200833016A

厚生労働科学研究費補助金

こころの健康科学研究事業

筋萎縮性側索硬化症に対する特異治療法の開発

平成20年度　総括研究報告書

主任研究者　　郭　伸

平成21年(2009年)4月

目　　次

別紙３

厚生労働科学研究費補助金（こころの健康科学研究事業）
（総括）研究報告書

筋萎縮性側索硬化症に対する特異的治療法の開発に関する研究

（主任）研究者 郭　伸　東京大学准教授
東京大学大学院医学系研究科脳神経医学専攻 神経内科学准教授

［研究趣旨］

孤発性 ALS の運動ニューロンで疾患特異的、部位選択的分子変化として我々が見出した GluR2 Q/R 部位の RNA 編集異常は、神経細胞死を引き起こす一次原因であり、疾患特異的な分子異常である。GluR2 Q/R 部位の RNA 編集は，RNA 編集酵素 adenosine deaminase acting on RNA type 2 (ADAR2) により触媒されることより，孤発性 ALS 運動ニューロンでは ADAR2 活性が低下していると考えられる．したがって，ADAR2 活性の賦活により GluR2 Q/R 部位の RNA 編集が正常化し、孤発性 ALS の神経細胞死を抑制する効果が期待される。本年度の研究において、１）ADAR2 活性賦活物質のスクリーニング，２）一次スクリーニングで得た候補物質の野生型マウス運動ニューロンでの評価，３）モデルマウス運動ニューロンにおける GluR2 Q/R 部位における ADAR2 活性賦活作用の検討，を行い，マウス運動ニューロンで ADAR2 活性を賦活する物質を複数同定した．

分担研究者氏名・所属機関名及び所属機関における職名

相澤仁志　旭川医科大学　講師

A. 研究目的

我々は孤発性 ALS 脊髄運動ニューロンにおいてグルタミン酸受容体である AMPA 受容体の GluR2 サブユニット mRNA の Q/R 部位における RNA 編集が不完全であること，この分子異常は ALS 運動ニューロンに疾患特異的かつ細胞選択的な変化であることを報告した(*1, 2*)。大多数の AMPA 受容体は Ca^{2+}透過性が低いが，これは，サブユニットに編集型 GluR2 (Q/R 部位がアルギニン R) を含むことに依る．ところが，未編集型 GluR2 (Q/R 部位がグルタミン Q) を含む AMPA 受容体は GluR2 を含まない AMPA 受容体同様 Ca^{2+}透過性が高い．正常のニューロンは編集型 GluR2 のみを発現し，未編集型 GluR2 を発現しないので，AMPA 受容体の大多数は Ca^{2+}非透過性である．GluR2 mRNA の Q/R 部位は、RNA 編集酵素 ADAR2 (adenosine deaminase acting on RNA 2) により特異的に編集され、ADAR2 のノックアウト動物は幼弱期に死亡する(*3*)。したがって， ADAR2 活性が低下すると未編集型 GluR2 が増加し、Ca^{2+}透過性 AMPA 受容体が増え，細胞内 Ca^{2+}濃度上昇を通じて神経細胞死を引き起こすと考えられる。実際，孤発性 ALS の脊髄前角組織では，ADAR2 mRNA 発現レベルが低下しており、ADAR2 活性が低下していることが未編集型 GluR2 の増加を引き起こしていると考えられる．この仮説が正しければ，ADAR2 活性を上げることにより運動ニューロン死を阻止し、孤発性 ALS の進行を抑止する治療法の開発が可能であると考えられる。本研究では、孤発性 ALS の特異治療法の開発のため、ADAR2 活性賦活を測定するための in vitro および in vivo システムを立ち上げることを目的とする。

B. 研究方法

1. 培養細胞を用いた ADAR2 活性賦活物質のスクリーニング

昨年度までに確立した TetHeLaG2m 細胞を用いて，0.1ー10μM 濃度の薬剤に 24 時間暴露後の GluR2 Q/R 部位編集率の変化から，ADAR2 活性賦活作用のスクリーニングを行った．

2．ADAR2 活性賦活物質の in vivo での活性の検討

TetHeLaG2m 細胞による in vitro スクリーニン

グで得られた ADAR2 活性賦活作用のある物質を野生型マウスに全身投与し，脳脊髄組織，単一運動ニューロン組織で，ADAR2 活性賦活作用を検討した．薬剤を 1 週間全身投与後に脳・脊髄を取り出し，レーザーミクロディセクターにより単一ニューロンを切り出した．各組織から定法に従い，昨年度報告した，新たに見出した ADAR2 基質である cytoplasmic FMRP interacting protein 2 (CYFIP2) mRNA の K/E 部位の編集率を測定した．対照として，薬剤の溶解に用いた生理食塩水，エタノールを投与した．

ヘテロ接合体 ADAR2 コンディショナルノックアウトマウスでは，ADAR2 活性が 50%程度に低下し，約 20%の運動ニューロンで GluR2 Q/R 部位の RNA 編集率が低下している．これらの物質が，GluR2 Q/R 部位の RNA 編集異常を改善するかどうかをこのヘテロ接合体マウスを用いて，同様の方法により検討した．

（倫理面への配慮）

遺伝子操作に関しては、第二種使用等拡散防止措置における承認を得、全ての遺伝子操作は本学 DNA 組換え実験指診に従い行った。また動物実験については、東京大学医学部動物委員会の承認を得、実験方法については同動物実験指針に従い動物愛護面に十分配慮した．

C. 研究結果及び考察

1. Tet-HeLaG2m 細胞系を用いて、50 種以上の物質につきスクリーニングを行い、ADAR2 活性賦活作用のある薬剤を数種類得た。これらの作用メカニズムは、ADAR2 mRNA 発現量の増加によるもの、ADAR2 mRNA/GluR2 pre-mRNA 比の増加によるものの他、これらの ADAR2 活性に関連しうる分子には影響を与えないものがあり、異なる分子メカニズムに依ることが明らかになった(5)。

2．TetHeLaG2m 細胞で ADAR2 活性を賦活し ADAR2 mRNA 発現量を増加させた物質 3 種が，野生型マウス運動ニューロンの CFIP2 K/E 部位の RNA 編集率を有意に上昇させた．また，ヘテロ接合体 ADAR2 コンディショナルノックアウトマウスへの全身投与で，脳脊髄組織，運動ニューロンの GluR2 Q/R 部位の RNA 編集率を上昇させた．

D. 結論

本研究で開発した in vitro、in vivo スクリーニングシステムにより，GluR2 Q/R 部位の RNA 編集異常を改善する ADAR2 活性賦活物質のスクリーニングが可能であることを明らかにした．ADAR2 活性の賦活による孤発性 ALS の治療薬の開発研究に有用であることが明らかになった．

（文献）

1. Kawahara, Y *et al.*, *Nature* 427, 801 (2004).

2. Kwak & Kawahara, *J Mol Med* 83, 110-120 (2005)

3. Higuchi, M *et al.*, *Nature* 406, 78-81 (2000).

4. Nishimoto Y et al. *Neurosci Res* 61, 201-206 (2008)

5. Sawada J, et al. *Neurosci Res,* in press.

E. 健康危険情報

なし

F. 研究発表

1. 論文発表

1. Nishimoto Y, Yamashita T, Hideyama T, Tsuji S, Suzuki N, Kwak S: Determination of editors of mRNAs with site-selective A-to-I editing positions, *Neurosci Res* 61:201-206, 2008.

