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ALS2CL, the novel protein highly homologous to the carboxy-terminal half of ALS2, binds to Rab5 and modulates endosome dynamics ☆

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Abstract ALS2, the causative gene product for juvenile recessive amyotrophic lateral sclerosis (ALS2), is a guanine-nucleotide exchange factor for the small GTPase Rab5. Here, we report a novel *ALS2* homologous gene, ALS2 C-terminal like (*ALS2CL*), which encodes a 108-kD ALS2CL protein. ALS2CL exhibited a specific but a relatively weak Rab5-GEF activity with accompanying rather strong Rab5-binding properties. In HeLa cells, co-expression of ALS2CL and Rab5A resulted in a unique tubulation phenotype of endosome compartments with significant colocalization of ALS2CL and Rab5A. These results suggest that ALS2CL is a novel factor modulating the Rab5-mediated endosome dynamics in the cells.

Keywords: ALS2 carboxy-terminal like; Amyotrophic lateral sclerosis 2; Guanine-nucleotide exchange factor; Rab5; Endosome dynamics

1. Introduction

Amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), and hereditary spastic paraplegia (HSP) are neurological disorders, which are characterized by a progressive motor neuronal degeneration [1,2]. Recently, we and other groups have identified the loss-of-functional mutations in the *ALS2* gene, accounting for a number of juvenile recessive motor neuron diseases (MND) including a type of juvenile ALS (ALS2), juvenile-onset PLS (PLSJ), and infantile-onset ascending hereditary spastic paralysis (IAHSP) [3–7].

The *ALS2* gene encodes a novel 184 kD protein, termed ALS2 or alsin, comprising three predicted guanine-nucleotide exchange factor (GEF) domains [3,4]; i.e., regulator of chromosome condensation (RCC1) [8]-like domain (RLD), the Dbl homology and pleckstrin homology (DH/PH) domains [9], and a vacuolar protein sorting 9 (VPS9) domain [10,11]. In addition, eight consecutive membrane occupation and recognition nexus (MORN) motifs [12] are noted in the region between DH/PH and VPS9 domains [13]. Recently, we have identified the ALS2-associated Rab5-specific GEF activity that is mediated by the carboxy-terminal MORN/VPS9 domain of ALS2 and also shown that ALS2 localizes preferentially onto the early endosome compartments in neuronal cells [13]. Since Rab5 plays crucial roles in clathrin-mediated vesicle endocytosis, trafficking, and early endosomal membrane fusion [14], ALS2 may implicate in the vesicle/membrane dynamics in the cells through the activation of Rab5.

Here, we report the identification and characterization of the novel *ALS2* homologous gene, ALS2 C-terminal like (*ALS2CL*/*Als2cl*), and its gene product ALS2CL.

2. Materials and methods

2.1. Cloning of the ALS2 homologous genes

To identify the potential homologs/paralogs for *ALS2*/*Als2* genes, we conducted BLAST searches [15] on the public databases. Cloning of the full-length transcripts for the identified homologous genes, i.e., *ALS2CL* and *Als2cl*, was performed by a reverse transcriptase (RT)-polymerase chain reaction (PCR)-based method. Analysis of the predicted amino acid sequences, including motif/domain identification and multiple protein sequence alignment was conducted as described [3].

2.2. Northern blotting

Multiple human adult tissue Northern (MTN) blot (Clontech) was hybridized with [α-^{32}P]dCTP-labeled open reading frame (ORF) probes generated from the *ALS2CL* or *ACTB* (β-actin) cDNA in PerfectHyb hybridization solution (Toyobo) at 68 °C. Membranes were washed with 0.1× SSC containing 1% SDS at 65 °C and exposed to X-ray film (Bio-MAX, Kodak). The mouse adult tissue MTN blot (Clontech) was also hybridized with the *Als2cl* or glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) cDNA.

☆ The cDNA sequences of *ALS2CL* and *Als2cl* have been deposited in DDBJ/EMBL/GenBank Database under Accession Nos. AB107015 and AB107016.

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Abbreviations: ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis; HSP, hereditary spastic paraplegia; MND, motor neuron disease; ALS2CL, ALS2 carboxy-terminal like; GEF, guanine-nucleotide exchange factor; RLD, regulator of chromosome condensation-like domain; DH, Dbl homology; PH, pleckstrin homology; MORN, membrane occupation and recognition nexus; VPS9, vacuolar protein sorting 9; EEA1, early endosome antigen-1; EGFP, enhanced green fluorescent protein

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2.3. Expression constructs

All cDNA expression constructs were generated by subcloning the RT-PCR amplified fragments into the appropriate vectors. For the GEF assay, four cDNA fragments; i.e., human ALS2_1018-1657aa (ALS2_1018-1657), human *ALS2CL* ORF (ALS2CL_human), and mouse *Als2cl* ORF (ALS2CL_mouse), were subcloned into pCI-neo Mammalian Expression Vector (Promega). For the subcellular localization studies, human and mouse ALS2CLs, which were fused amino-terminally with enhanced green fluorescent protein (EGFP), were generated using pEGFP-C1 (Clontech). The bacterial expression plasmids encoding the small GTPases and FLAG-tagged human Rab5A, which have been previously generated [13], were also utilized.

