RNA editing and death of motor neurons

There is a glutamate-receptor defect in patients with amyotrophic lateral sclerosis.

The aetiology of sporadic amyotrophic lateral sclerosis (ALS), a fatal paralytic disease, is largely unknown. Here we show that there is a defect in the editing of the messenger RNA encoding the GluR2 subunit of glutamate AMPA receptors in the spinal motor neurons of individuals affected by ALS. This failure to swap an arginine for a glutamine residue at a crucial site in the subunit, which occurs normally in the affected brain areas of patients with other neurodegenerative diseases, will interfere with the correct functioning of the glutamate receptors and may be a contributory cause of neuronal death in ALS patients.

In RNA editing, gene-specified codons are altered by a post-transcriptional modification of the base sequence of mRNA. The change from glutamine (Q) to arginine (R) at the Q/R site in the putative second membrane domain of GluR2 results from RNA editing and affects the properties of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors, for example by decreasing their permeability to calcium ions[1].

Editing at the GluR2 Q/R site in neurons occurs with virtually 100% efficiency throughout life from the embryonic stage. The premature demise of mice that have defective GluR2 mRNA editing is caused by neuronal death[2] but can be rescued by restoring the RNA-editing function[3], indicating that this GluR2 modification is crucial for neuronal survival.

We extracted RNA from single motor neurons isolated with a laser microdissector[4] from five individuals with ALS and from five normal control subjects (see supplementary information). (Written informed consent was obtained from all subjects or from the bereaved family; the Ethics Committee of the University of Tokyo approved the experimental procedures.)

Editing efficiency was calculated by measuring the difference in digestion patterns of nested GluR2 mRNA products from the polymerase chain reaction with reverse transcription, obtained by using the restriction enzyme *Bbv*I, which cuts only the edited mRNA[5,6] (see supplementary information). The editing efficiency in cerebellar Purkinje cells was also quantified for individuals with ALS or with dentatorubral–pallidoluysian atrophy (DRPLA), and the results compared with those from normal subjects.

The frequency of GluR2 mRNA positivity was not significantly different between the ALS and the control groups (two-sample test for equality of proportions, $P > 0.05$).

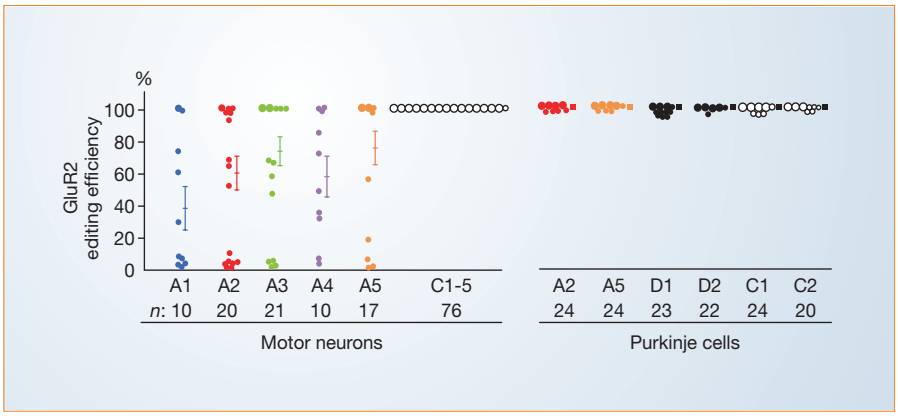

Figure 1 Editing efficiency at the Q/R site of GluR2 messenger RNA in single neurons. Small circles represent the editing efficiency of one cell; each large circle represents five cells in which editing was complete. The mean ± s.e.m. and the number (*n*) of cells examined per person are shown. Left, GluR2 mRNA editing is significantly decreased in motor neurons from all patients with ALS (A1–A5) and showed high variability even within individuals (from 75.3 ± 9.9% in A5 to 38.1 ± 13.1% in A1), whereas editing was complete in all control motor neurons (C1, $n = 28$; C2, $n = 12$; C3, $n = 13$; C4, $n = 12$; C5, $n = 11$) (Mann–Whitney *U*-test, $P < 0.001$). Right, editing efficiency in Purkinje cells from individuals with ALS (A2, 99.9 ± 0.04%; A5, 99.9 ± 0.06%) and individuals with DRPLA (D1, 98.8 ± 0.5%; D2, 99.8 ± 0.2%) was the same as that of control cells (C1, 99.8 ± 0.1%; C2, 99.9 ± 0.05%) ($P > 0.05$).

However, the editing efficiency varied between 0% and 100% in the motor neurons from each individual with ALS, and was incomplete in 44 of them (56%); all 76 of the control motor neurons examined showed 100% editing efficiency (Fig. 1).

The editing efficiency in Purkinje cells was virtually complete in the ALS, DRPLA and normal groups (Fig. 1). Other factors that might influence the properties of AMPA receptors, including the absolute amount of expression and the relative proportion of GluR2 mRNA to total AMPA receptor mRNA, were the same for ALS motor neurons and control motor neurons[4].

In agreement with our results, mice transgenic for GluR2 made artificially permeable to calcium ions develop motor-neuron disease late in life[7], indicating that motor neurons may be specifically vulnerable to defective RNA editing. An unedited GluR2 subunit is incorporated into functional AMPA receptors and transported to the cell surface more efficiently than an edited GluR2 subunit[8], implying that a moderate reduction in GluR2 RNA editing may induce an ALS-like syndrome.

At the tissue level, GluR2 Q/R-site editing is preserved in the severely pathologically affected brain areas of patients with other neurodegenerative diseases[9]. In addition, GluR2 editing is virtually complete in Purkinje cells (Fig. 1) and in the motor cortex[5] of ALS patients, indicating that the defect in GluR2 editing is likely to be specific to ALS spinal motor neurons.

Further investigation should determine the molecular mechanism underlying the degeneration of upper motor neurons. If the marked reduction that we observe in GluR2 RNA editing at the Q/R site is relevant to ALS aetiology, elucidation of this mechanism, including the dysfunction of the RNA-editing enzymes involved, could reveal a therapeutic target that is specific to ALS, which may turn out to be a disease of RNA processing[10].

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