2. Pan W, Soma R, Kwak S, Yamamoto Y: Improvement of motor functions by noisy vestibular stimulation in central neuro-degenerative disorders. *J Neurol* 255:1657-1661, 2008.

3. Buckingham SD, Kwak S, Jones AK, Blackshaw SE, Sattelle DB: Edited GluR2, a gatekeeper for motor neuron survival? *BioEssays* 30:1185-1192, 2008.

4. Kwak S, Nishimoto Y, Yamashita T: Newly identified ADAR2-mediated editing positions as a useful tool for ALS research. *RNA Biology* 5:193-197, 2008.

5. Sawada J*, Yamashita T*, Aizawa H, Aburakawa Y, Hasebe N, Kwak S: Effects of antidepressants on GluR2 Q/R site-RNA editing in a modified HeLa cell line. *Neurosci Res* accepted

他 6 編

2. 学会発表

1. 日出山拓人、西本祥仁、山下雄也、伊藤杏子、辻省次、高橋良輔、三澤日出巳、鈴木岳之、郭　伸：RNA 編集異常による孤発性 ALS モデルマウスの開発. 第49回日本神経学会総会、横浜、15-17 May 2008
2. 澤田　潤、相澤仁志、山下雄也、油川陽子、長谷部直幸、郭　伸：AMPA 受容体サブユニット GluR2 の Q/R 部位 RNA 編集率に及ぼす各種薬剤の効果. 第 49 回日本神経学会総会、横浜、15-17 May 2008
3. 山下雄也、只見智恵子、西本祥仁、日出山拓人、木村大輔、鈴木岳之、郭　伸：培養細胞による GluR2Q/R 部位の編集制御機構 Regulatory mechanism of GluR2 Q/R site-editing in cultured cell lines. 第 31 回神経科学大会、neuro2008、東京　9-11 July, 2008.
4. 郭　伸、日出山拓人、山下雄也、辻省次、高橋良輔、三澤日出巳、木村大輔、鈴木岳之: RNA 編集異常による孤発性筋萎縮性側索硬化症モデルマウスの作製. Mouse model of sporadic ALS by abnormality of RNA editing enzyme. 第 31 回神経科学大会、neuro2008、東京　July 9-11, 2008.
5. Hideyama T, Yamashita T, Tsuji S, Takahashi R, Misawa H, Suzuki T, Kwak S: Sporadic ALS model mice by RNA editing enzyme abnormality. *6th Forum of European Neuroscience Forum 2008* Geneve, July 12-16, 2008
6. 郭　伸：「TDP43 異常と運動ニューロン死を結ぶ分子異常」シンポジウム S3 前頭側頭葉変性症（FTLD）と ALS における TDP-43 をめぐる最近の話題. 第 27 回日本認知症学会、前橋、Oct 10-11, 2008
7. Hideyama T, Yamashita T, Tsuji S, Takahashi R, Misawa H, Suzuki T, Kwak S: Slow death of motor neurons in sporadic ALS mouse model by conditional targeting of RNA editing enzyme ADAR2 *The 19th International Symposium on MND/ALS. 2008*, Birmingham, 3-5 Nov, 2008.
8. Kwak S: A-to-I RNA editing of GluR2 and ALS. Trinity Term 2008 Departmental Seminar. Department of Physiology, Anatomy and Genetics, Oxford Univ., Oxford, UK, Nov 7, 2008.
9. Yamashita T, Tadami C, Nishimoto Y, Hideyama T, Kimura D, Suzuki T, Kwak S: Regulatory mechanism of GluR2 Q/R site-editing in cultured cell lines, *38th Annual Meeting Society for Neuroscience*, Washington, 15-19 November 2008
10. Hideyama T, Yamashita T, Tsuji S, Takahashi R, Misawa H, Suzuki T, Kwak S: Slow neuronal death of motor neurons in sporadic ALS mouse model by RNA editing enzyme ADAR2 knockout. *38th Annual Meeting Society for Neuroscience*, Washington, 15-19 November 2008
11. 郭　伸：「ALS の臨床と研究の現状」. 平成 20 年度日本神経学会関東地区生涯教育講演会, 東京, Nov 30, 2008.
12. 日出山拓人、鈴木岳之, 郭　伸：シンポジウム「システムとしての神経疾患――病因解明と治療への新たな戦略」第 82 回薬理学会年会、横浜、March 16-18, 2009.
13. 木村 大輔、日出山 拓人、鈴木 岳之、郭 伸：Neuronal death in tamoxifen-driven conditional ADAR2 knockout mice. 第 82 回薬理学会年会、横浜、March 16-18, 2009.

他　5 編

G. 知的財産権の出願・登録状況

1. 特許取得：なし
2. 実用新案登録：なし
3. その他：なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
日出山拓人、郭　伸	筋萎縮性側索硬化症の AMPA 受容体仮説.	柳澤信夫 他	Annual Review 神経　2008	中外医学社	東京	2008	212-221
Kwak S, Hideyama T, Yamashita T	AMPA receptor-mediated neuronal death in motor neuron diseases.		Amino Acid Receptor Research	Nova Science Publishers Inc		2008	293-310
郭　伸	Kugelberg-Welander 病.		今日の診断指針第 6 版	医学書院	東京		印刷中

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Iwata NK, Aoki S, Okabe S, Arai N, Terao Y, Kwak S, Abe O, Kanazawa I, Tsuji S, Ugawa Y	Evaluation of corticospinal tracts in ALS with diffusion tensor MRI and brainstem stimulation.	*Neurology*	70	528-532	2008
Nishimoto Y, Yamashita T, Hideyama T, Tsuji S, Suzuki N, Kwak S	Determination of editors of mRNAs with site-selective A-to-I editing positions.	*Neurosci Res*	61	201-206	2008
Ishiura H, Morikawa M, Hamada M, Watanabe T, Kako S, Chiba S, Motokura T, Hangaishi A, Shibahara J, Akahane M, Goto J, Kwak S, Kurokawa M, Tsuji S	Lymphomatoid Granulomatosis Involving Central Nervous System Successfully Treated with Rituximab Alone.	*Arch Neurol*	65	662-665	2008
Massie A, Cnops L, Smolders I, McCullumsmith R, Kwak S, Arckens L, Michotte Y	High-affinity Na+/K+-dependent glutamate transporter EAAT4 is expressed throughout the rat fore- and midbrain.	*J comp Neurol*	511	155-172	2008
Buckingham SD, Kwak S, Jones AK, Blackshaw SE, Sattelle DB	Edited GluR2, a gatekeeper for motor neuron survival?	*BioEssays*	30	1185-1192	2008
Kwak S, Nishimoto Y, Yamashita T	Newly identified ADAR2-mediated editing positions as a useful tool for ALS research.	*RNA Biology*	5	193-197	2008