2.4. GEF assay and in vitro binding

GEF assay was conducted as previously described [13]. In vitro binding experiments were also performed as described [13] with several modifications. In brief, purified Rab5A (4 pmol), which was pre-loaded with either GDP or GTPγS, or nucleotide-free, was mixed with FLAG-M2 beads conjugating 4 pmol equivalent of the immunoprecipitated FLAG-tagged ALS2CL or ALS2 in 100 μl of a modified GEF buffer [25 mM Tris–HCl, pH 7.4, 100 mM NaCl, 20 mM $MgCl_2$, 1.5 mM CHAPS, and 0.1% (w/v) skimmed milk] containing 50 μM of either GDP or GTPγS, or without nucleotides, for 2 h at 30 °C. After washing with 4 × 1 ml of the same buffer, bound Rab5A was co-eluted with FLAG-ALS2CL protein by the addition of SDS–PAGE sample buffer and detected by Western blotting analysis using appropriate antibodies.

2.5. Cell culture, transfection, and microscopic observations

HeLa and COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were transfected with plasmid constructs using Effectene Transfection Reagent (Qiagen). Immunocytochemical detection and image analysis were conducted by Leica TCS_NT confocal-microscope systems (Leica) as previously described [13].

3. Results

3.1. Identification of the human ALS2CL and mouse Als2cl genes

A BLAST search of the GenBank/DDBJ/EMBL database for potential *ALS2*/*Als2* paralogs and/or homologs revealed the presence of the genomic segments, mRNAs, and EST sequences, which encode a novel protein highly homologous to the carboxy-terminal half of the human and mouse ALS2 proteins, in human, mouse, and rat genome. Computational analyses of the DNA sequences by assembling the mRNA and EST sequences, followed by the comparison with genomic DNA sequences and mapping, demonstrated that human and mouse *ALS2*/*Als2* homologous genes comprised 26 exons and resided within approximately 24.5 kb of the genomic region on human chromosome 3p21.31 and the 23.2 kb genomic region on mouse chromosome 9F3, respectively (Fig. 1A). We designated these human and mouse genes as ALS2 C-terminal like; *ALS2CL* (HGNC approved symbol) and *Als2cl* (MGD nomenclature committee approved symbol), respectively. The sequences of the *ALS2CL* and *Als2cl* transcripts encompassed 4741 nt (ORF; 2862-nt long) (AB107015) and 5081 nt (ORF; 2859-nt long) (AB107016), and matched with the sequences for human transcript LOC259173_isoform 1 (NM_147129) and mouse RN49018 (NM_146228) [16], respectively (Fig. 1A and B).

3.2. Alternative splicing variants for ALS2CL and Als2cl

In the course of our database searches and RT-PCR based cloning of the transcribed DNA sequences, an extensive alternative splicing of the transcripts, giving rise to at least 17 minor variants of *ALS2CL*, was noted in human tissues (Fig. 1B), in addition to the major *ALS2CL* transcript. Analyses of the flanking DNA sequences for these variants identified the cryptic acceptor and donor consensus sequences (data not shown), suggesting that all these variants arose by alternative splicing. It was also evident that all these alternative splicing, resulting in premature stop codons, disrupted the coding frame for full-length *ALS2CL* (Fig. 1B). Physiological significances of these extensive splicing events were currently unknown.

We also identified five minor murine variants as follows: (1) alternative 5′-UTR exons '**a**' and '**b**' proceeded by exon 2 (Fig. 1A), (2) cryptic three nucleotides insertion (-27_-26insTAG), (3) transcript with intron 13, (4) transcripts with skipping exon 19, and (5) alternative exon '**c**' between exons 7 and 8 (RIKEN_5830412B02: with skipping exon 19, Fig. 1A).

3.3. Deduced amino acid sequences

Computational predictions have shown that major transcripts for both *ALS2CL* and *Als2cl* encode 108-kD proteins, ALS2CL [953 and 952 amino acids (aa), respectively], comprising several domains and motifs including PH, MORN, and VPS9 (Fig. 2A). Human and mouse ALS2CL proteins showed a high degree of sequence conservation to each other (81% identity and 88% similarity), and with the corresponding C-terminal region of human ALS2 (33% identity and 51% similarity) (Fig. 2B). Interestingly, phylogenetical analysis revealed that amino acid sequences for ALS2CL were much closer to that for fugu ALS2 than those for human and mouse ALS2 (data not shown).

3.4. Tissue distribution of the ALS2CL/Als2cl mRNA

Northern blot analysis revealed the *ALS2CL* transcript of an approximately 5 kb of the major mRNA in various adult human tissues with higher expression in both heart and kidney (Fig. 3A). It is also noted that the band-signals for the *ALS2CL* transcript were rather blurred, consistent with the presence of multiple alternative splicing variants. In contrast, a single 5 kb discrete band for the mouse *Als2cl* transcript was observed in variety of tissues in adult mice with highest in liver (Fig. 3B).

