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Defective relocalization of ALS2/alsin missense mutants to Rac1-induced macropinosomes accounts for loss of their cellular function and leads to disturbed amphisome formation

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ABSTRACT

Loss of ALS2/alsin function accounts for several recessive motor neuron diseases. ALS2 is a Rab5 activator and its endosomal localization is regulated by Rac1 via macropinocytosis. Here, we show that the pathogenic missense ALS2 mutants fail to be localized to Rac1-induced macropinosomes as well as endosomes, which leads to loss of the ALS2 function as a Rab5 activator on endosomes. Further, these mutants lose the competence to enhance the formation of amphisomes, the hybrid-organelle formed upon fusion between autophagosomes and endosomes. Thus, Rac1-induced relocalization of ALS2 might be crucial to exert the ALS2 function associated with the autophagy-endolysosomal degradative pathway.

Structured summary:
Rac1 physically interacts with **ALS2** by pull down (View interaction)
Rab5A physically interacts with **ALS2** by pull down (View Interaction 1, 2)
ALS2 and **EEA1** colocalize by fluorescence microscopy (View Interaction 1, 2, 3)
ALS2 physically interacts with **ALS2** by anti tag coimmunoprecipitation (View interaction)

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1. Introduction

Mutations in the *ALS2* gene account for several recessive motor neuron diseases (MNDs), including amyotrophic lateral sclerosis 2 [1], demonstrating that the ALS2 protein (ALS2 or alsin) is essential for motor neurons. ALS2 contains three predicted guanine nucleotide exchange factor (GEF) domains [2,3], i.e., the regulator of chromosome condensation 1 (RCC1)-like domain (RLD), the Dbl-homology and pleckstrin-homology (DH/PH) domain, and the vacuolar protein sorting 9 (VPS9) domain [2,3]. In addition, eight consecutive membrane occupation and recognition nexus (MORN) motifs are noted in the region between the DH/PH and VPS9 domains.

We and others have reported that ALS2 acts as a GEF for Rab5 (Rab5GEF) [4–6]. Rab5 is a key regulator in endocytosis, endosome fusion, and endosome trafficking [7]. Indeed, it has been shown that ALS2 regulates endosome fusion and trafficking by activating Rab5 [4,5]. Further, we have demonstrated that ALS2 is a novel Rac1 effector [8] rather than a Rac1GEF [5], and plays a role in macropinocytosis and the following endosome maturation [8]. Activated Rac1 interacts with ALS2 and induces the relocalization of ALS2 from cytoplasm to membranous compartments (e.g., membrane ruffle, macropinosome, and endosome) [8]. This Rac1-mediated relocalization of ALS2 is required for the ALS2-mediated Rab5 activation on the membranous compartments.

As almost all mutations identified in the *ALS2* gene result in the truncation of the C-terminal Rab5GEF domain of ALS2, loss of the ALS2-associated Rab5GEF activity may underlie the pathogenesis of *ALS2*-linked MNDs [1]. Recently, two missense mutations in the *ALS2* gene were identified [9,10]. Both mutations result in the mutant proteins (ALS2^{C157Y} and ALS2^{G540E}) carrying a single

Abbreviations: ALS, amyotrophic lateral sclerosis; GEF, guanine nucleotide exchange factor; RLD, regulator of chromosome condensation 1-like domain; DH, Dbl-homology; PH, pleckstrin-homology; VPS9, vacuolar protein sorting 9; MORN, membrane occupation and recognition nexus

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amino-acid substitution in the N-terminal RLD of ALS2 (ALS2-RLD) with a preservation of the C-terminal Rab5GEF domain [9,10]. These missense mutations might lead to loss of the ALS2 function, because the pedigrees carrying these mutations are consistent with an autosomal recessive inheritance [9,10]. However, the molecular mechanism by which the missense mutations cause loss of the ALS2 function is still unknown. To directly address this issue, we here investigated the molecular and cellular functions of these two pathogenic mutants.

2. Materials and methods

2.1. Quantification of cellular phenotypes

HeLa cells were cotransfected with pCMV10-2XHA-Rac1^{Q61L} and either pCIneo-FLAG-ALS2^{WT}, -ALS2^{C157Y}, -ALS2^{G540E}, -ALS2^{T510A}, or -ALS2^{P1603A} as previously described [8]. Twenty-four hours after transfection, the cells were fixed, permeabilized, and co-stained with anti-ALS2 (HPF1-680) and anti-Rac1 antibodies (Abs). Two hundred cells expressing FLAG-tagged ALS2 with HA-tagged Rac1^{Q61L} were examined. The phenotypes of the cells were categorized into two groups; i.e., 'cytoplasmic' and 'macropinosome (at least four vesicles/cell were over 2 μm in diameter)', based on the localization of ALS2. Each value represents the mean and standard deviation of at least five independent experiments. Statistical significance was evaluated by ANOVA with Bonferroni's post hoc test.