Pan W, Soma R, Kwak S, Yamamoto Y	Improvement of motor functions by noisy vestibular stimulation in central neuro-degenerative disorders.	*J Neurol*	255	1657-1661	2008
Saito T, Amakusa Y, Kimura T, Yahara O, Aizawa H, Ikeda Y, Day JW, Ranum LP, Ohno K, Matsuura T	Myotonic dystrophy type 2 in Japan: ancestral origin distinct from Caucasian families.	*Neurogenetics*	9	61-3	2008
Sawada J, Yamashita T, Aizawa H, Aburakawa Y, Hasebe N, Kwak S	Effects of antidepressants on GluR2 Q/R site-RNA editing in a modified HeLa cell line.	*Neurosci Res*			accepted
郭　伸、Struzik, ZR、相馬りか、大橋恭子、潘衛東、山本義春	電気的前庭神経刺激による神経疾患の治療の試み.	*Equilibrium Res*	67	58-64	2008
日出山拓人、郭　伸	孤発性 ALS と興奮性アミノ酸.	*Clinical Neuroscience*	26	303-305	2008

In: Amino Acid Receptor Research
Editors: B.F. Paley and T.E. Warfield, pp. 293-310

ISBN: 978-1-60456-283-5

Chapter 10

AMPA Receptor-Mediated Neuronal Death in Motor Neuron Disease

Shin Kwak*, Takuto Hideyama and Takenari Yamashita
Department of Neurology, Graduate School of Medicine, The University of Tokyo
7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Abstract

AMPA receptor-mediated excitotoxicity has been proposed to play a major role in death of motor neurons in amyotrophic lateral sclerosis (ALS), the most common motor neuron disease. We demonstrated that RNA editing of GluR2 mRNA at the Q/R site was decreased in autopsy-obtained spinal motor neurons of patients with sporadic ALS. This molecular change did not occur in the other regions of central nervous system (CNS), and has been demonstrated as a molecular change inducing neuronal death in mutant mice, hence may be highly relevant to ALS pathogenesis. We found that the extent of GluR2 Q/R site-editing was reduced to various extents in motor neurons of all the sporadic ALS cases irrespective of the phenotype. On the contrary, although AMPA receptor-mediated neurotoxicity plays a role in SOD1-associated familial ALS (ALS1), the underlying molecular mechanism is an up-regulation of GluR2-lacking Ca^{2+}-permeable AMPA receptors but not GluR2 Q/R site-underediting as seen in motor neurons of sporadic ALS. The molecular mechanism increasing the GluR2-lacking AMPA receptors in ALS1 model rats may likely result from up-regulation of GluR3 mRNA as occurred in kainic acid-induced ALS model rats that we recently developed, hence over-activation of AMPA receptors might participate in death of motor neurons in ALS1. On the contrary, AMPA receptor-mediated neuronal death mechanism did not seem play a role in dying motor neurons of spinal and bulbar muscular atrophy (SBMA).
Because GluR2 Q/R site-editing is specifically catalyzed by adenosine deaminase acting on RNA type 2 (ADAR2), it is likely that regulatory mechanism of ADAR2 activity does not work well in the motor neurons of sporadic ALS. Although ADAR2-null mice die young from status epilepticus, conditional ADAR2 knockout mice that we newly developed exhibited slow progressive death of motor neurons as seen in transgenic mice for artificial Ca^{2+}-permeable GluR2 (GluR-B(N)). Therefore, it is likely that deficient ADAR2 activity induces neuronal death via underediting of GluR2 Q/R site. Indeed, ADAR2 expression level was significantly decreased in the spinal ventral gray matter of sporadic ALS as compared to

* E-mail address: kwak-tky@umin.ac.jp, Tel: +81-3-5800-8672, Fax:+81-3-5800-6548

normal control subjects. Taken together, it is likely that ADAR2 underactivity selective in motor neurons induces deficient GluR2 Q/R site-editing and resulting neuronal death in sporadic ALS.

Thus, there seem multiple different molecular mechanisms underlying death of motor neurons in various motor neuron diseases, and the molecular mechanism generating Ca^{2+}-permeable AMPA receptors that mediated neuronal death may not be uniform among death of motor neurons; an increase of GluR2-lacking Ca^{2+}-permeable AMPA receptors plays a role in ALS1, whereas an increase of Q/R site-unedited GluR2-contatining Ca^{2+}-permeable AMPA receptors in sporadic ALS.

Key words:ALS, AMPA receptor, GluR2, RNA editing, ADAR2, ALS1, SOD1, SBMA, MND

ALS Etiology: the AMPA Receptor-Mediated Neuronal Death Hypothesis

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease characterized by progressive weakness and muscle wasting leading to death within a few years after onset due to the destruction of both upper and lower motor neurons. The majority of ALS cases are sporadic, with a variety of phenotypes including limb-onset classical ALS, progressive bulbar palsy (PBP), and ALS with dementia (ALS/FTD). Cases with familial ALS accounts for less than 10% of all ALS cases, and among the causative genes so far identified, mutations in the copper-zinc superoxide dismutase 1 (SOD1) are the most common, accounting for approximately 20% of familial ALS cases.

The molecular mechanism leading motor neurons to death has not been elucidated even in SOD1-associated familial ALS (ALS1) despite the most intensively investigated ALS, and none of the causative genes so far identified in familial cases has been demonstrated to play any causal role in sporadic cases. Therefore, it is likely that there are multiple different death pathways in motor neurons and that the mechanism producing ALS-phenotype may not necessarily be the same. Several hypotheses have been proposed to explain the etiology of ALS, among which excitotoxicity mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, a subtype of ionotropic glutamate receptors, has been attracted much attention due to the fact that motor neurons are particularly vulnerable to AMPA receptor-mediated neurotoxicity in cultured spinal cord neurons. Although mechanisms of excitotoxic neuronal injury are complex and incompletely understood, intracellular Ca^{2+} overload is an important trigger and an increased influx of Ca^{2+} through activated AMPA receptor-coupled channels appears to play a key role in slow death of motor neurons in culture [1,2].

Ca^{2+} and the AMPA Receptor

Functional AMPA receptors are homo- or hetero-tetrameric assemblies that are composed of four subunits, GluR1, GluR2, GluR3 and GluR4, in various combinations. The Ca^{2+} conductance of AMPA receptors differs markedly depending on whether the receptor has the GluR2 subunit in its subunit assembly. AMPA receptors that contain at least one GluR2

subunit have low Ca^{2+} conductance (Fig. 1a), whereas those lacking a GluR2 subunit are Ca^{2+} permeable [3-6]. These properties of GluR2 are generated by a single nucleotide conversion from adenosine (A) to inosine (I) by posttranscriptional RNA processing called RNA editing, during which as I is recognized as G during translation, the glutamine (Q) codon (CAG) is substituted by an arginine (R) codon (CIG; CGG) at the position called the Q/R site in the putative second membrane domain (M2) (Fig. 1b) [4,5,7]. Analyses of adult rat, mouse, and human brain RNA have demonstrated that almost all GluR2 mRNA *in vivo* has R at the Q/R site, whereas Q remains at this critical position in the GluR1, GluR3 and GluR4 subunits. Thus, AMPA receptor-mediated neuronal death occurs after an increase in Ca^{2+} influx through AMPA receptor-coupled ion channels, in which either a decrease in GluR2 expression or inefficient RNA editing at the GluR2 Q/R site is the possible causative molecular change.

