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In Vivo Function of the ER-Golgi Transport Protein LMAN1 in Photoreceptor Homeostasis

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Abstract

LMAN1 is a type I transmembrane protein that selectively transports its cargo proteins from ER to ER-Golgi intermediate compartment (ERGIC) and Golgi. *Lman1* is a direct target of the transcription factor NRL in mouse retina. Therefore, we examined the in vivo function of LMAN1 in retina. Although *Lman1*$^{-/-}$ mouse eyes did not show abnormality in histology and electroretinogram analysis at 3 months, *Lman1*$^{-/-}$ retina at 6 months showed a decrease in cis-Golgi markers GM130 and GRASP65. We also observed abnormal level and location of Rhodopsin in these mice. Taken together, LMAN1 may play a role in photoreceptor gene transport and homeostasis.

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Keywords

LMAN1; NRL; Photoreceptor; Transport; Homeostasis

50.1 Introduction

LMAN1 (also known as ERGIC-53) is a type I transmembrane protein that is located at ER, ER-Golgi intermediate compartment (ERGIC) and cis-Golgi [1]. LMAN1 facilitates transport of several cargo proteins including factors critical to the coagulation cascade from ER to Golgi [1, 2]. Mutations in *LMAN1* cause combined deficiency of factor V and factor VIII, a genetic bleeding disorder [1]. We previously identified *Lman1* as a direct target of the transcription factor neural retina leucine zipper (NRL) in mouse retina [3]. The rod photoreceptor specific transcription factor NRL regulates the expression of genes that are important for the rod photoreceptor development and homeostasis, and mutations in over 20 of NRL target genes have been associated with retinal diseases [4]. We hypothesize that LMAN1 facilitates transport of photoreceptor genes and is important for the functional maintenance of mature rod photoreceptors. In this study, we tested this hypothesis using the *Lman1*$^{-/-}$ mice.

50.2 Materials and Methods

50.2.1 Animal Care and Use

Lman1$^{-/-}$ mice were generated previously using a gene-trap strategy [5]. The Institutional Animal Care and Use Committees at the Cleveland Clinic Foundation and the National Eye Institute approved the animal care and use procedures. PCR analysis for genotyping was performed as previously described [5].

50.2.2 Immunohistochemistry

Retinas were dissected, fixed in 4 % paraformaldehyde, and cryoprotecetd in 30 % sucrose. Retinal sections were probed with the primary antibodies overnight. The slides were stained with secondary antibody AlexaFluor 568 (Invitrogen) and counterstained with DAPI (1 μg/mL). Sections were visualized using an Olympus FluoView FV1000 confocal laser scanner and BX61WI microscope (Center Valley).

50.3 Results

Hematoxylin and eosin stain did not reveal gross abnormalities in histology of *Lman1*$^{-/-}$ eyes at 3 months of age (data not shown). To examine whether photoreceptor function was compromised, we measured the electroretinogram (ERG) of these mice. No abnormalities in either scotopic or photopic ERGs were observed at 3 months, in line with the normal histology observed at this age (data not shown). These data prompted us to examine *Lman1*$^{-/-}$ eyes at a later stage.

To test the involvement of LMAN1 in ER to Golgi trafficking in photoreceptors, we examined the effect of LMAN1 deficiency on cis-Golgi markers GM130 and GRASP65,

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using immunohistochemistry on retina sections. We observed a small decrease in GM130 and GRASP65 signal in *Lman1*$^{-/-}$ retina section compared to wild type litter-mates at 6 months (Fig. 50.1). To test the potential role of LMAN1 in photoreceptor gene transport, we examined the effect of LMAN1 deficiency on Rhodopsin (Rho). We observed a small decrease in Rho signal in outer segment and an increased staining of Rho in outer nuclear layer at 6 months, suggesting abnormal transport of Rho (Fig. 50.2a). Increased GFAP staining in *Lman1*$^{-/-}$ retina indicates that these retinas are under stress (Fig. 50.2b).

50.4 Discussion

Rod and cone photoreceptors are light sensing neurons responsible for vision under dim light and bright light, respectively. Photoreceptor outer segment (OS), the specialized apical cellular extension housing the phototransduction components, undergoes a complete renewal over the course of 10 days [6]. As a result, photoreceptors are under high demands for protein synthesis and transport. Abnormal expression or trafficking of the rod photoreceptor photo-pigment, Rho, has been associated with photoreceptor degeneration [7]. The mechanism of Rho transport is not clear.