3.5. Assays of the ALS2CL-associated GEF activity

To analyze the ALS2CL-associated GDP/GTP exchanging activities, i.e., so-called GEF activities, on the small GTPases, in vitro GDP dissociation assays were conducted using nine bacterially produced small GTPases including Rab4A, Rab5A, Rab5B, Rab5C, Rab7, Rab11A, Rac1, RhoA, and Cdc42. The ALS2_1018-1657 peptide, which spans the minimum region associating with Rab5-GEF activity [13], was used as a positive control. The results showed that human ALS2CL exclusively catalyzed GDP dissociation on Rab5A, Rab5B, and Rab5C, as in the cases for ALS2_1018-1657 (Fig. 4A), suggesting that humanALS2CL retained selective catalytic activity with all the members of Rab5 family. However, the degree of activities for ALS2CL was significantly lower than that for ALS2_1018-1657 (Fig. 4A). Interestingly, mouse ALS2CL exhibited almost no Rab5-GEF activities (Fig. 4A).

To define the enzymatic kinetics for the Rab5GEF activity, we next carried out the GDP dissociation assay on Rab5A using a wide range of concentrations for either ALS2CLs or

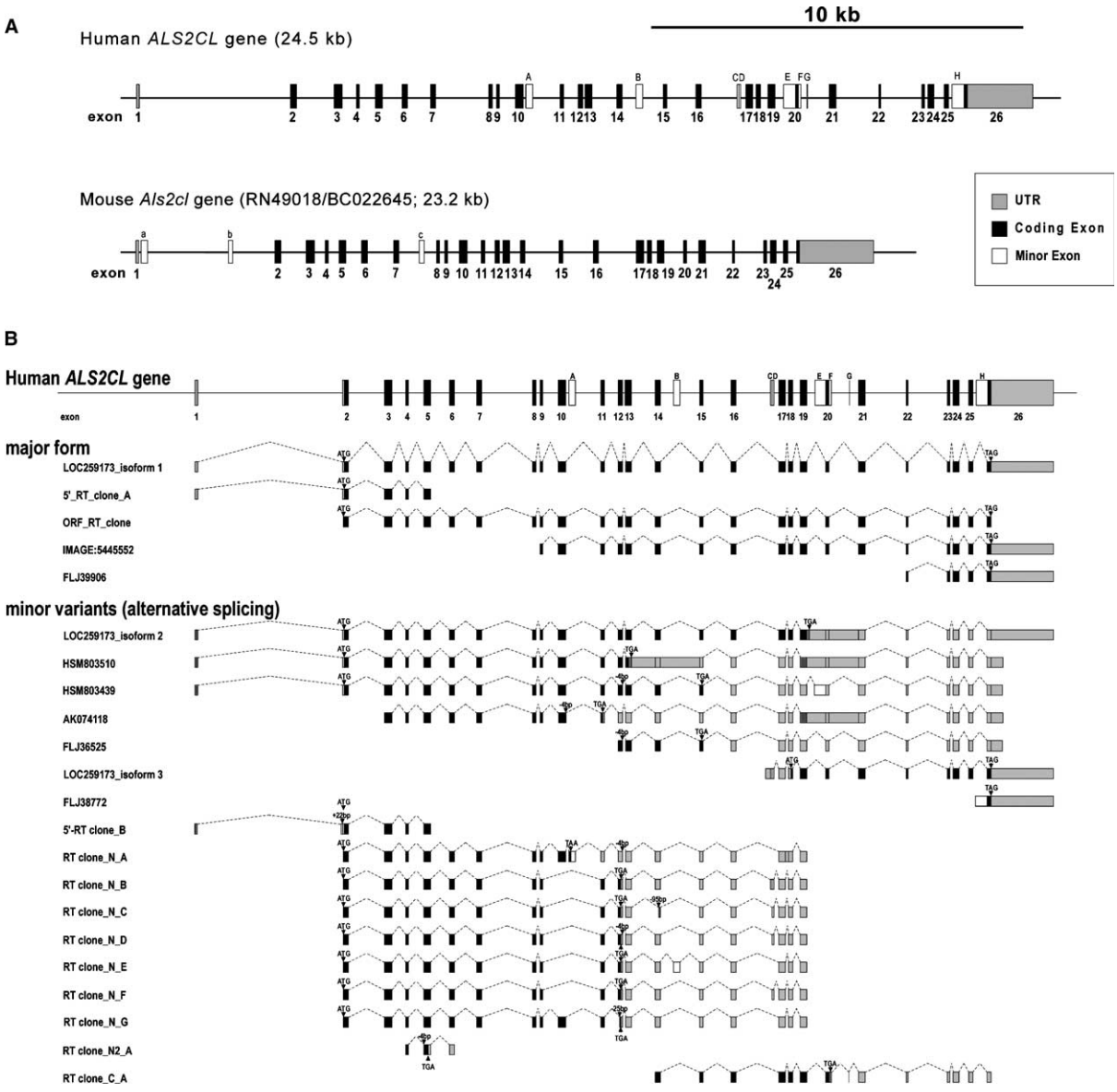

Fig. 1. Genomic organization of the human *ALS2CL* and mouse *Als2cl* genes. (A) Schematic representation of genomic organizations for human *ALS2CL* (upper) and mouse *Als2cl* (lower) genes. *ALS2CL* spans approximately 24.5 kb of the genomic region on human chromosome 3p21.31, while *Als2cl* covers approximately 23.2 kb of the region on chromosome 9F3. Both genes comprise 26 major exons. Black and gray boxes represent coding (translated) and non-coding (untranslated) region of major exons (exons 1–26), respectively. Minor alternative exons (A–H for human; a–c for mouse) are also shown as white boxes. (B) Exon organization of splicing variants for the human *ALS2CL* transcripts. A single major form and 17 differentially spliced minor variants are shown. The positions of translation initiation (ATG) and termination (TGA, TAG, or TAA) codons are shown.

