別紙２

Results report

１．Title of Research and Development : Pathogenic mechanism underlying neurodevelopmental disorder in schizophrenia

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４．Results of Research and Development:

Schizophrenia is a chronic brain disease, which imposes one of the greatest burdens on patients, their relatives and public health care. However, the molecular mechanisms underlying the pathophysiology of this disease are poorly defined and the diagnosis is still done by clinical interviews without the assistance of objective biological tests. Nevertheless, neurodevelopmental abnormalities have been considered as part of the pathophysiology of schizophrenia. The project combines an interdisciplinary approach of scientists from Japan and Israel, where the Japan-research group (JPN group) has identified rare missense single nucleotide variants (namely *NDE1*-S214F) in *NDE1* in patients as well as rare exonic duplications in *NDE1* and the small GTPase regulators such as ARHGAP26 and RAPGEF1. The JPN group generated the mutated constructs, expressed them in primary hippocampal neurons and analyzed differential protein complexes from different subcellular fractions by Mass spectrometry. The overexpression and knockdown studies revealed that RAPGEF1 was involved in the dendritogenesis of immature neurons. The expression of constitutive active Rap1 altered the dendritic complexicity. These findings suggest that Rap1 signaling is implicated in the pathology of schizophrenia via neurodevelopemnt. The JPN group found that expression of *NDE1*-S214F allele inhibited axonal outgrowth of hippocampal neurons (Kimura et al, Schizophrenia Bull, 41(3), 2015). To clarify the molecular pathology caused by *NDE1*-S214F allele, the JPN group tried to investigate NDE1-interactome using a NDE1-affinity column chromatography and identified more than 100 of NDE1-interactors. Furthermore, the pulldown samples from the immobilized with wildtype NDE1 or NDE1-S214F protein were subjected to the quantitative mass spectrometry. We found that more than 10 molecules were affected in the binding to NDE1 by the NDE-1S214F mutation.

In this joint project, the Israel-research group (ISR group) employed in the *in utero* electroporation and the genome-editing techniques for studying neuronal proliferation, migration and connectivity. To evaluate the pathophysiological meaning of *NDE1*-S214F expression in the developing brain, ISR group introduced the NDE1-S214F construct by in utero electroporation. The neurons expressing NDE1-S214F were impaired in cortical migration compared to the neurons expressing wildtype NDE1. This result suggests that the *NDE1*-S214F allele is involved in the cortical development. Recently, ISR group generated a knock-in mouse carrying *NDE1*-S214F allele by a genome-editing technique. When ISR group performed the immunoblotings of NDE1 using the brain lysates of wildtype or the mutant mice, there is little difference in the expression levels of NDE1 from both mice. In this collaborative project, we indicated that Rap1 signaling and the NDE1-interactome are implicated in the pathophysiology of schizophrenia.