The rod photoreceptor specific transcription factor NRL is the master regulator of rod photoreceptor development and homeostasis [4]. We previously identified global NRL target genes and validated the functional importance of many of the targets by in vivo shRNA knockdown experiments [3]. Although LMAN1 is expressed in numerous tissues, the levels of expression vary greatly, with strong expression observed in mouse retina [5]. In addition, *Lman1* expression in rod photoreceptors is specifically regulated by NRL [3]. These observations suggest that LMAN1 may be of functional importance to rod homoeostasis.

LMAN1 is known to cycle between the ER and ERGIC to transport selective cargo proteins from ER [1]. GM130 continuously cycles between the ERGIC and the *cis*-Golgi compartments and is tethered to the *cis*-Golgi by GRASP65 [8]. Vesicles formed from ERGIC undergo tethering and fusion at the *cis*-Golgi region to form larger membrane units and get incorporated into the Golgi stacks. Both GM130 and GRASP65 are required for this process, and hence are important for the maintenance of Golgi [9, 10]. We observed a small decrease in GM130 and GRASP65 in *Lman1*$^{-/-}$ eye. It is possible that LMAN1 deficiency affected the vesicle trafficking from ER to ERGIC, and the downstream trafficking from ERGIC to Golgi.

We observed a small decrease of Rho in outer segment and an increase of Rho in outer nuclear layer *Lman1*$^{-/-}$ eye. This suggests that LMAN1 could play a role in Rho transport. As abnormal trafficking of Rho has been associated with photoreceptor degeneration [7], we observed an increase in GFAP in *Lman1*$^{-/-}$ eye, suggesting that the *Lman1*$^{-/-}$ retina is under stress. Additional cargos and molecular mechanism of LMAN1 function in photoreceptors need to be investigated.

In summary, the ER-Golgi transport protein LMAN1 may play a role in photoreceptor gene transport. The global NRL target genes may serve as an excellent resource to facilitate the identification of retinal disease genes.

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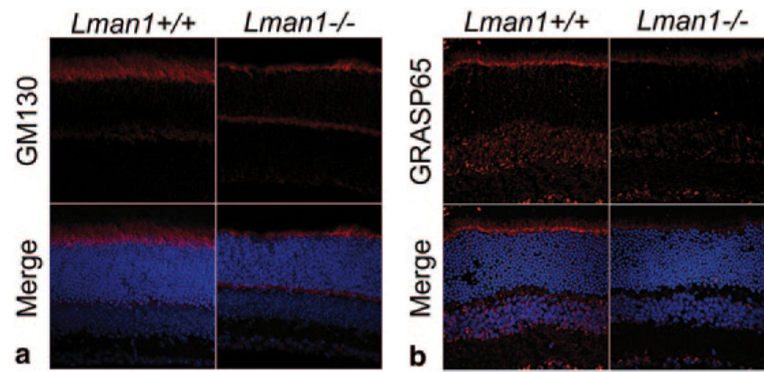

Fig. 50.1.

The effect of LMAN1 deficiency on GM130 and GRASP65. Representative images of immunohistochemistry staining of GM130 (**a**), and GRASP65 (**b**) in retinal sections of *Lman*$^{+/+}$and *Lman*$^{-/-}$ mice at 6 months. Antibody staining is shown in *red* and DAPI staining is shown in *blue*

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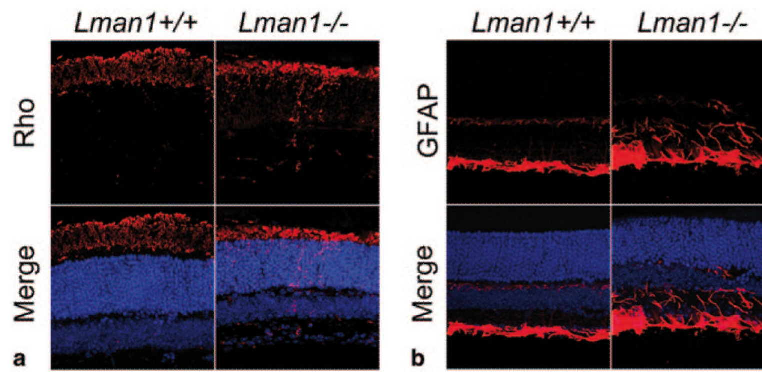

Fig. 50.2.

The effect of LMAN1 deficiency on Rhodopsin (*Rho*) and GFAP. Representative images of immunohistochemistry staining of Rho (**a**) and GFAP (**b**) in retinal sections of *Lman*$^{+/+}$and *Lman*$^{-/-}$ mice at 6 months. Antibody staining is shown in *red* and DAPI staining is shown in *blue*