ALS2_1018-1657 (final 0–1.6 μM) (Fig. 4B). Both ALS2_1018-1657 and human ALS2CL revealed the protein concentration-dependent Rab5-GEF activities with approximately eightfold lower dissociation constant with human ALS2CL (ALS2_1018-1657; ~25 nM vs. human ALS2CL; ~200 nM). However, mouse ALS2CL did not show any significant Rab5-GEF activities at any concentrations of the protein (Fig. 4B).

3.6. Interaction of ALS2CL and Rab5

To examine whether the ALS2CL proteins directly interact with Rab5, we conducted the in vitro binding assays using the FLAG-M2 pull-down experiments. The amino-terminally FLAG-tagged ALS2_1018-1657, human ALS2CL, and mouse ALS2CL were immunoprecipitated in the presence of recombinant Rab5A, which was pre-loaded with either GDP or GTPγS, or left unloaded with any nucleotides. Despite the fact that the ALS2CL proteins showed a low or negligible Rab5-GEF activity, they bound rather strongly to Rab5A (Fig. 5). Notably, the ALS2_1018-1657 peptide, which revealed the higher Rab5-GEF activity (Fig. 4), bound preferentially to the nucleotide-free form of Rab5A, whereas mouse ALS2CL, which exhibited no Rab5-GEF activity, interacted non-selectively with any forms of Rab5A. The Rab5A-binding properties for human ALS2CL demonstrated an intermediate preferentiality.

3.7. Subcellular localization of ALS2CL

To investigate the subcellular localization of ALS2CL, HeLa cells were transfected with plasmid expressing either human or mouse ALS2CL. Ectopically expressed amino-terminally