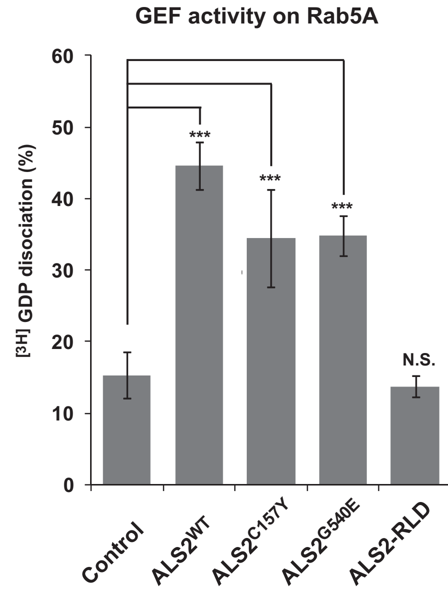

Fig. 2. In vitro Rab5GEF activity of the ALS2 mutants. GDP-loaded recombinant Rab5A were incubated with a variety of purified proteins (control; without proteins, FLAG-ALS2^{WT}, FLAG-ALS2^{C157Y}, FLAG-ALS2^{G540E}, and FLAG-ALS2_RLD) for 1 h. Percentage of dissociated GDP is shown. Values are means ± SD (each sample; n = 5–6). There are significant differences in the GDP dissociation activity between control and indicated samples (***P < 0.001).

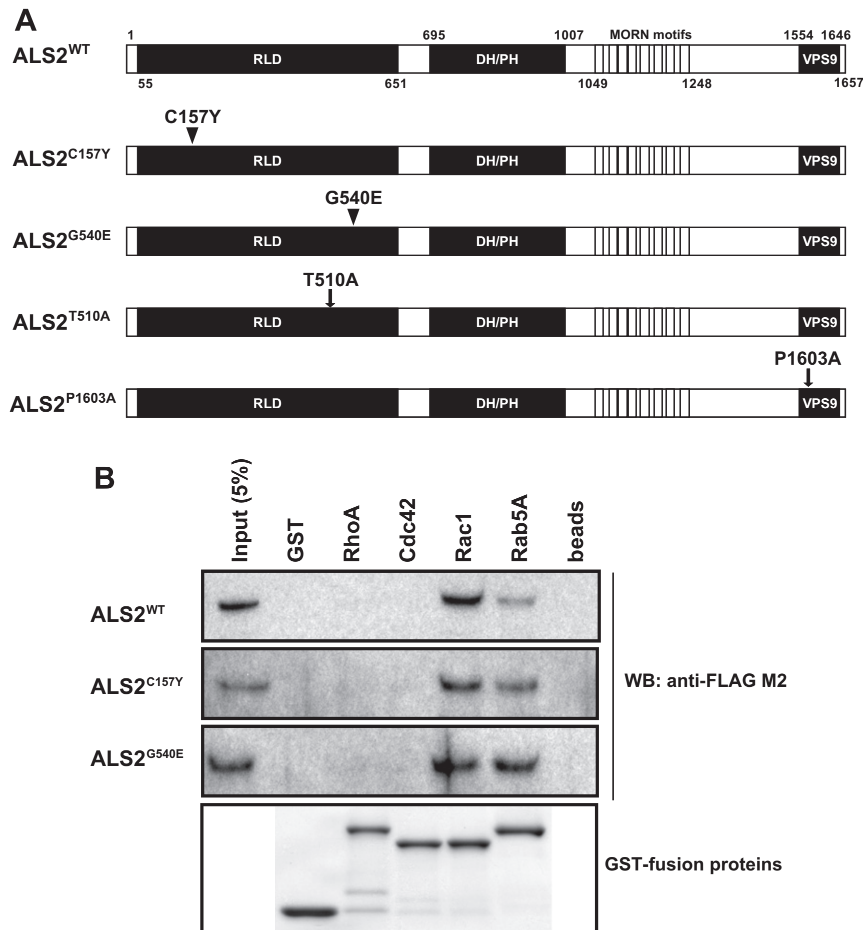

Fig. 1. (A) Schematic diagram of ALS2 and its mutants. The pathogenic mutations identified in the RLD are indicated by arrowheads, and the other mutations are indicated by arrows. It has been shown that Threonine-510 is phosphorylated [12]. Proline-1603 is a crucial amino acid residue to the GEF activity on Rab5 [4]. (B) ALS2 interacts with Rac1 and Rab5. Recombinant GST and GST-fused proteins were mixed with lysates from the cells expressing FLAG-ALS2 or its mutants, and pulled down by glutathione-conjugated beads. Pulled down samples were immunoblotted and probed with anti-FLAG M2 Ab. Amounts of GST-fusion proteins used in these experiments are shown by CBB staining.