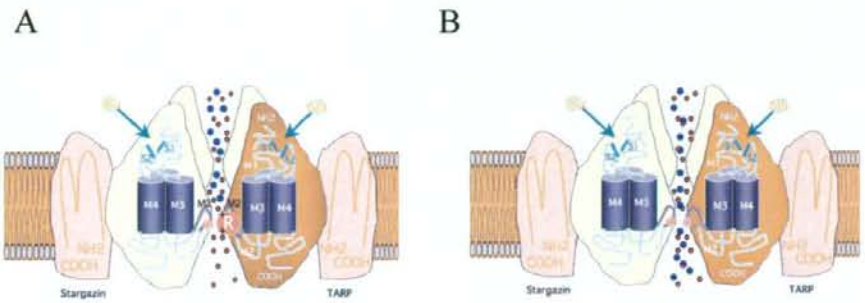

Figure 1. AMPA receptor and Ca^{2+}-permeability. A: AMPA receptor containing GluR2 subunit edited at the Q/R site (orange) in M2 (curved line in dark gray). Due to the positive charge of arginin (R) residue at the Q/R site, Ca^{2+} (blue circle) cannot pass through the channel pore, while Na^{+} (red circle) can pass through it. Majority of the AMPA receptors expressed on neurons is Q/R site-edited GluR2-containing AMPA receptors. B: Q/R site-unedited GluR2-containing or GluR2-lacking AMPA receptors. When none of the subunits have R at the Q/R position, AMPA receptors are Ca^{2+}-permeable. GluR1, 3, 4 subunits are in yellow.

In mammals, the majority of RNA editing occurs as the base conversion of A-to-I, which is most active in the central nervous system and has been found to occur predominantly in non-coding RNAs and has been considered to play a role in RNA processing including efficiency of splicing, stability, etc [8,9]. The best example of A-to-I RNA editing in non-coding RNAs is the recent finding on miRNAs that targeted different mRNAs depending on whether the miRNAs are edited or unedited [10]. On the other hand, A-to-I editing also occurs in the coding regions of mRNA encoding receptors and ion channels, including ionotropic glutamate receptor subunits [11-13], the serotonin-2C receptor ($5\text{-}HT_{2C}R$) [14], the voltage-dependent potassium channel Kv1.1 [15], and the RNA editing enzyme ADAR2 [16]. RNA editing alters the properties of these functional proteins by changing single amino acid at the specific position including the channel pore of the receptors, thereby regulating the strength of each synapse and function of neuronal networks. The regionally highly variable extent of kv1.1 I/V site-editing in the human, mouse and rat brains [15] may be a reflection of

such fine tuning of synaptic strength. In particular, the change in amino acid residue at the Q/R site of GluR2 results in marked alterations in channel properties, including Ca^{2+} permeability [3,4,12,17-22], trafficking [23], subunit assembly [24] and kinetic aspects of channel gating [13] of AMPA receptors. Furthermore, failure of GluR2 Q/R site-editing led mice to fatal status epilepticus [25], hence alterations in the AMPA receptor properties by GluR2 Q/R site-editing play pivotal role in excitation and death of neurons.

GluR2 Q/R Site-Editing and Sporadic ALS

Because several lines of evidence have indicated that molecular changes increasing Ca^{2+}-permeability of AMPA receptors may be relevant to neuronal death, particularly of motor neurons, we have investigated the molecular changes that alter the Ca^{2+}-permeability of AMPA receptors in neurons of sporadic ALS patients. In order to detect the molecular changes selective in motor neurons, we dissected motor neurons from the autopsy-obtained spinal cord of patients with a laser microdissecter (Fig. 2). Although the role of GluR2-lacking AMPA receptors in neuronal death has been implicated in ALS and in delayed neuronal death after brain ischemia, we could find no evidence supporting an increase of

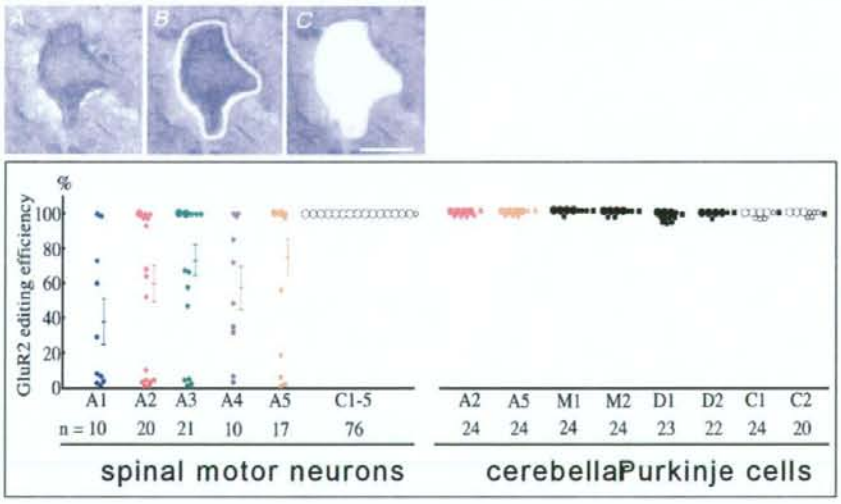

Figure 2. Upper panel: Dissection of single motor neurons with a laser microdissector. Before dissection; demargination with a narrow laser beam; after capturing the neuronal tissue. Bar = 40 μm. Lower panel: Editing efficiency at the GluR2 Q/R site in single neurons of ALS, disease control and normal control subjects (modified from Fig. 1 in [28]). The editing efficiency varied greatly, from 0% to 100% (mean: 38.1-75.3%), among the motor neurons of each individual with ALS, and was not complete in 44 of them (56%); this was in marked contrast to the control motor neurons, of which all 76 examined showed 100% editing efficiency. The editing efficiency in Purkinje cells was virtually complete (greater than 99.8%) in the ALS, disease control (DRPLA, MSA) and the normal control groups. Adopted from ref [28].

GluR2-lacking AMPA receptors in motor neurons of sporadic ALS [26]. On the contrary, GluR2 Q/R site-editing was variably decreased (from 0% to 100%) in spinal motor neurons of sporadic ALS, which was in marked contrast to the control motor neurons, of which all showed invariably 100% Q/R site-editing efficiency (Fig. 2) [27,28]. Above results indicate that an increase of Ca^{2+}-permeable AMPA receptors may occur in a subset of dying motor neurons due to a reduced extent of GluR2 Q/R site-editing but not due to a reduced proportion of GluR2-containing AMPA receptors in motor neurons of sporadic ALS. Because mice deficient for GluR2 Q/R site-editing died young from status epilepticus [25], a marked reduction of GluR2 Q/R site-editing in a subset of motor neurons may likely be the death-inducing molecular change in sporadic ALS.