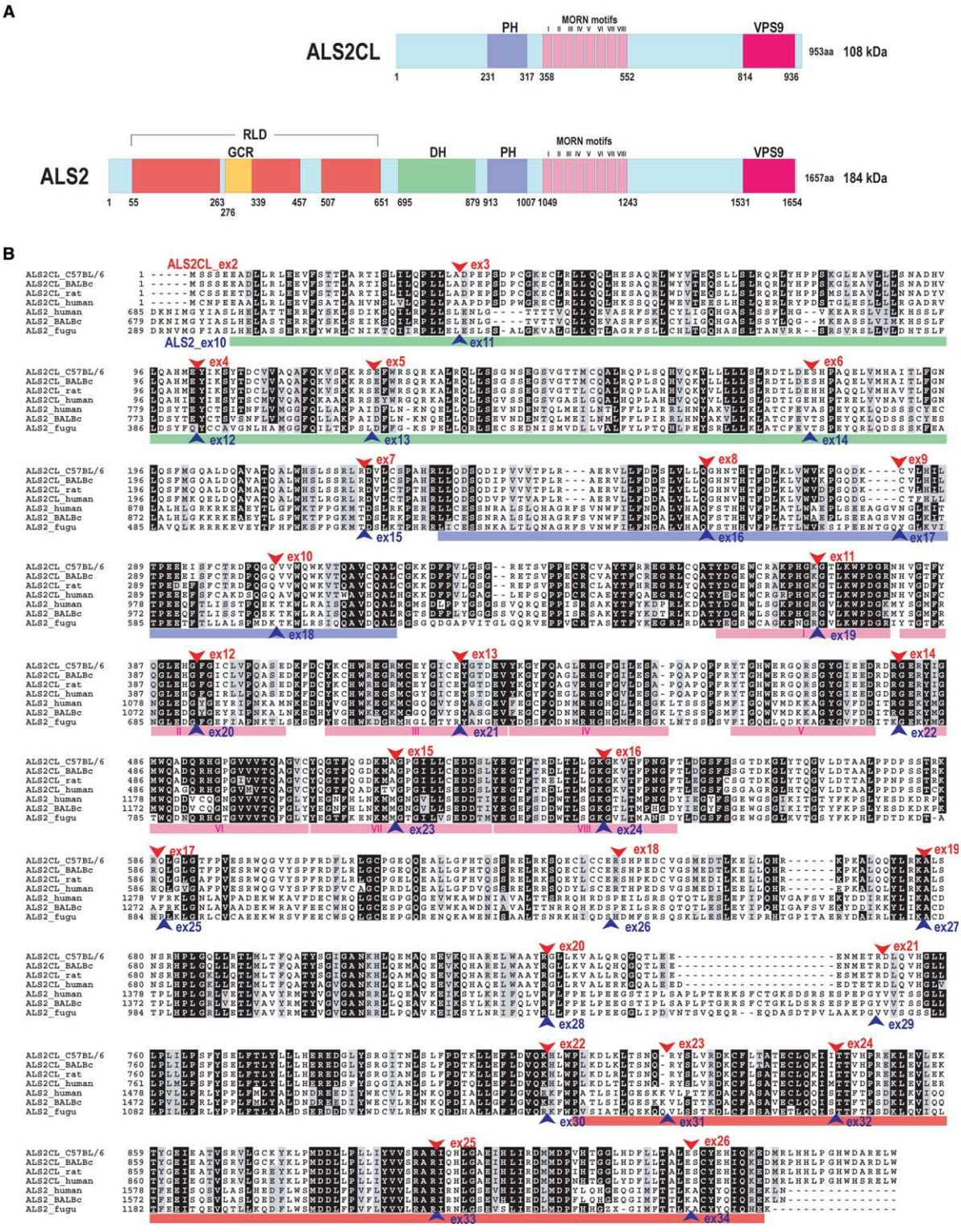

Fig. 2. Amino acid sequence analysis. (A) Schematic representation of the domains and motifs identified in the predicted human ALS2CL and ALS2 proteins. (B) ClustalW multiple amino acid sequence alignment of mouse (ALS2CL_C57BL/6; NP_666340, and ALS2CL_BALBc; this study), rat (ALS2CL_rat; XP_236654), and human (ALS2CL_human; this study) ALS2CL proteins, and the C-terminal portions of human (ALS2_human; NP_065970), mouse (ALS2_BALBc; NP_082993), and fugu (ALS2_fugu; SINFRUP00000067515) ALS2 proteins. Intron–exon boundaries for human *ALS2CL* and *ALS2* are also shown. Sequences are numbered with the initiation codon of each protein as #1.

FLAG-tagged (Fig. 6A) and EGFP-fused (Fig. 6B) human ALS2CL proteins localized throughout the cells with strong punctated stainings in cytoplasm. EGFP-fused mouse ALS2CL also showed similar distribution pattern (Fig. 6C). Co-localization analyses revealed that human ALS2CL partially overlapped with early endosome antigen 1 (EEA1) [17] immunopositive vesicles and/or early endosomes (Fig. 6D–F), but not with the late-endosomal/lysosomal markers, LAMP-1

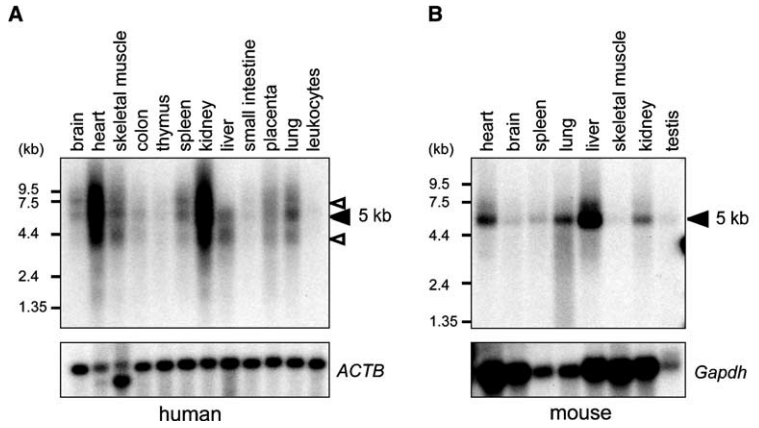

Fig. 3. Northern blot analysis. MTN blots were hybridized with *ALS2CL*/*Als2cl* (upper panel) cDNA clones. Filled and open arrowheads indicate the position of major and minor transcripts, respectively. The lower panel represents the same blots hybridized with (A) the human *ACTB* (β-actin) cDNA or (B) the mouse glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) cDNA to confirm RNA quality and relative loading. Positions of size-markers are shown on the left.

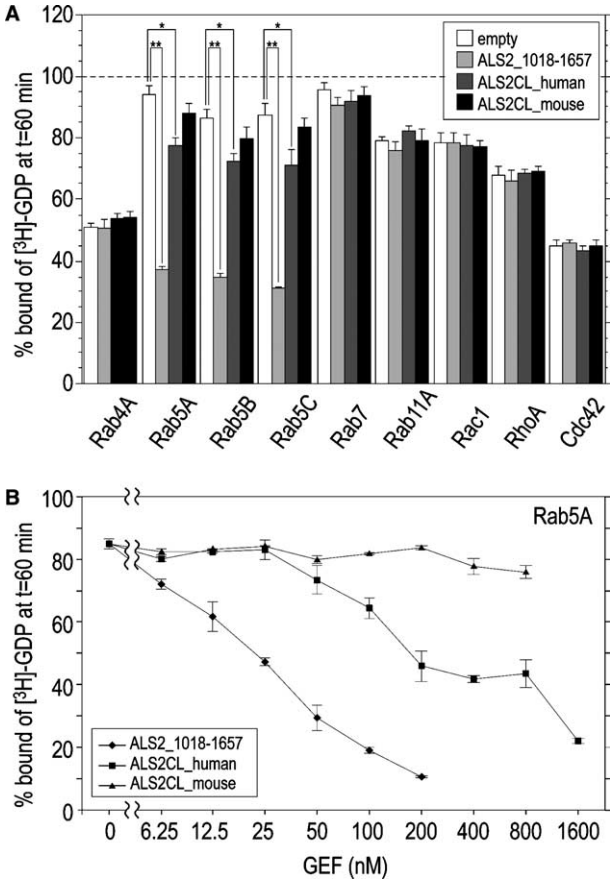

Fig. 4. GEF activity assays. (A) In vitro [^{3}H]GDP dissociation assay for FLAG-tagged ALS2 peptide (ALS2_1018-1657aa; light gray bars), human ALS2CL (ALS2CL_human; dark gray bars), mouse ALS2CL (ALS2CL_mouse; black bars), and FLAG-tag alone (empty; as a control, open bars) on nine different small GTPases. Each value represents the mean and standard deviation of at least three independent assays. Double asterisk, $P < 0.001$; asterisk, $P < 0.01$ in t tests. (B) In vitro [^{3}H]GDP dissociation assay on Rab5A in the presence of increasing amount of recombinant ALS2CL and ALS2 proteins. Each value represents means ± S.D. ($n = 3$) of the percentage of [^{3}H]-GDP bound to Rab5A after 60 min in the presence of given amount either of ALS2_1018-1657 (diamonds), ALS2CL_human (squares), or ALS2CL_mouse (triangles).