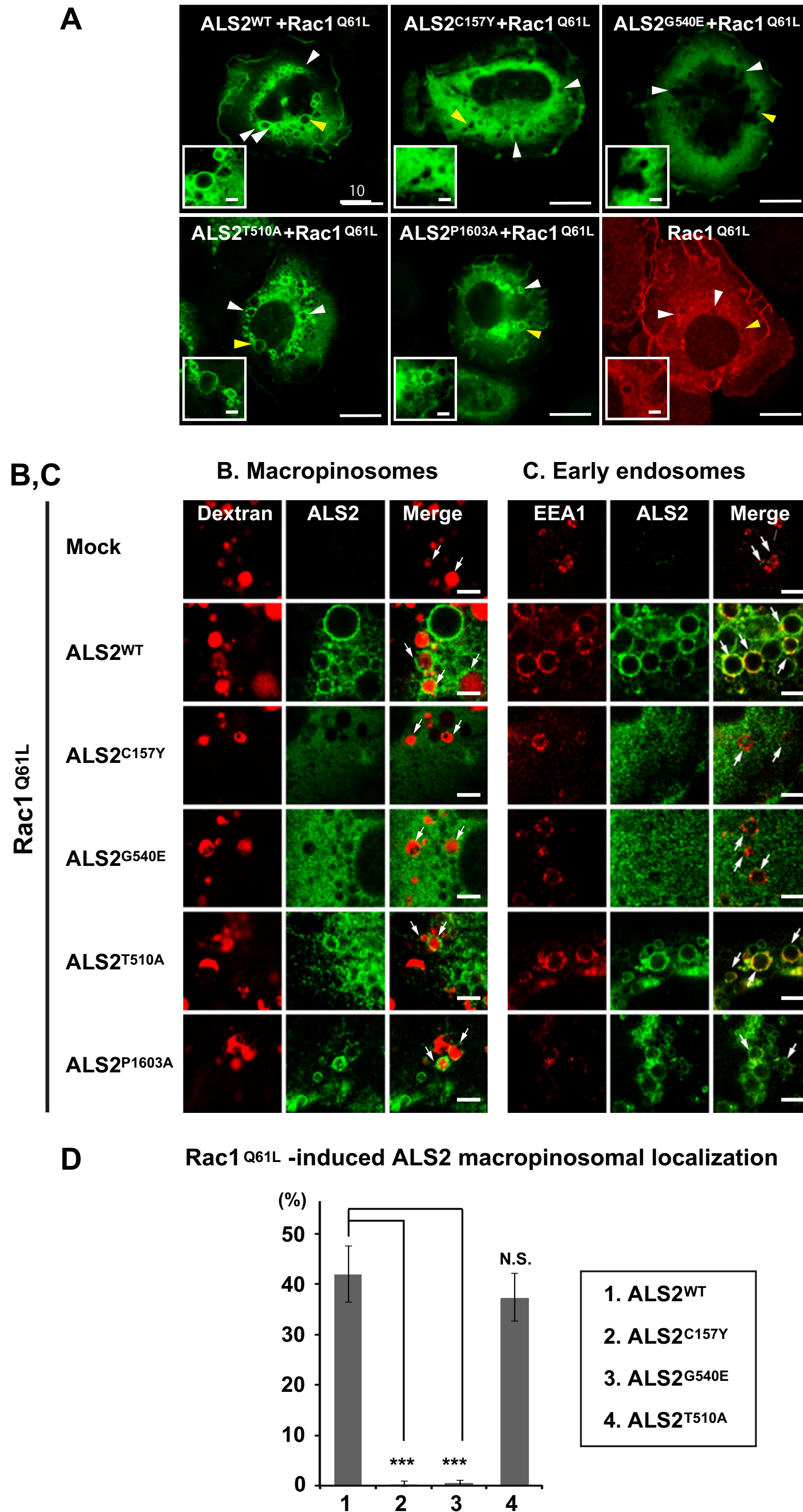

Fig. 3. Pathogenic ALS2 mutants fail to be localized to Rac1-induced macropinosomes and endosomes. (A) Confocal microscopic images represent the localization of FLAG-ALS2^{WT}, FLAG-ALS2^{C157Y}, FLAG-ALS2^{G540E}, FLAG-ALS2^{T510A}, and FLAG-ALS2^{P1603A} in 2XHA-Rac1^{Q61L} expressing cells. Both white and yellow arrowheads indicate Rac1^{Q61L}-induced macropinosomes. The insets represent the typical Rac1-induced macropinosomes (corresponding to yellow arrowheads) with a higher magnification. (B and C) FLAG-ALS2^{WT}, FLAG-ALS2^{T510A}, and FLAG-ALS2^{P1603A}, but not pathogenic mutants FLAG-ALS2^{C157Y} and FLAG-ALS2^{G540E}, are localized to Rac1-induced dextran-positive nascent macropinosomes (B, arrows) and macropinosome-derived EEA1-positive endosomes (C, arrows). (D) Quantification of Rac1-mediated macropinosomal localization of ALS2. The values are shown as mean ± S.D. (each genotype; $n = 6–7$). There are significant differences in the percentage of the cells showing macropinosomal localization of ALS2 between indicated genotypes ($^{***}P < 0.001$). Scale bars shown in (A) in the main and inset images indicate 10 and 2 μm, respectively. Scale bars shown in (B) and (C) indicate 5 μm.

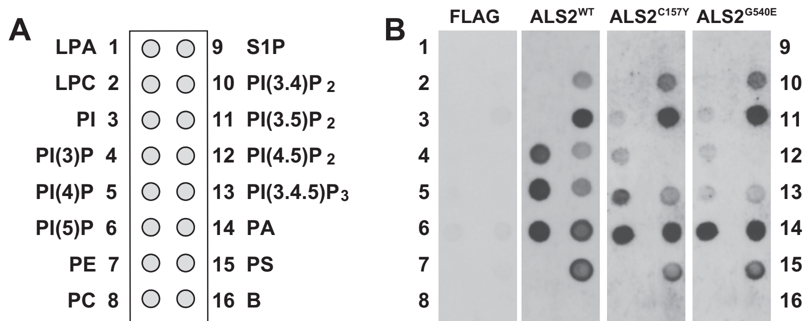

Fig. 4. ALS2^{C157Y} and ALS2^{G540E} show lower affinities to PI(3)P and PI(4)P. (A) Layout of strips is as follows. Spot 1: Lysophosphatidic acid (LPA); 2: Lysophosphatidylcholine (LPC); 3: Phosphatidylinositol (PI); 4: PI(3)P; 5: PI(4)P; 6: PI(5)P; 7: Phosphatidylethanolamine (PE); 8: Phosphatidylcholine (PC); 9: Sphingosine 1-phosphate (SP1); 10: PI(3,4)P_2; 11: PI(3,5)P_2; 12: PI(4,5)P_2; 13: PI(3,4,5)P_3;14: Phosphatidic acid (PA); 15: Phosphatidylserine (PS); 16: Blank. (B) Both ALS2^{C157Y} and ALS2^{G540E} show lower affinities to PI(3)P and PI(4)P. Association between ALS2 and PIPs was analyzed by protein-lipid overlay assay. PIP strips™ was incubated with purified FLAG-peptide, FLAG-ALS2^{WT}, or the mutants, followed by probing with anti-ALS2 Ab.

Other information concerning the materials and methods including antibodies, plasmid constructs, Western blot analysis and gel staining, cell culture and transfection, in vitro GST pull down assay, co-immunoprecipitation, in vitro GEF assay, immunocytochemistry, confocal microscopy, dextran-uptake assay, and protein-lipid overlay assay are available in the Supplementary data.

3. Results

3.1. Pathogenic ALS2 mutants interact with Rac1 and Rab5

ALS2 interacts with both Rac1 and Rab5 [4,5,8]. Thus, we first analyzed whether the pathogenic mutants (Fig. 1A) interacted with Rac1 and Rab5. Consistent with the previous report [8], FLAG-ALS2^{WT} was pulled down with GST-Rac1 and GST-Rab5, but not with GST, GST-Cdc42, and GST-RhoA (Fig. 1B). Notably, both mutants were also efficiently pulled down with either GST-Rac1 or GST-Rab5 but not with GST, GST-Cdc42, and GST-RhoA (Fig. 1B), as was ALS2^{WT}.

3.2. Pathogenic ALS2 mutants retain the Rab5GEF activity

Next, we analyzed the Rab5GEF activities of the pathogenic mutants by in vitro GDP dissociation assay. Both mutants stimulated the GDP dissociation from Rab5A, although they showed slightly lower activities than ALS2^{WT} (Fig. 2A). Consistently, neither pathogenic missense mutations abolished the ALS2 self-association competence (Supplementary Fig. 1) [6]. These results indicate that the pathogenic ALS2 mutants retain the ability to activate Rab5 in vitro.