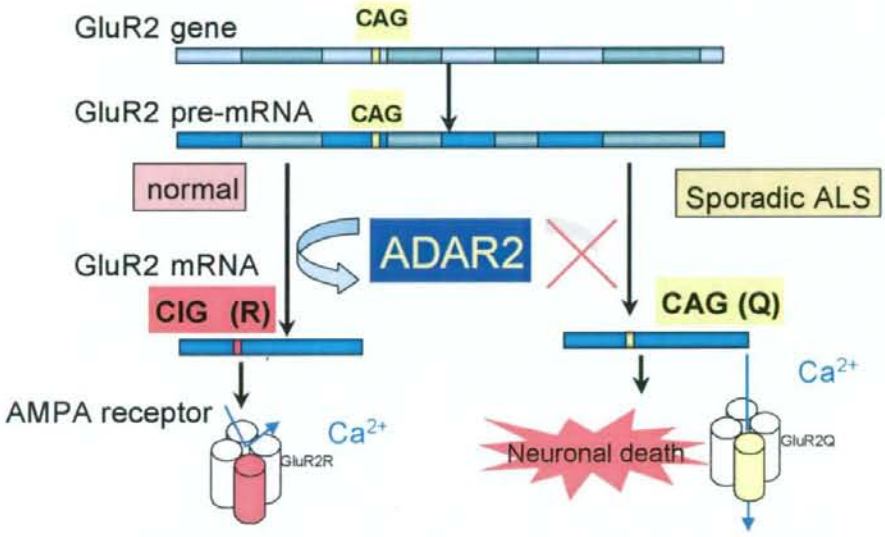

Figure 3. RNA editing and neuronal death in sporadic ALS. Codon for the Q/R site is CAG in the GluR2 gene, whereas A-to-I conversion due to ADAR2, codon for the Q/R site in mRNA is CIG, which is translated as CGG (arginine; R) in normal neurons. By contrast, due to ADAR2-underactivity, codon for the Q/R site remains CAG, which is translated as glutamin (Q) in motor neurons of sporadic ALS. An increase of Q/R site-unedited GluR2Q-containig Ca^{2+}-permeable AMPA receptors mediate neuronal death due to an increase of cytoplasmic Ca^{2+} concentration.

Progressive degeneration occurs selectively in motor neurons in ALS and other neuronal subsets undergo degeneration at a much later disease stage, if ever. We found that the reduction of GluR2 Q/R site-editing did not occur in the cerebellar Purkinje cells of the patients with sporadic ALS despite that the motor neurons expressed a high proportion of Q/R site-unedited GluR2 [28]. Additionally, we found that dying cerebellar Purkinje cells of patients with spinocerebellar degeneration (ie. dentatorubral pallidoluysian atrophy (DRPLA) and multiple system atrophy (MSA-c)) expressed only Q/R site-edited GluR2 mRNA [28].

Moreover, albeit at a tissue level, GluR2 Q/R site-editing has been reported to be preserved even in the brain areas severely affected in several other neurodegenerative diseases including the striatum of Huntington disease, the neocortex and hippocampus of Alzheimer and Pick diseases, and the cerebellum of diseases of spinocerebellar degeneration [29-32]. Therefore, the defect in GluR2 Q/R site-editing occurs in motor neurons of sporadic ALS in a disease-specific and dying neuron-selective manner.

GluR2 Q/R Site-Editing and Neuronal Death

Mutant mice deficient for GluR2 Q/R site-editing exhibited fatal status epilepticus [25], suggesting that cortical neurons including those in the hippocampus underwent exaggerated excitation due to an increase of Ca^{2+} influx through AMPA receptors containing Q/R site-unedited GluR2. On the other hand, although GluR2-lacking AMPA receptors are also Ca^{2+}-permeable, neither GluR2 knockout mice [33] nor GluR2/GluR3 double-knockout mice [34] exhibited only minor behavioral changes but no demonstrable seizure activity or neuronal death. Therefore, it seems likely that there may be additional influence of Q/R site-unedited GluR2Q other than increasing Ca^{2+}-permeabililty on the properties of AMPA receptors.

Recently, it has been reported that the Q/R site-unedited GluR2Q subunit was more readily incorporated into functional AMPA receptors than the Q/R site-edited GluR2R subunit at the stage of tetramer (dimer of dimers) formation [24]. In addition, AMPA receptors containing the Q/R site-unedited GluR2Q subunit were more readily transported from the endoplasmic reticulum (ER) to the cell surface than those containing the Q/R site-edited GluR2R subunit during receptor trafficking [23]. Therefore, if the extent of Q/R site-RNA editing were decreased, even to a small extent, the density of Ca^{2+}-permeable AMPA receptors containing Q/R site-unedited GluR2Q may increase in the postsynaptic membrane. Indeed, neuronal death was enhanced after transfection of exogenous GluR2Q in cultured rat hippocampal neurons, while it was attenuated when the AMPA receptor trafficking was blocked, suggesting that an exaggerated increase of Ca^{2+}-permeable AMPA receptor density was required to induce neuronal death [35]. Therefore, neuronal death seems to be induced only when the density of Ca^{2+}-permeable functional AMPA receptors were significantly increased in the functioning synapses, and the difference of phenotype severity between mutant mice deficient for GluR2 Q/R site-editing [25] and GluR2-null mutant mice [33,34] may be due to the difference in the density of Ca^{2+}-permeable functional AMPA receptors at the excitatory synapse.

The role of Ca^{2+}-permeable functional AMPA receptors in neuronal death has been implicated in delayed neuronal death of pyramidal cells in the hippocampal pyramidal cells after brain ischemia. Previously, a decrease in GluR2 mRNA prior to neuronal death has been demonstrated [36], whereas a decrease in GluR2 Q/R site-editing was not demonstrated in ischemic brain [29-31] or brains of animals under conditions inducing neuronal death after cerebral hypoxia [37] or kindling [38]. However, it was recently demonstrated that dying hippocampal pyramidal cells after transient ischemia expressed increasing amount of Q/R site-unedited GluR2 mRNA and restoration of Q/R site-editing inhibited the neuronal death after ischemia [39]. Because CA1 pyramidal cells in the hippocampus undergo delayed neuronal death after ischemia [40], these observations suggest that neurons may die with a slow temporal profile when GluR2 Q/R site-editing was diminished. The time-course of slow

progression of neuronal death is the characteristics in ALS as well, lending further support to the relevance of a reduction of GluR2 Q/R site-editing to death of motor neurons in sporadic ALS. The reduction of GluR2 Q/R site-editing has been demonstrated only in the CA1 pyramidal cells after ischemia despite that ischemic insult occurred in entire forebrain area. Therefore, it is likely that a reduction of GluR2 Q/R site-editing occurs only in a subset of neurons that may have low potency keeping this site-editing and hence vulnerable to neuronal death that progresses with a slow process. It has not been elucidated, however, what factor(s) produced the motor neuron-selectivity.