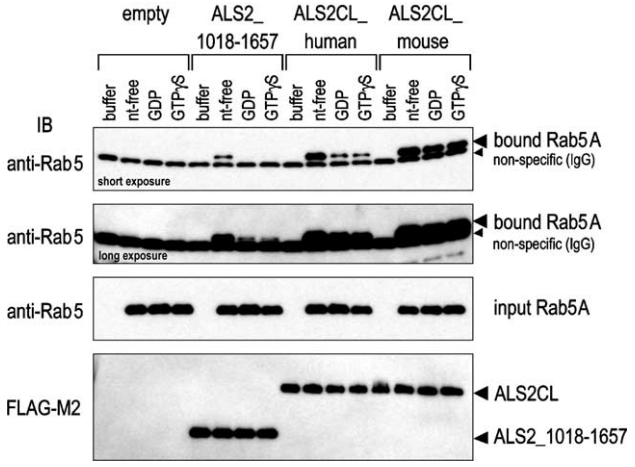

Fig. 5. In vitro Rab5A binding assay. Nucleotide free (nt-free), GDP bound (GDP), or GTPγS bound (GTPγS) forms of Rab5A were incubated with FLAG-M2 beads conjugating FLAG-tagged ALS2_1018-1657aa, human ALS2CL (ALS2CL_human), or mouse ALS2CL (ALS2CL_mouse), or with FLAG-M2 beads alone (empty) as a control. The bound and input Rab5A were detected by immunoblotting method using anti-Rab5 antibody. Western blotting analysis of the FLAG-tagged proteins was also conducted using the FLAG-M2 antibody.

and LAMP-2 [18], or Golgi marker GM130 [19] (data not shown). However, the degree of EEA1 overlapping was quite low (<10%), when compared with those for ALS2_660-1657, in which a high degree (~85%) of vesicular co-localization was observed [13].

3.8. Co-localization of ALS2CL and Rab5A

To investigate whether human ALS2CL co-localized with Rab5 in vivo, co-transfection of HeLa cells with expression constructs for EGFP-fused ALS2CL and FLAG-tagged Rab5A was conducted. The results showed that both proteins were significantly co-distributed onto the vesicular/membranous compartments in cytoplasm, particularly to the leading edges of the cells (Fig. 6G–I). Notably, co-expression of ALS2CL and Rab5A frequently (~40% of the co-transfected

