[課程—2]

審査の結果の要旨

氏名 陳 萱容

HLA class II DQA1*01:02-DQB1*06:02 is the strongest genetic risk factor for narcolepsy. Since it has been well-established that the level of hypocretin is significantly decreased in CSF of narcoleptic patient, I hypothesized that hypocretin peptide in hypocretin-producing cells might be presented by DQA1*01:02-DQB1*06:02, and induce autoimmune reactions. In the present study, I searched for hypocretin-derived peptides that can bind to HLA-DQA1*01:02-DQB1*06:02 protein by in vitro binding assay.

1. I constructed DQA1*01:02-DQB1*06:02, and other narcolepsy-associated HLA-DQ molecules. Expression of DQ proteins fused to His-tag or Strep-tag by NIH3T3 cells was confirmed by dot-blot analysis for DQA1*01:02-DQB1*06:02.

2. To determine the reagent with the lowest non-specific binding signal on the NIH3T3 cell surface, the non-specific binding affinity of various manufacturers' reagents on the NIH3T3 cell surface was examined. The nonspecific binding signals of NeutrAvidin DyLight 550 were weakest even at 500 μM.

3. I examine synthetic peptides designed for prepro-hypocretin as potential autoantigens associated with the onset of narcolepsy. The preliminary binding assay confirmed the binding of hypocretin signal peptide to narcolepsy-associated proteins. Peptides derived from other regions of hypocretin were tested on DQB1*06:02 and other allele products. The binding affinities of different risk- and protective-HLA-DQ proteins with different hypocretin-derived peptides were confirmed.

4. Insulin B_{1-15} binds to DQA1*01:02-DQB1*06:02. Insulin B_{1-15} and insulin B_{1-20} (which contains the insulin B_{1-15} binding motif) as positive controls were analyzed in this experiment. However, these assays were unsuccessful in detecting specific

binding of insulin B_{1-15} and insulin B_{1-20}, i.e., the positive control peptides, in NIH3T3 cells. In the cell-based peptide-binding assay, the assay system exhibited high non-specific binding under a variety of conditions.

5. Eight new hypocretin candidate peptides were evaluated in the plate-based peptide binding assay. In particular, the binding intensity of the hypocretin_{1-13} to DQA1*01:03-DQB1*06:03 (resistant haplotype) DQA1*01:03-DQB1*06:03 (resistant haplotype), and DQA1*01:02-DQB1*06:04 (neutral haplotype) were addressed.

6. The binding of hypocretin-derived peptides to DQ molecules was examined in this study. For $\text{hypocretin}_{1-13,}$ DQA1*01:02-DQB1*06:02 and DQA1*01:03-DQB1*06:03 differ at Phe9β in DQB1*06:02, Tyr9β in DQB1*06:03, Tyr30β in DQB1*06:02, and His30β in DQB1*06:03. 9β and 30β are involved in accommodating P6 in the binding pocket. The signal intensities of hypocretin_{1-13} binding to DQA1*01:03-DQB1*06:03 protein appeared different from DQA1*01:02-DQB1*06:02, differences at 9β and 30β may not affect the accommodation of P6 valine.

The results of the plate-based peptide-binding assay were reproducible for HLA DQ protein and hypocretin peptides. Screening a large number of candidate peptides for diverse HLA allele products might facilitate the detection of potential epitopes such as TRIB2. The results described in this thesis need to be verified by using T cells from narcoleptic patients in future studies. The results provide new insights into the epitopes and molecular mechanisms underlying narcolepsy and other autoimmune diseases.