3.3. Missense mutations in the ALS2-RLD result in mislocalization of ALS2

Rac1-induced relocalization of ALS2 is necessary to proceed with the ALS2-mediated activation of Rab5 on membranous compartments. Thus, we assume that the mutants fail to be relocalized to membranous compartments irrespective of their Rac1 binding properties, which results in loss of the ALS2 function as a Rab5GEF on membranous compartments. To verify this, we examined whether the Rac1-dependent subcellular relocalization of the mutants were affected. We expressed ALS2^{WT} or its mutant proteins with constitutively activated-Rac1 mutant (Rac1^{Q61L}) in HeLa cells, and observed the localization of ALS2 in Rac1^{Q61L} expressing cells. Expression of Rac1^{Q61L} induced macropinosomes (large phase bright organelle, over 2 μm in diameter, indicated by arrowheads in Fig. 3A) arisen from peripheral and/or dorsal membrane ruffles as previously reported [11].

Although ALS2^{WT} was widely distributed throughout the cytoplasm (Supplementary Fig. 2), it was redistributed to membrane ruffles and dextran-positive nascent macropinosomes in a Rac1^{Q61L} dependent manner (Fig. 3A and B). Further, a significant proportion of ALS2 was colocalized with EEA1 (endosome marker) (Fig. 3C), suggesting that ALS2-localizing macropinosomes matured into early endosomes (EEs). Interestingly, unlike ALS2^{WT}, both ALS2^{C157Y} and ALS2^{G540E} failed to be localized to both Rac1^{Q61L}-induced macropinosomes and macropinosome-derived EEs, and yet sequestered in the cytoplasm (Fig. 3A–C). Quantitative analysis revealed that ALS2^{WT} was localized to macropinosomes in over 40% of the Rac1^{Q61L}/ALS2^{WT}-expreeessing cells, whereas both ALS2^{C157Y} and ALS2^{G540E} were almost devoid of macropinosomal localization [ALS2^{WT}, 42.00 ± 4.76%; ALS2^{C175Y}, 0.28 ± 0.48%; ALS2^{G540E}, 0.50 ± 0.54%] despite that a comparable number of macropinosomes was observed (Fig. 3A and B). Conversely, both mutants were widely distributed throughout the cytoplasm without localizing to small endosomes and membrane ruffles in over 80% of the cells expressing Rac1^{Q61L} (Supplementary Fig. 3). By contrast, the ALS2 mutants carrying a single amino-acid substitution on a phosphorylation site [12] of the ALS2-RLD (ALS2^{T510A}) or a critical site for Rab5GEF activity in the VPS9 domain (ALS2^{P1603A}) [4] were localized to Rac1^{Q61L}-induced macropinosomes and EEs (Fig. 3A–C), as was ALS2^{WT}, although expression of ALS2^{P1603A} slightly inhibited EEA1 recruitment [8]. These results suggest that the seemingly non-pathogenic amino-acid substitution in the RLD may not disturb the Rac1^{Q61L}-dependent macropinosomal localization of ALS2. Collectively, our data demonstrate that the disease causing missense mutations in the ALS2-RLD specifically result in the mislocalization of ALS2 in cell.

3.4. Pathogenic ALS2 mutants show a lower affinity to phosphatidylinositol phosphates

To clarify the mechanisms of mislocalization of the ALS2 mutants, we tested whether the ALS2 mutants could associate with phosphoinositide phosphates (PIPs). Since ALS2 has putative lipid association domains and motifs; i.e., RLD [8], DH/PH [2,3] and MORN [2,3] (Fig. 1A), we assume that macropinosomal localization of ALS2 is regulated by the lipid association. We performed PIP-Strip overlay assay using purified FLAG-ALS2^{WT} and the mutants. FLAG-ALS2^{WT} showed strong affinities to several PIPs including phosphatidylinositol-3-phosphate [PI(3)P], phosphatidylinositol-4-phosphate [PI(4)P], phosphatidylinositol-5-phosphate [PI(5)P], and phosphatidylinositol-3,5-phosphate [PI(3,5)P_2] (Fig. 4). Notably, both FLAG-ALS2^{C157Y} and FLAG-ALS2^{G540E} exhibited lower affinities to PI(3)P and PI(4)P compared to FLAG-ALS2^{WT} (Fig. 4). These results, combined with a recent finding indicating that