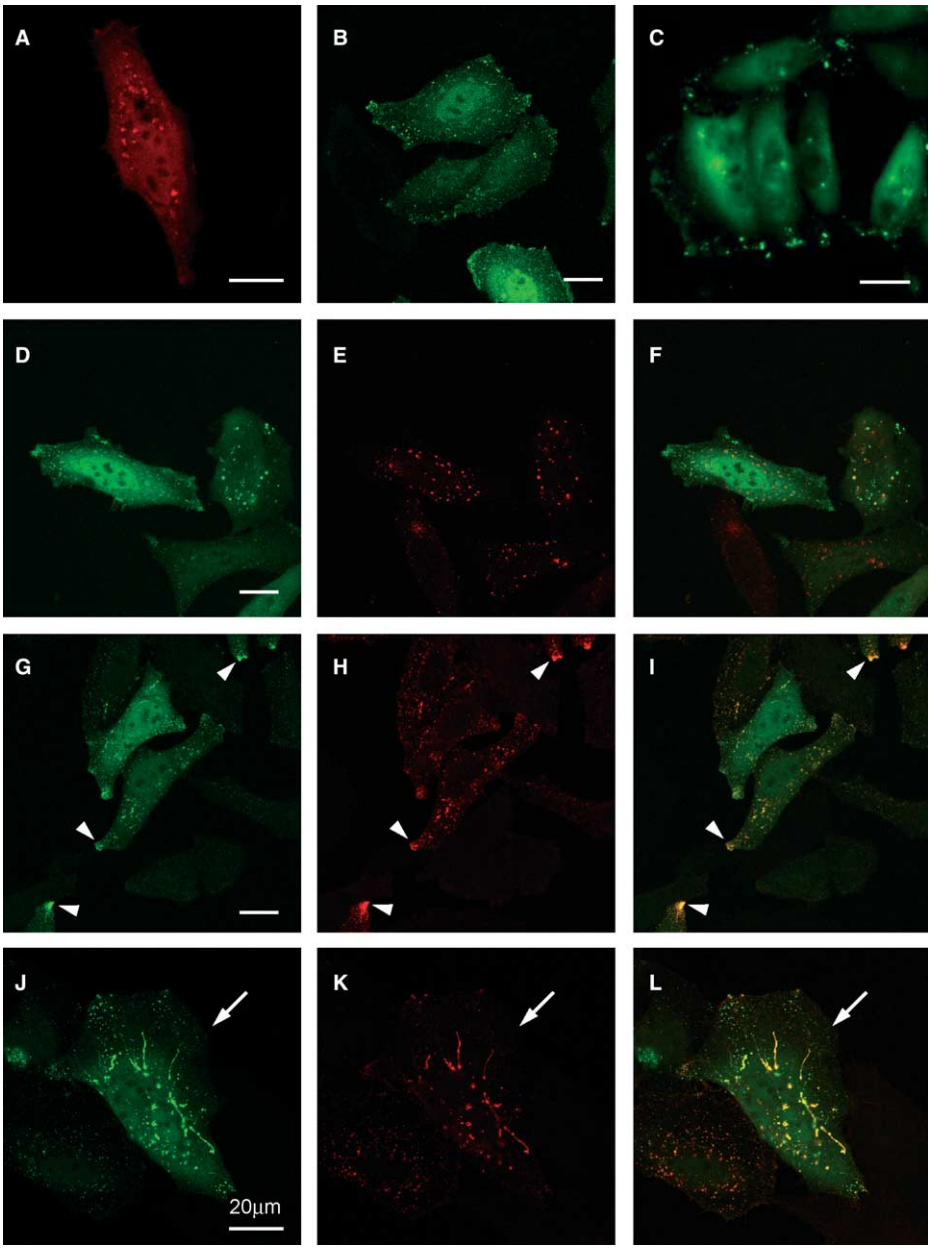

Fig. 6. Localization of ALS2CL in the cells. (A–C) Subcellular distribution of the ectopically expressed ALS2CL in HeLa cells (A, FLAG-tagged human ALS2CL; B, EGFP-fused human ALS2CL; C, EGFP-fused mouse ALS2CL). (D–F) Partial co-localization of the human ALS2CL onto EEA1-positive endosomes (D, EGFP-fused human ALS2CL; E, endogenous EEA1; F, merged image). (G–L) Co-transfection of the EGFP-fused human ALS2CL and FLAG-tagged Rab5A expression constructs in HeLa cells (G and J, EGFP-fused human ALS2CL; H and K, FLAG-tagged Rab5A; I and L, merged images). The representative images for the cells exhibiting vesicular (G–I) and tubular membranous (arrows; J–L) distributions are shown. Arrowheads indicate the leading edges of the cells.

cells) caused a striking effect on the morphology of endosomal compartments, showing a tubulation and/or elongation with accompanying extensive co-localization of ALS2CL and Rab5A (Fig. 6J–L).

4. Discussion

In this study, we newly identified the *ALS2* homologous gene, *ALS2CL* and *Als2cl*. *ALS2CL* and *Als2cl* map to the syntenic chromosomal regions on human chromosome 3p21 and mouse chromosome 9F3, respectively, both comprising 26 major exons. The intron–exon boundaries for *ALS2CL* and *Als2cl*, and those for the homologous regions of *ALS2*/*Als2* were nearly completely preserved. Further, analysis of the predicted amino acid sequences revealed a high level of sequence similarity throughout the entire region of ALS2CL and the C-terminal portion of the ALS2 proteins, indicating that *ALS2CL*/*Als2cl* and *ALS2*/*Als2* were evolutionally conserved genes evolved from a common ancestry of origin. Notably, *ALS2CL*/*Als2cl* lack several exons corresponding to those encoding N-terminal RLD for ALS2, suggesting that the N-

terminal portion of ALS2 can be independently evolved. In spite of such structural conservation, our results demonstrate that the molecular functions for ALS2CL and the C-terminal homologous region of ALS2 seem to be slightly different.

Previously, we have shown that enzymatic activity for the activation of Rab5 GTPase is retained in the C-terminal portion of human ALS2 comprising MORN/VPS9 domain, and that either deletion of MORN motifs or the mutation in the evolutionally conserved amino acid residues in VPS9 domain resulted in the loss of its catalytic activity [13]. In this study, we demonstrated that, although human ALS2CL showed a similar catalytic specificity on the small GTPases as that for human ALS2, the level of its Rab5-GEF activity was significantly lower than that for ALS2, or even at almost negligible levels. On the other hand, ALS2CL, especially an enzymatically almost-inactive mouse ALS2CL, exhibited a stronger binding to Rab5 than ALS2, in a nucleotide state-independent manner. These results suggest that ALS2CL may act as a functional modulator in the Rab5-mediated enzymatic reactions, whilst those actions are slightly different from that for ALS2.

We have previously also shown that overexpression of the C-terminal human ALS2 peptide containing 660–1657 aa, spanning a homologous region corresponding to entire ALS2CL, localizes onto the Rab5/EEA1-positive early endosomal compartments, and induces the striking enlargement of endosomes, which is mediated through the Rab5GEF activity inherent to the MORN/VPS9 region of ALS2 [13]. On the other hand, ectopically expressed ALS2CLs localize in both nucleus and cytoplasm with strong punctated stainings that are extensively overlapped with Rab5A but not with EEA1, suggesting that the ALS2CL proteins preferentially localize to the Rab5-positive/EEA1-negative vesicles and/or endosome sub-compartments. Further, co-expression of human ALS2CL and Rab5A caused unique morphological changes in endosomal compartments, which were characterized by membrane tubulation rather than enlargement. Taken together, these results again support the biochemical notion that ALS2CL and ALS2 mediate similar but slightly different molecular functions in the cells.