When the relative expression level of each AMPA receptor subunit in various neuronal subsets was investigated at an mRNA level, the motor neurons of control subjects expressed significantly lower levels of GluR2, as compared to other neuronal subsets in humans [26]. The lower relative proportion of GluR2 mRNA among AMPA receptor subunits mRNAs in motor neurons as compared to other neuronal subsets was also found in adult rats, while GluR2 shared much lower proportion as compared to human motor neurons [41]. Above results may imply that AMPA receptors in motor neurons have lower chance to include GluR2 in their subunits than those in other neuronal subsets, resulting in an increase of the proportion of GluR2-lacking Ca^{2+}-permeable AMPA receptors. Therefore, effects of GluR2 RNA editing deficiency on neuronal excitability may be more clearly expressed in neurons expressing relatively low GluR2 [26]. According to this scheme, a small increase in Q/R site-unedited GluR2Q will greatly increase the proportion of Ca^{2+}-permeable functional AMPA receptors, thereby promoting excitotoxicity in ALS motor neurons. It is of interest that rat hippocampal type-II neurons that undergo delayed neuronal death after ischemia, expressed GluR2 mRNA at the low level less than 50% of total AMPA receptor subunits [42], as seen in rat motor neurons [41].

GluR2 Q/R site-Editing in Motor Neuron Disease

In order to rule out the possibility that deficient GluR2 Q/R site-editing is non-specifically associated with death of motor neurons in sporadic ALS, we also investigated the extent of GluR2 Q/R site-editing in dying motor neurons of motor neuron diseases other than sporadic ALS. Because we found that GluR2 Q/R site-editing was low in various types of sporadic ALS in addition to classical limb-onset type and progressive bulbar palsy, including progressive muscular atrophy type ALS with very long course, early-onset ALS with rapid progression [43], and ALS with dementia (unpublished observation), analysis of the extent of GluR2 Q/R site-editing may provide novel aspect for the classification of motor neuron diseases in terms of the death-inducing factors.

ALS1 is the most frequent familial ALS [44], and mutated human SOD1 transgenic animals have been studied extensively as a disease model of ALS1 and sometimes of all ALS cases [45], yet the molecular mechanism how mutated SOD1 induces neuronal death has not been elucidated.

In contrast with the significant GluR2 Q/R site-underediting in motor neurons of sporadic ALS [28], GluR2 mRNA in all the motor neurons of the mutated human SOD1 transgenic rats with two different mutation sites (G93A and H46R) we examined was completely edited at the Q/R site (Fig. 4) [46]. We examined the motor neurons in the spinal cord segment corresponding to the hindlimbs of mutated human SOD1 transgenic rats after they manifested

paralysis of their hindlimbs, indicating that the motor neurons we examined were already pathologically affected. Because the pathogenic mechanism underlying ALS1 is considered to be the same as in mutant human SOD1 transgenic animals, motor neurons in affected ALS1 patients would be expected to have only edited GluR2 mRNA. Therefore, the death-inducing molecular mechanisms may be different between sporadic ALS and ALS1. Indeed, an association study of the SOD1 gene in a considerable number of patients with sporadic ALS reported no significant association with mutations of the SOD1 gene [47].

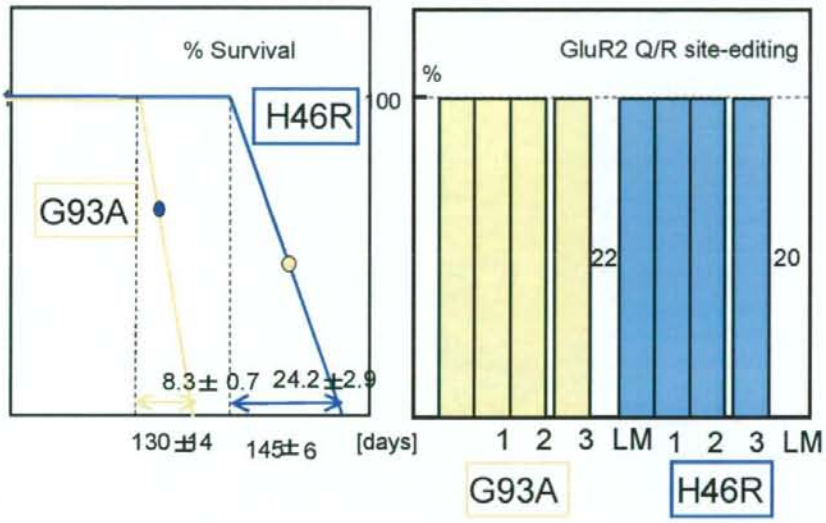

Figure 4. Extent of GluR2 Q/R site-editing was analyzed in laser-captured single motor neurons of the mutated human SOD1 transgenic rats with two different mutation sites (G93A and H46R). Analysis was conducted after transgenic rats manifested ALS signs (indicated as filled circle). More than 10 motor neurons were dissected from each rat (n=3 for each mutation) with their littermate rats. All the motor neuron examined (n=117 for transgenic rats and n=42 for littermate rats) expressed only Q/R site-edited GluR2 mRNA [46].

However, many recent studies support critical roles of Ca^{2+}-permeable AMPA receptors in motor neuron degeneration in ALS1. Transgenic animal studies have recently solidified the link between Ca^{2+}-permeable AMPA receptors and motor neuron loss in SOD1 linked familial forms of ALS. Specifically, crossing G93A SOD1 transgenic mouse models of ALS1 with either mice lacking GluR2 entirely [48] or mice with modified GluR2 encoding aspargine (N) at the Q/R site (GluR-B(N)), which is equivalent to Q/R site-unedited GluR2Q, [49] resulted in marked acceleration of the disease. Conversely, when mice with decreased numbers of Ca^{2+}-permeable AMPA receptors in their motor neurons (via targeted GluR2 overexpression) were crossed with the G93A mice, the disease was significantly delayed [50]. Because GluR2 Q/R site-editing was full in ALS1 model rats motor neurons, these lines of evidence may indicate an increase of GluR2-lacking Ca^{2+}-permeable AMPA receptors in

mutated SOD1 transgenic mice. On the other hand, an increase in GluR3 mRNA has been reported in the motor neurons of SOD1 G93A mice [51]. In addition, the survival of these mice can be prolonged by the administration of GluR3 antisense protein nucleic acid [52], which may reverse an up-regulation of GluR3 and ameliorate an increase of GluR2-lacking Ca^{2+} permeable AMPA receptors. However, as we have mentioned above, a lack of GluR2 *per se* did not induce neuronal death in GluR2 knockout mice [33]. In this regard, our recent observation in rats continuously infused with kainic acid may be worthy of note. When kainic acid was infused into the rat subarachnoid space of the caudal lumbar vertebral segments continuously for more than 1 month, Wistar and Fischer rats displayed progressive and selective motor dysfunction with neuronal death selective in the spinal motor neurons [53]. There was an up-regulation of total AMPA receptor subunit mRNAs due to a selective increase of GluR3 mRNA in motor neurons after the initiation of kainic acid infusion, suggesting that an increase in the density of GluR2-lacking Ca^{2+} permeable AMPA receptors [53]. This study implies that AMPA receptors in motor neurons underwent exaggerated activation in ALS1 model mice, leading to GluR3 up-regulation resulting in an increase in the synaptic density of GluR2-lacking Ca^{2+} permeable AMPA receptors, and ultimately in neuronal death. However, the precise mechanism underlying GluR3 up-regulation after continuous AMPA receptor activation remains elusive.

