SIMPLE: A new regulator of endosomal trafficking and signaling in health and disease

Lih-Shen Chin, Samuel M. Lee and Lian Li*
Department of Pharmacology; Emory University School of Medicine; Atlanta, GA USA

SIMPLE, also known as LITAF, EET1 and PIG7, was originally identified based on its transcriptional upregulation by estrogen, p53, lipopolysaccharide or a microbial cell-wall component. Missense mutations in SIMPLE cause Charcot-Marie-Tooth disease (CMT), and altered SIMPLE expression is associated with cancer, obesity and inflammatory bowel diseases. Despite increasing evidence linking SIMPLE to human diseases, the biological function of SIMPLE is unknown and the pathogenic mechanism of SIMPLE mutations remains elusive. Our recent study reveals that SIMPLE is a functional partner of the endosomal sorting complex required for transport (ESCRT) machinery in the regulation of endosome-to-lysosome trafficking and intracellular signaling. Our results indicate that CMT-linked SIMPLE mutants are loss-of-function mutants which act dominantly to impair endosomal trafficking and signaling attenuation. We propose that endosomal trafficking and signaling dysregulation is a key pathogenic mechanism in CMT and other diseases that involve SIMPLE dysfunction.

Charcot-Marie-Tooth disease (CMT) is the most common hereditary peripheral neuropathy with no effective treatment.1 Human genetic studies reveal that missense mutations in SIMPLE, a protein of unknown function, cause autosomal dominant CMT type 1C (CMT1C).2-6 SIMPLE, also known as LITAF, EET1 and PIG7, was originally identified as a gene product whose transcription is upregulated by estrogen,7 p53 protein,8 lipopolysaccharide (LPS)9 and a microbial cell-wall component.10 Reduced SIMPLE expression is associated with several types of cancer, including breast cancer,11 lymphoma,12 leukemia13 and thyroid carcinoma,14 whereas increased SIMPLE expression is linked to obesity15 and inflammatory bowel diseases, such as Crohn’s disease and ulcerative colitis.16 The connection of SIMPLE to multiple human diseases underscores the importance of understanding the biochemical function and cellular role of this enigmatic protein.

**SIMPLE Functions in the Regulation of Endosome-to-Lysosome Trafficking and Cell Signaling**

Endosome-to-lysosome trafficking is a crucial cellular process that not only controls protein degradation but also regulates intracellular signaling.17,18 Receptors at cell surface are internalized in response to ligand binding and delivered to the early endosome, where they are either recycled to the cell surface or sorted to intralumenal vesicles of multivesicular bodies for transport to the lysosome for degradation. The core machinery for mediating endosomal cargo sorting to the lysosomal pathway comprises ESCRT-0, -I, -II and -III complexes.17 The ESCRT accessory factors and mechanisms that confer temporal and spatial control to the endosomal trafficking process remain largely unknown.

SIMPLE is a 161-amino-acid protein with widespread expression in a variety of tissues and cells.2,10,19,20
Although a distinct transcript coding for a 228-amino-acid protein was reported to be encoded by the *SIMPLE* gene, it is now clear that this larger transcript is the result of a DNA sequencing error.\(^5\),\(^6\),\(^10\),\(^19\),\(^22\) Accumulating evidence\(^5\),\(^10\),\(^19\) indicates that *SIMPLE* is unlikely to be a transcription factor as initially proposed.\(^9\) The function of *SIMPLE* is unknown, although it contains binding sites for TSG101 and NEDD4 (Fig. 1A).\(^9\) *SIMPLE* also contains a cysteine-rich (C-rich) domain (Fig. 1A) that was hypothesized to be a putative RING finger with E3 ubiquitin-protein ligase activity.\(^9\),\(^22\) However, our analysis shows that the *SIMPLE* C-rich domain is not a RING finger because it lacks a key His residue and is interrupted by an embedded transmembrane domain (Fig. 1A).\(^9\) Furthermore, the results of our biochemical experiments reveal that *SIMPLE* protein has no E3 ligase activity either in vitro or in vivo.\(^24\) By using highly specific anti-*SIMPLE* antibodies, we found that endogenous *SIMPLE* is an early endosomal membrane protein,\(^9\) rather than a nuclear protein\(^9\),\(^25\) or a lysosomal/late endosomal membrane protein,\(^10\) as previously suggested.

In a recent study,\(^24\) we examined the cellular function of *SIMPLE* and found that *SIMPLE* is a novel regulator of endosome-to-lysosome trafficking. Our results indicate that *SIMPLE* participates in the recruitment of ESCRT components STAM1, Hrs and TSG101 to the early endosomal membrane and functions with the ESCRT machinery in controlling endosomal sorting and lysosomal degradation of cargo proteins, such as ErbB receptors. In addition, we found that *SIMPLE* is required for efficient attenuation of signaling events downstream of ligand-activated ErbB receptors. Our data support that *SIMPLE* regulates cell signaling by promoting endosome-to-lysosome trafficking and degradation of signaling receptors.\(^24\) Given its widespread expression pattern,\(^10\),\(^19\),\(^20\) our findings suggest that *SIMPLE* may regulate endosomal trafficking and signaling processes in many different cells, including LPS-induced inflammatory signaling in macrophages.\(^26\)

