mediated by misfolded proteins. These drugs could be evaluated as potential treatments for demyelinating CMT. Our recent finding that autophagy activation by rapamycin promotes autophagic degradation of misfolded SIMPLE, together with the report of rapamycin in promoting myelination in explant cultures from neuropathic mouse models of CMT1A, suggest that autophagy activation could be efficacious for treating demyelinating peripheral neuropathy.

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