Several recent studies demonstrated that the similar tubular endosome phenotypes were elicited under certain experimental conditions, such as in the presence of wortmannin, an inhibitor for phosphatidylinositol-3 kinase [20], or brefeldin A, a potent inhibitor of trafficking through the secretory pathway [21], as well as by the overexpression of dominant positive Rab4 small GTPase [22]. As each of these treatments leads to the impairment of normal endosomal membrane trafficking [20–22], overexpression of human ALS2CL may also disturb the normal vesicle/membrane trafficking possibly through the actions associating with a lower Rab5-GEF activity with a higher Rab5 binding property for the ALS2CL proteins.

In summary, our results demonstrate that *ALS2CL*/*Als2cl* and *ALS2*/*Als2* are evolutionally conserved genes, and that their products, ALS2CL and ALS2, play overlapping but slightly distinctive roles on the Rab5-mediated vesicle/membrane trafficking and dynamics in the cells. Thus, it is possible that ALS2CL modulates the ALS2-mediated molecular and cellular functions either directly or indirectly, thereby implicating in the phenotypic variations seen in patients with ALS2/PLSJ/IAHSP. Further dissection of molecular and cellular functions for ALS2CL as well as those for ALS2 will give us additional insights into the pathogenesis for a number of MNDs caused by the *ALS2* mutations.

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References

[1] Cleveland, D.W. and Rothstein, J.D. (2001) Nat. Rev. Neurosci. 2, 806–819.
[2] Fink, J.K. (2001) Semin. Neurol. 21, 199–207.
[3] 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) Nat. Genet. 29, 166–173.
[4] 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) Nat. Genet. 29, 160–165.
[5] Eymard-Pierre, E., Lesca, G., Dollet, S., Santorelli, F.M., di Capua, M., Bertini, E. and Boespflug-Tanguy, O. (2002) Am. J. Hum. Genet. 71, 518–527.
[6] Gros-Louis, F., Meijer, I.A., Hand, C.K., Dube, M.P., MacGregor, D.L., Seni, M.H., Devon, R.S., Hayden, M.R., Andermann, F., Andermann, E. and Rouleau, G.A. (2003) Ann. Neurol. 53, 144–145.
[7] Devon, R.S., Helm, J.R., Rouleau, G.A., Leitner, Y., Lerman-Sagie, T., Lev, D. and Hayden, M.R. (2003) Clin. Genet. 64, 210–215.
[8] Ohtsubo, M., Kai, R., Furuno, N., Sekiguchi, T., Sekiguchi, M., Hayashida, H., Kuma, K., Miyata, T., Fukushige, S. and Murotsu, T. (1987) Genes. Dev. 1, 85–593.
[9] Schmidt, A. and Hall, A. (2002) Genes Dev. 16, 1587–1609.
[10] Burd, C.G., Mustol, P.A., Schu, P.V. and Emr, S.D. (1996) Mol. Cell. Biol. 16, 2369–2377.
[11] Horiuchi, H., Lippe, R., McBride, H.M., Rubino, M., Woodman, P., Stenmark, H., Rybin, V., Wilm, M., Ashman, K., Mann, M. and Zerial, M. (1997) Cell 90, 1149–1159.
[12] Takeshima, H., Komazaki, S., Nishi, M., Iino, M. and Kangawa, K. (2000) Mol. Cell 6, 11–22.
[13] 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) Hum. Mol. Genet. 12, 1671–1687.
[14] Zerial, M. and McBride, H. (2001) Nat. Rev. Mol. Cell Biol. 2, 107–117.
[15] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Nucl. Acids Res. 25, 3389–3402.
[16] Mitchem, K.L., Hibbard, E., Beyer, L.A., Bosom, K., Dootz, G.A., Dolan, D.F., Johnson, K.R., Raphael, Y. and Kohrman, D.C. (2002) Hum. Mol. Genet. 11, 1887–1898.
[17] Mu, F.T., Callaghan, J.M., Steele-Mortimer, O., Stenmark, H., Parton, R.G., Campbell, P.L., McCluskey, J., Yeo, J.P., Tock, E.P. and Toh, B.H. (1995) J. Biol. Chem. 270, 13503–13511.
[18] Fukuda, M. (1994) Subcell. Biochem. 22, 199–230.
[19] Barr, F.A., Nakamura, N. and Warren, G. (1998) EMBO J. 17, 3258–3268.
[20] Shpetner, H., Joly, M., Hartley, D. and Cotvera, S. (1996) J. Cell Biol. 132, 595–605.
[21] de Figueiredo, P., Doody, A., Polizotto, R.S., Drecktrah, D., Wood, S., Banta, M., Strang, M.S. and Brown, W.J. (2001) J. Biol. Chem. 276, 47361–47370.
[22] McCaffrey, M.W., Bielli, A., Cantalupo, G., Mora, S., Roberti, V., Santillo, M., Drummond, F. and Bucci, C. (2001) FEBS Lett. 495, 21–30.