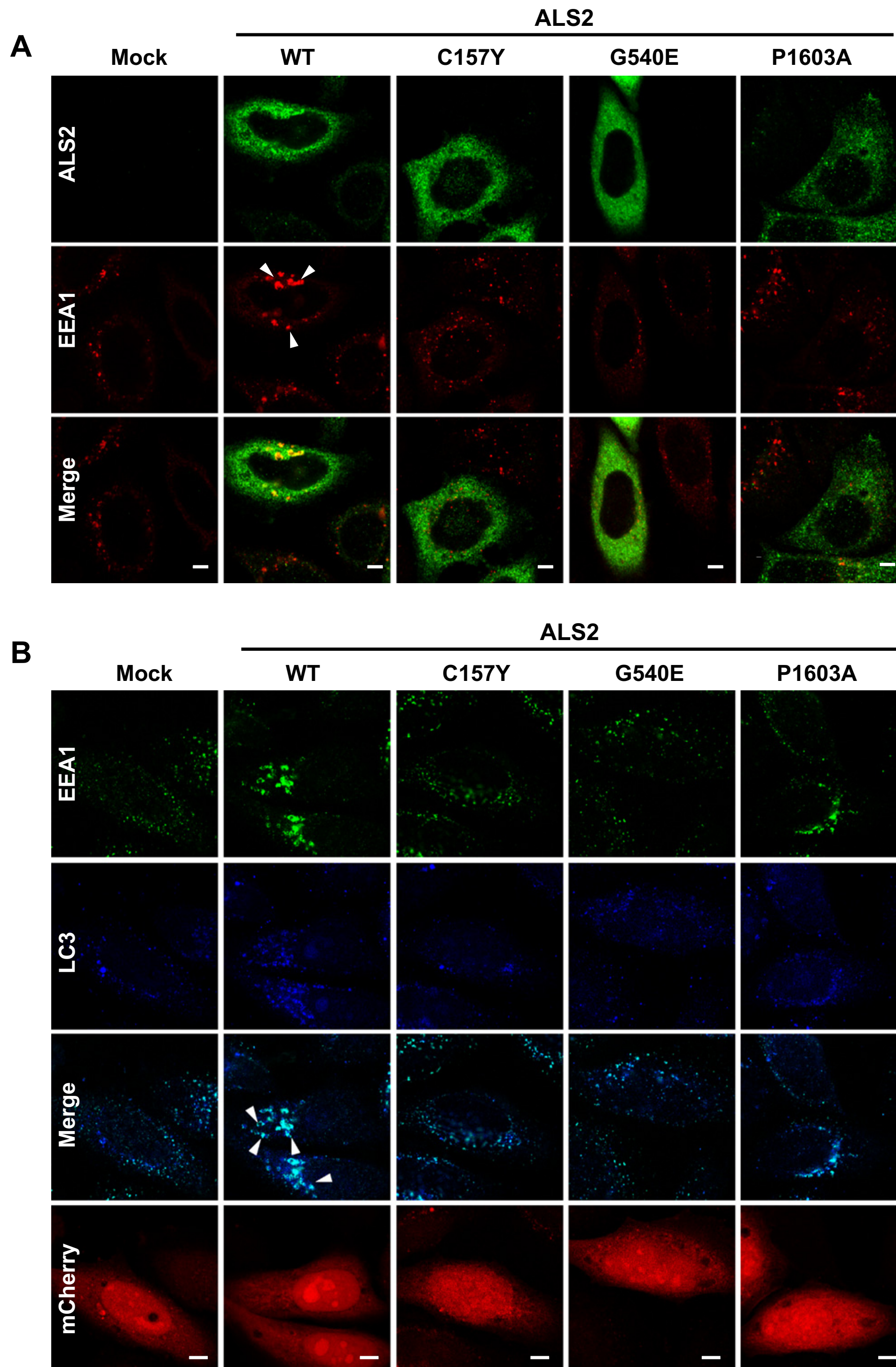

Fig. 5. (A) Expression of FLAG-ALS2^{WT} induces endosome enlargement. Green and red fluorescent signals indicate the localization of ALS2 and EEA1, respectively. Expression of FLAG-ALS2^{WT} resulted in the enlargement of EEA1-positive endosomes (arrowheads), while none of the ALS2 mutants including FLAG-ALS2^{C157Y}, FLAG-ALS2^{G540E}, and FLAG-ALS2^{P1603A} led to change the endosome morphology. (B) Enlarged endosomes are colocalized with LC3. The construct expressing mCherry was cotransfected with ALS2 to visualize the cells expressing either FLAG-ALS2^{WT} or the mutants. We confirmed that the ALS2/mCherry double transfection efficiency was over 97%. Green and blue fluorescent signals represent EEA1 and LC3, respectively. In FLAG-ALS2^{WT} expressing cells, EEA1 was colocalized with LC3 onto the enlarged endosomes (arrowheads). Scale bars indicate 5 μm.

PI(3)P is an important signaling lipid involved in macropinosomal maturation and traffficking after macropinocytosis [13], suggest that binding to specific PIPs, particularly PI(3)P, might regulate macropinosomal localization of ALS2, and that loss of such association in pathogenic ALS2 mutants may explain the preferential cytosolic distribution of these proteins.