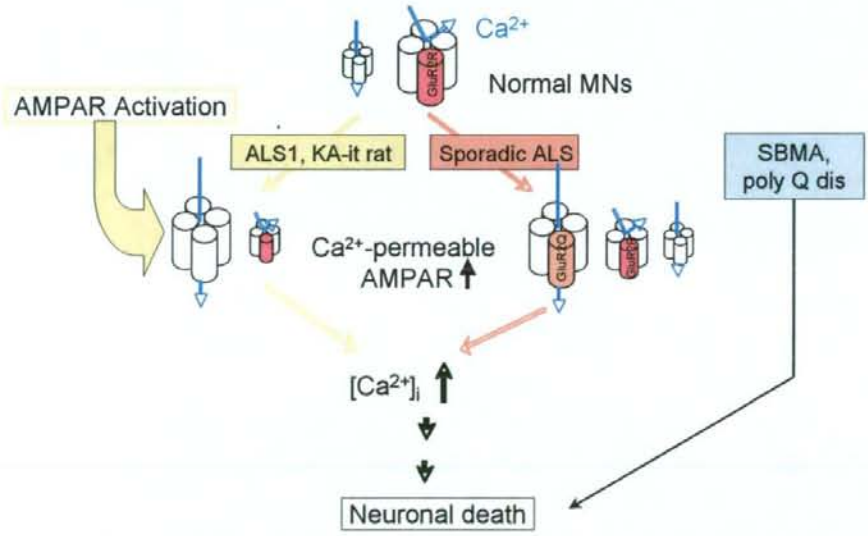

Figure 5. Under basal conditions, motor neurons possess a substantial number of Ca^{2+}-permeable as well as Ca^{2+}-impermeable AMPA receptors. Motor neurons in sporadic ALS express considerable Q/R site-unedited GluR2 mRNA, probably resulting in an increased number of Ca^{2+}-permeable AMPA channels containing unedited GluR2. By contrast, motor neurons in SOD1-associated familial ALS (ALS1) and ALS model rats by continuous intrathecal kainic acid infusion express reduced proportion of GluR2-containing AMPA receptors due to up-regulation of GluR3. Although an increase of GluR2-lacking AMPA receptors per se does not induce neuronal death, neuronal death may be resulted when

AMPA receptors are continuously exaggerated. AMPA receptor-mediated neuronal death does not play a role in death of motor neurons in SBMA, a CAG-repeat expansion disease.

Therefore, AMPA receptor-mediated neuronal death plays a pivotal role in both sporadic ALS and SOD1-associated familial ALS, but the molecular mechanisms are different between the two; deficient GluR2 Q/R site-editing increases Ca^{2+} permeable AMPA receptors in the former, whereas a relative decrease of GluR2 increased GluR2-lacking Ca^{2+} permeable AMPA receptors in the latter (Fig. 5). Difference of molecular mechanisms between sporadic ALS and ALS1 were also suggested by recent findings that abnormally phospohrylated and fragmented TDP-43 was localized in ubiquitin-positive cytoplasmic inclusion bodies of motor neurons of sporadic ALS and cortical neurons of FTLD [54,55] but not in inclusion bodies of SOD1-associated familial ALS [56,57]. Investigation into the link between abnormal TDP-43 and GluR2 Q/R site underediting may promote our understanding of the ALS pathogenesis.

Another example of non-ALS motor neuron disease is spinal and bulbar spinal atrophy (SBMA), in which lower motor neurons were predominantly affected remaining upper motor neurons intact with a relatively slow clinical course. Since the CAG-repeat expansion in the androgen receptor gene has been demonstrated in SBMA [58,59], and pharmacological castration is therapeutically effective in animal models [60], the death cascade responsible for SBMA is likely different from sporadic ALS. In contrast to ALS1 model animals, it has been reported that NMDA receptor-mediated neurotoxicity but not AMPA receptor-mediated neurotoxicity plays a role in the neuronal death in model mice of Huntington disease, another CAG-repeat expansion disease [61]. In agreement with these reports, we found that GluR2 Q/R site-editing was complete in dying motor neurons in the autopsied SBMA spinal cord [46]. Therefore, it is likely that there are multiple different death pathways in motor neurons, and motor neurons in sporadic ALS, ALS1 and SBMA die by different death cascades (Fig. 5).

Role of ADAR2 in Death of Motor Neurons in Sporadic ALS

The GluR2 Q/R site is almost completely edited in various brain regions, including white matter in neonatal and adult rodent brains [17,62-64]. GluR2 mRNA in human brains including white matter is, however, not always completely edited at the Q/R site [26,27,29,31,38,65-67]. We have demonstrated that neurons in various human brain nuclei express only Q/R site-edited GluR2 mRNA, but adult, but not in immature, human white matter express Q/R site-unedited GluR2 mRNA to an extent up to one-thirds of total GluR2 mRNA [26,67]. It seems likely that human glial cells physiologically express Ca^{2+}-permeable AMPA receptors: oligodendrocytes express those containing unedited GluR2 subunits, and astrocytes and Bergmann's glial cells express those lacking edited GluR2 subunits [5,18,68].

Q/R site-editing of GluR2 is an enzymatic reaction. Enzymes responsible for the A-to-I conversion have been termed adenosine deaminases acting on RNA (ADARs), and three structurally related ADARs (ADAR1 to ADAR3) have been identified in mammals [69-74]. ADAR1 and ADAR2 are expressed in most tissues [69,71,75,76] and ADAR2 recognize the adenosine residue to be edited in the Q/R site of GluR2 through the structure of the duplex

that is formed between the editing site and its editing site complementary sequence (ECS), which is located in the adjacent downstream intron of the precursor (pre-) mRNA [77-79] and catalyzes conversion of A-to-I at the Q/R site of GluR2 mRNA exon 11 most efficiently. ADAR1 and ADAR2 catalyze A-to-I conversion in various RNAs in both coding and non-coding regions. There are two isoforms generated by alternative splicing in ADAR1, with molecular weight 110 kDa and 150 kDa, respectively. There are at least 48 different variants in ADAR2 mRNA and two ADAR2 protein isoforms generated by either including or excluding Alu repeats in human brains [80]. ADAR3 is expressed exclusively in the brain, but is catalytically inactive on both extended double-stranded RNA and known pre-mRNA editing substrates [72,74].

It has been shown that the mRNA expression of ADAR2 is regulated in a cell-specific manner throughout development in rodent brain; first detected by *in situ* hybridization in the thalamic nuclei formation at embryonic day 19 (E19), with more extensive and widespread distribution by the third postnatal week when the expression is highest in the thalamic nuclei and very low in white matter [81]. RNA editing at several editing sites of GluRs was developmentally up-regulated in accordance with the increase of ADAR2 mRNA levels in rat [62,82-85] and human [67] brains, in a cultured human teratocarcinoma cell line [86], and in surgically excised hippocampus from patients affected with refractory epilepsy [87]. In addition, the extent of GluR2 Q/R site-RNA editing was determined in part by the expression level of ADAR2 mRNA in human white matter [26]. However, the ADAR2 expression level does not seem to be corresponding to the extent of RNA editing at various A-to-I positions among different tissues. Thus, it has not been precisely elucidated how ADAR2 activity is regulated in neurons.