**Endosomal Trafficking and Signaling Dysregulation: A Key Mechanism in CMT Pathogenesis**

Despite the identification of eight distinct point mutations in *SIMPLE* (Fig. 1A) as the genetic defects for causing CMT1C,\(^2\),\(^6\) the pathogenic mechanisms of these mutations are unknown. We have shown that *SIMPLE* is a post-translationally inserted, C-tail-anchored membrane protein that uses its TMD for anchoring to the early endosomal membrane.\(^10\),\(^24\) Interestingly, all of the identified disease-causing *SIMPLE* mutations map in and around the TMD (Fig. 1A). We found that CMT1C-linked *SIMPLE* W116G and P135T mutations promote *SIMPLE* misfolding and impair its membrane insertion, causing *SIMPLE* to mislocalize from the endosomal membrane to the cytosol.\(^9\),\(^27\) Our recent results indicate that *SIMPLE* W116G and P135T are loss-of-function mutants that exert dominant pathogenic effects to impair endosome-to-lysosome trafficking and signaling attenuation in cells.\(^24\) The dominant pathogenic role of *SIMPLE* mutation is further supported by our finding of a CMT1C-like peripheral neuropathy phenotype in transgenic mice expressing *SIMPLE* W116G mutant\(^28\) and the lack of a neuropathy phenotype in *SIMPLE* knockout mice.\(^20\)

Our work reveals a critical role of dysregulated endosome-to-lysosome trafficking in the pathogenesis of demyelinating CMT1C.\(^24\) Previous studies have shown that mutations in MTMR2 and MTMR13, which are also involved in regulation of endosomal trafficking, cause demyelinating CMT4B1 and CMT4B2.\(^1\),\(^29\) Thus, endosomal trafficking dysregulation appears to be a common
Pathogenic mechanism in several demyelinating CMT diseases. The fact that mutations in these ubiquitously expressed proteins cause demyelinating peripheral neuropathy suggests that, compared with other cell types, Schwann cells are particularly susceptible to defects in endosomal trafficking. We found that SIMPLE is highly abundant in Schwann cells and that SIMPLE W116G and P135T mutations cause dysregulation of neural-regulon 1 (NRG1)-ErbB2/ErbB3 signaling, a key pathway for controlling peripheral nerve myelination by Schwann cells. In our transgenic CMT1C mouse model, SIMPLE W116G mutation-induced peripheral neuropathy is accompanied by myelin infolding—the focally infolded myelin loops that protrude into the axon. Together, our findings suggest a pathogenic pathway (Fig. 1B) by which SIMPLE mutation disrupts endosome-to-lysosome trafficking and signaling attenuation of NRG1-activated ErbB2/ErbB3 receptors in Schwann cells, causing prolonged activation of downstream signaling pathways, thereby leading to myelin infolding and demyelinating peripheral neuropathy.

Conclusions

Our recent study revealing the function of SIMPLE as a regulator of endosome-to-lysosome trafficking and intracellular signaling has provided new insights into the mechanisms of SIMPLE action in health and disease. The evidence obtained from our work indicates that SIMPLE mutation-induced endosomal trafficking and signaling dysregulation in Schwann cells play a key role in CMT1C pathogenesis. Our findings also raised the possibility that altered SIMPLE expression found in cancer, obesity and inflammatory bowel diseases may contribute to the pathogenesis or progression of these diseases by altering SIMPLE-dependent endosomal trafficking and signaling dysregulation in Schwann cells play a key role in CMT1C pathogenesis. Our findings also raised the possibility that altered SIMPLE expression found in cancer, obesity and inflammatory bowel diseases may contribute to the pathogenesis or progression of these diseases by altering SIMPLE-dependent endosomal trafficking and signaling dysregulation in Schwann cells play a key role in CMT1C pathogenesis. Our findings also raised the possibility that altered SIMPLE expression found in cancer, obesity and inflammatory bowel diseases may contribute to the pathogenesis or progression of these diseases by altering SIMPLE-dependent endosomal trafficking and signaling dysregulation in Schwann cells play a key role in CMT1C pathogenesis. Our findings also raised the possibility that altered SIMPLE expression found in cancer, obesity and inflammatory bowel diseases may contribute to the pathogenesis or progression of these diseases by altering SIMPLE-dependent endosomal trafficking and signaling dysregulation in Schwann cells play a key role in CMT1C pathogenesis. Our findings also raised the possibility that altered SIMPLE expression found in cancer, obesity and inflammatory bowel diseases may contribute to the pathogenesis or progression of these diseases by altering SIMPLE-dependent endosomal trafficking and signaling dysregulation in Schwann cells play a key role in CMT1C pathogenesis. Our findings also raised the possibility that altered SIMPL
25. Tang X, Fenton MJ, Amar S. Identification and functional characterization of a novel binding site on TNF-alpha promoter. Proc Natl Acad Sci USA 2003; 100:4096-101; PMID:12655064; http://dx.doi.org/10.1073/pnas.0630562100

26. Tang X, Metzger D, Leeman S, Amar S. LPS-induced TNF-alpha factor (LITAF)-deficient mice express reduced LPS-induced cytokine: Evidence for LITAF-dependent LPS signaling pathways. Proc Natl Acad Sci USA 2006; 103:13777-82; PMID:16954198; http://dx.doi.org/10.1073/pnas.0605988103

27. Lee SM, Chin LS, Li L. Protein misfolding and clearance in demyelinating peripheral neuropathies: Therapeutic implications. Commun Integr Biol 2012; 5:107-10; PMID:22482025; http://dx.doi.org/10.4161/cib.18638

28. Lee SM, Sha D, Mohammed AA, Axress S, Glass JD, Chin LS, et al. Motor and sensory neuropathy due to myelin infolding and paranodal damage in a transgenic mouse model of Charcot-Marie-Tooth disease type 1C. Hum Mol Genet 2013; In press; PMID:23359569; http://dx.doi.org/10.1093/hmg/ddt022

29. Quintes S, Goebbels S, Saher G, Schwab MH, Nave KA. Neuron-glia signaling and the protection of axon function by Schwann cells. J Peripher Nerv Syst 2010; 15:10-6; PMID:20433601; http://dx.doi.org/10.1111/j.1529-8027.2010.00247.x