3.5. Pathogenic ALS2 mutants do not act as an activator for Rab5 in cells

We next clarified the effect of ALS2 mislocalization in cells. To investigate whether the mislocalization of ALS2 causes a change in the activity of ALS2-mediated early endosomes (EEs) fusion

[4], we expressed ALS2^{WT} or the mutants in HeLa cells, and analyzed the morphology of EEA1-positive EEs. Consistent with the previous reports [4], expression of ALS2^{WT} induced EEs enlargement (Fig. 5A, white arrowheads). Remarkably, both ALS2^{C157Y} and ALS2^{G540E} expression failed to induce EEs enlargement, as did the Rab5-GEF activity deficient mutant (ALS2^{P1603A}) (Fig. 5A). These results indicate that mislocalization of ALS2 leads to loss of ALS2 competence to act as an activator for Rab5 in cells.

3.6. Pathogenic ALS2 mutants lose the competence to enhance the amphisome formation

Finally, we examined whether expression of ALS2 or its mutants regulate the autophagy-endolysosomal system. Recently, we have reported that ALS2 deficiency causes the delay of autophago-endolysosome mediated protein degradation [14]. Since a subpopulation of autophagosomes are fused with EEs and form hybrid-organelle called amphisomes before they fuse with lysosomes [15], we hypothesize that ALS2 plays a role in the autophagy-endolysosomal pathway by regulating the formation of amphisomes through its Rab5 GEF activity. To verify this, we examined the colocalization of EEA1 (endosome marker) and microtubule-associated protein 1A/1B-light chain 3 (LC3) (autophagosome marker) in vesicular compartments. Expression of ALS2^{WT} induced enlarged EEA1-positive vesicles that were colocalized with LC3 (Fig. 5B, white arrowheads), indicating an enhanced formation of amphisomes. By contrast, expression of ALS2^{P1603A} did not induce enlarged amphisomes (Fig. 5B). Notably, expression of neither ALS2^{C157Y} nor ALS2^{G540E} enhanced the formation and/or enlargement of amphisomes (Fig. 5B). These results indicate that like Rab5-GEF activity-defective ALS2, relocalization-defective ALS2 mutants lead to the decreased formation of amphisomes in cells.

4. Discussion

In this study, we demonstrated that the pathogenic missense mutations in the ALS2-RLD impaired Rac1-induced relocalization of ALS2, probably by decreasing the affinities to specific lipid molecules in membranes. Further, these mutants lost the competence to enhance the formation of amphisomes in cells. Based on these findings, mislocalization of ALS2 leads to loss of the ALS2 function as a Rab5 activator on macropinosomes/endosomes, resulting disturbance of the autophagosome and endosome maturation, might account for the *ALS2*-linked MNDs.

Previously, we have shown that loss of the ALS2-associated Rab5GEF activity underlies the pathogenesis of *ALS2*-linked MNDs [1,4]. Further, the ALS2-associated Rab5GEF activity is involved in macropinocytosis and the following early endocytic pathway [4,5,8]. Although the physiological role of macropinocytosis is still unclear, macropinocytosis may link cargo molecules for the incorporation into macropinosomes to the degradative pathway [16]. Since ALS2 activates Rab5 on macropinosomes and endosomes, it may enhance endosome trafficking leading to the degradation of certain cargo molecules that are endocytosed via macropinocytosis. Indeed, we have shown that mouse embryonic fibroblasts derived from *Als2*-knockout mice displays slower degradation rate of endocytosed epidermal growth factor [17]. Further, loss of ALS2 in a mutant Cu/Zn-superoxide dismutase (SOD1^{H46R})-expressing ALS mouse model results in the delay of autophago-lysosome mediated protein degradation and accelerates neurodegeneration [14]. Moreover, we here demonstrated that ALS2-mediated Rab5 activation was involved in the amphisome formation, which may link the endocytosed molecules to autophago-lysosome mediated protein degradation. Collectively, ALS2 is implicated in macropinocytosis, endosome trafficking, and the following degradative pathways. Thus, loss of ALS2 might promote the accumulation of misfolded proteins in particular groups of motor neurons and neurodegeneration.

Future studies on the molecular basis of ALS2 may uncover the roles for macropinocytosis in selective cargo sorting and degradation, which bring new insights into the molecular pathogenesis for *ALS2*-linked MNDs and other neurodegenerative diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.01.045.

References

[1] Hadano, S., Kunita, R., Otomo, A., Suzuki-Utsunomiya, K. and Ikeda, J.E. (2007) Molecular and cellular function of ALS2/alsin: implication of membrane dynamics in neuronal development and degeneration. Neurochem. Int. 51, 74–84.

[2] Hadano, S., Hand, C.K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R.S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., Figlewicz, D.A., Kwiatkowski, T., Hosler, B.A., Sagie, T., Skaug, J., Nasir, J., Brown Jr, R.H., Scherer, S.W., Rouleau, G.A., Hayden, M.R. and Ikeda, J.E. (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat. Genet. 29, 166–173.