ADAR2 knockout mice died young from status epilepticus with expressing markedly high proportion of Q/R site-unedited GluR2, but the ADAR2 knockout mice additionally expressed Q/R site-edited GluR2 without ADAR2 (GluR-B^R) exhibited normal phenotype without developing seizure activity [88]. Although whether death of motor neurons occurred in ADAR2-null mice remains unclear due to their early death, mice transgenic for an artificial Ca^{2+}-permeable GluR2 that has aspargine (N) at the Q/R position (GluR-B(N)) developed motor neuron disease late in life without developing seizure activity [49,89], suggesting that motor neurons are specifically vulnerable to neuronal death resulting from deficient GluR2 Q/R site-editing. These results suggest that ADAR2-deficiency *per se* may not be neurotoxic provided abundant Q/R site-edited GluR2 without ADAR2. Indeed, seizure-inducing phenotype of the ADAR2 knockout mice suggest that neuronal excitation was exaggerated due to an increase of functional Ca^{2+}-permeable AMPA receptors. Although histological demonstration was lacking, the neurons may have undergone seizure-related degeneration in ADAR2 knockout mice, particularly in the limbic brains including the hippocampus, as seen in epileptic rats after kainic acid injection. Therefore, we need ADAR2-deficient mice that do not exhibit seizure activity to investigate whether a lack of ADAR2 induces ALS-like slow progressive neuronal death when the mice lived long without developing status epilepticus. In order to avoid non-specific neuronal injury associated with seizure activity, we developed a mouse line in which ADAR2 was conditionally targeted in the motor neurons using Cre/LoxP system, and found that motor neurons devoid of ADAR2 underwent ALS-like slow neuronal death with expression of only Q/R site-unedited GluR2 (unpublished observation). Because we found that the expression level of ADAR2 mRNA was lower in the ventral gray matter of the autopsy-obtained spinal cord of sporadic ALS cases than in control subjects [90], the

conditional ADAR2 knockout mice underwent slow neuronal death under the molecular mechanism in common with sporadic ALS.

We lend further support to our hypothesis that deficient GluR2 Q/R site-editing resulting from a reduction in ADAR2 activity is death-causing molecular change in sporadic ALS but not in SOD1-associated familial ALS. In addition, in agreement with our results, recent reports demonstrated that abnormal TDP-43 accumulation in the inclusion bodies of sporadic ALS and FTLD but not in those of SOD1-associated familial ALS [56,57]. Therefore, due to the difference in disease-specific molecular changes, we may not properly evaluate the therapeutic efficacy on patients with ALS by using mouse model transgenic for the mutated SOD1 gene. Because GluR2 Q/R site-underediting is likely the direct cause of sporadic ALS, restoring deficient ADAR2 activity in motor neurons may be a specific therapeutic strategy for sporadic ALS, and the conditional ADAR2 knockout mouse line will provide a novel tool for developing specific therapy.

Acknowledgement

This study was supported, in part, by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (S.K. & T.Y.), a grant-in-aid for Scientific Research from the Ministry of Health, Labor, and Welfare of Japan (S.K.), a grant from The Nakabayashi Trust for ALS Research (T.H.) and a grant from the Japan ALS Association (T.H.).

References

[1] Carriedo SG, Yin HZ, Weiss JH. Motor neurons are selectively vulnerable to AMPA/kainate receptor-mediated injury in vitro. *J Neurosci* 1996; 16: 4069-4079.

[2] Lu YM, Yin HZ, Chiang J, Weiss JH. Ca^{2+}-permeable AMPA/kainate and NMDA channels: high rate of Ca^{2+} influx underlies potent induction of injury. *J Neurosci* 1996; 16: 5457-5465.

[3] Hollmann M, Hartley M, Heinemann S. Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science* 1991; 252: 851-853.

[4] Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B. Structural determinants of ion flow through recombinant glutamate receptor channels. *Science* 1991; 252: 1715-1718.

[5] Burnashev N, Monyer H, Seeburg P, Sakmann B. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* 1992; 8: 189-198.

[6] Koh DS, Burnashev N, Jonas P. Block of native Ca^{2+}-permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification. *J Physiol* 1995; 486: 305-312.

[7] Hume RI, Dingledine R, Heinemann SF. Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science* 1991; 253: 1028-1031.

[8] Levanon EY, Eisenberg E, Yelin R, Nemzer S, Hallegger M, Shemesh R *et al.* Systematic identification of abundant A-to-I editing sites in the human transcriptome. *Nat Biotechnol* 2004; 22: 1001-1005.

[9] Nishikura K. Editor meets silencer: crosstalk between RNA editing and RNA interference. *Nat Rev Mol Cell Biol* 2006; 7: 919-931.

[10] Kawahara Y, Zinshteyn B, Sethupathy P, Iizasa H, Hatzigeorgiou AG, Nishikura K. Redirection of silencing targets by adenosine-to-inosine editing of miRNAs. *Science* 2007; 315: 1137-1140.

[11] Sommer B, Kohler M, Sprengel R, Seeburg PH. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell* 1991; 67: 11-19.

[12] Köhler M, Burnashev N, Sakmann B, Seeburg P. Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing. *Neuron* 1993; 10: 491-500.

[13] Lomeli H, Mosbacher J, Melcher T, Hoger T, Geiger JR, Kuner T *et al.* Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. *Science* 1994; 266: 1709-1713.

[14] Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E *et al.* Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature* 1997; 387: 303-308.

[15] Hoopengardner B, Bhalla T, Staber C, Reenan R. Nervous system targets of RNA editing identified by comparative genomics. *Science* 2003; 301: 832-836.

[16] Rueter SM, Dawson TR, Emeson RB. Regulation of alternative splicing by RNA editing. *Nature* 1999; 399: 75-80.

[17] Burnashev N, Khodorova A, Jonas P, Helm P, Wisden W, Monyer H *et al.* Calcium-permeable AMPA-kainate receptors in fusiform cerebellar glial cells. *Science* 1992; 256: 1566-1570.

[18] Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P *et al.* Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* 1995; 15: 193-204.

[19] Puchalski R, Louis J, Brose N, Traynelis S, Egebjerg J, Kukekov V *et al.* Selective RNA editing and subunit assembly of native glutamate receptors. *Neuron* 1994; 13: 131-147.

[20] Burnashev N, Zhou Z, Neher E, Sakmann B. Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. *J Physiol* 1995; 485: 403-418.

[21] Swanson GT, Feldmeyer D, Kaneda M, Cull-Candy SG. Effect of RNA editing and subunit co-assembly single-channel properties of recombinant kainate receptors. *J Physiol* 1996; 492: 129-142.

[22] Swanson G, Kamboj S, Cull-Candy S. Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. *J Neurosci* 1997; 17: 58-69.

[23] Greger IH, Khatri L, Ziff EB. RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. *Neuron* 2002; 34: 759-772.

[24] Greger IH, Khatri L, Kong X, Ziff EB. AMPA receptor tetramerization is mediated by Q/R editing. *Neuron* 2003; 40: 763-774.