別紙２

Results report

１． Title of Research and Development： RNA-based novel approaches for discovery of ALS biomarker

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４． Results of Research and Development:

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease affecting middle-aged individuals in their motor functions and lack of effective therapy leads them to eventual death by respiratory muscle weakness within a few years of onset. More than 90% of ALS occurs in a sporadic fashion, and there are no reliable biomarkers for the disease. Recently it has been demonstrated that deficient adenosine to inosine (A-I) conversion (RNA editing) of pre-mRNA resulting from down-regulation of RNA editing enzyme called adenosine deaminase acting on RNA 2 (ADAR2) occurs in the motor neurons in the majority of patients with ALS in a disease-specific manner. Notably, this molecular abnormality is a cause of both death of motor neurons and TDP-43 pathology, the most reliable neuropathological hallmark of ALS, in the mouse model （ADAR2$^{flox/flox}$/VChAT-Cre, or AR2 mice）mimicking these ALS-specific molecular abnormalities. These lines of evidence indicate that ADAR2 down-regulation is involved in the pathogenesis of sporadic ALS.

Because demonstration of ADAR2 down-regulation in peripherally accessible organs would be a pathomechanism-associated ALS biomarker, we aim to search for a biomarker that reflects down-regulation of ADAR2 in the patients' body fluids. RNAs and proteins are secreted as such or packed in exosomes from individual cells into the body fluids, including blood and cerebro-spinal fluid (CSF) and RNAs in exosomes and circlar RNAs (circRNA) in body fluids are known to be very stable. A-I conversion occurs actively at numerous positions in the mammalian central nervous system and ADAR2 is the major executive enzyme. Down-regulation of ADAR2 in the ALS motor neurons results in the failure of RNA editing at numerous ADAR2-mediated positions in RNAs, including both coding and non-coding regions. If we could detect some of these RNAs with unedited at ADAR2 positions in the body fluids, they likely become biomarkers for sporadic ALS because of the selectivity of ADAR2 down-regulation in sporadic ALS.

Conditional ADAR2 knockout mice（AR2 mice）mimic the molecular abnormalities seen in the motor neurons of ALS patients and exhibit ALS phenotype. ADAR2-lacking motor neurons in the AR2 mice express RNAs devoid of RNA editing at the ADAR2-mediated positions. We therefore started by isolating single motor neurons from AR2 mice and wild-type mice with a laser microdisecter to extract motor neuron-specific RNAs. We dissected more than 2,000 motor neurons from an individual mouse (n=3 for each group). We extracted RNAs from the single motor neuron tissues using the SMARTer Stranded RNA-Seq Kit（Clontech Laboratories, Inc）to prepare samples for RNA-seq.

After removing all variants present in catalogues of DNA differences in SNP databases, those caused by sequence mapping artifacts such as read alignment across splice junctions, gene duplications, and homopolymer stretches and rare SNPs, research collaborators in Israel scrutinized the RNA-seq data on A-I positions. We then searched

for ADAR2 positions among these A-I positions and selected candidate RNAs by tentative criteria that the proportion of edited RNA was 1) more than 10% of the transcript in the wild-type mice and 2) significantly lower in the AR2 mice than in the wild-type mice.

We then searched for the human homolog of these candidate mouse RNAs and tested whether human homolog of RNAs have ADAR2 positions in the human-derived cultured cells by siRNA knockdown and overexpression of ADAR2. We are now examining the presence of these candidate RNAs in human body fluids.