[3] Yang, Y., Hentati, A., Deng, H.X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W.Y., Ouahchi, K., Yan, J., Azim, A.C., Cole, N., Gascon, G., Yagmour, A., Ben-Hamida, M., Pericak-Vance, M., Hentati, F. and Siddique, T. (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat. Genet. 29, 160–165.

[4] Otomo, A., Hadano, S., Okada, T., Mizumura, H., Kunita, R., Nishijima, H., Showguchi-Miyata, J., Yanagisawa, Y., Kohiki, E., Suga, E., Yasuda, M., Osuga, H., Nishimoto, T., Narumiya, S. and Ikeda, J.E. (2003) ALS2, a novel guanine nucleotide exchange factor for the small GTPase Rab5, is implicated in endosomal dynamics. Hum. Mol. Genet. 12, 1671–1687.

[5] Topp, J.D., Gray, N.W., Gerard, R.D. and Horazdovsky, B.F. (2004) Alsin is a Rab5 and Rac1 guanine nucleotide exchange factor. J. Biol. Chem. 279, 24612–24623.

[6] Kunita, R., Otomo, A., Mizumura, H., Suzuki, K., Showguchi-Miyata, J., Yanagisawa, Y., Hadano, S. and Ikeda, J.E. (2004) Homo-oligomerization of ALS2 through its unique carboxyl-terminal regions is essential for the ALS2-associated Rab5 guanine nucleotide exchange activity and its regulatory function on endosome trafficking. J. Biol. Chem. 279, 38626–38635.

[7] Stenmark, H. (2009) Rab GTPases as coordinators of vesicle traffic. Nat. Rev. Mol. Cell Biol. 10, 513–525.

[8] Kunita, R., Otomo, A., Mizumura, H., Suzuki-Utsunomiya, K., Hadano, S. and Ikeda, J.E. (2007) The Rab5 activator ALS2/alsin acts as a novel Rac1 effector through Rac1-activated endocytosis. J. Biol. Chem. 282, 16599–16611.

[9] Eymard-Pierre, E., Yamanaka, K., Haeussler, M., Kress, W., Gauthier-Barichard, F., Combes, P., Cleveland, D.W. and Boespflug-Tanguy, O. (2006) Novel missense mutation in ALS2 gene results in infantile ascending hereditary spastic paralysis. Ann. Neurol. 59, 976–980.

[10] Panzeri, C., De Palma, C., Martinuzzi, A., Daga, A., De Polo, G., Bresolin, N., Miller, C.C., Tudor, E.L., Clementi, E. and Bassi, M.T. (2006) The first ALS2 missense mutation associated with JPLS reveals new aspects of alsin biological function. Brain 129, 1710–1719.

[11] Kerr, M.C. and Teasdale, R.D. (2009) Defining macropinocytosis. Traffic 10, 364–371.

[12] Tudor, E.L., Perkinton, M.S., Schmidt, A., Ackerley, S., Brownlees, J., Jacobsen, N.J., Byers, H.L., Ward, M., Hall, A., Leigh, P.N., Shaw, C.E., McLoughlin, D.M. and Miller, C.C. (2005) ALS2/Alsin regulates Rac-PAK signaling and neurite outgrowth. J. Biol. Chem. 280, 34735–34740.

[13] Yoshida, S., Hoppe, A.D., Araki, N. and Swanson, J.A. (2009) Sequential signaling in plasma-membrane domains during macropinosome formation in macrophages. J. Cell Sci. 122, 3250–3261.
[14] Hadano, S., Otomo, A., Kunita, R., Suzuki-Utsunomiya, K., Akatsuka, A., Koike, M., Aoki, M., Uchiyama, Y., Itoyama, Y. and Ikeda, J.E. (2010) Loss of ALS2/Alsin exacerbates motor dysfunction in a $SOD1^{H46R}$-expressing mouse ALS model by disturbing endolysosomal trafficking. PLoS ONE 5, e9805.
[15] Eskelinen, E.L. (2005) Maturation of autophagic vacuoles in mammalian cells. Autophagy 1, 1–10.
[16] Bryant, D.M., Kerr, M.C., Hammond, L.A., Joseph, S.R., Mostov, K.E., Teasdale, R.D. and Stow, J.L. (2007) EGF induces macropinocytosis and SNX1-modulated recycling of E-cadherin. J. Cell Sci. 120, 1818–1828.
[17] Hadano, S., Benn, S.C., Kakuta, S., Otomo, A., Sudo, K., Kunita, R., Suzuki-Utsunomiya, K., Mizumura, H., Shefner, J.M., Cox, G.A., Iwakura, Y., Brown Jr, R.H. and Ikeda, J.E. (2006) Mice deficient in the Rab5 guanine nucleotide exchange factor ALS2/alsin exhibit age-dependent neurological deficits and altered endosome trafficking. Hum. Mol. Genet. 15, 233–250.