論 文 の 内 容 の 要 旨

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Studies on the polymorphisms of major histocompatibility complex (MHC) class II gene in common chimpanzees (*Pan troglodytes*)

(チンパンジーにおける主要組織適合性遺伝子複合体クラス II 遺伝子の多型性に関する研究)

Nonhuman primates have been used extensively as models for human disease. In particular, the common chimpanzee (*Pan troglodytes*), which belongs to the superfamily *Hominoidea*, has been used as a human model for the study of diseases such as human hepatitis C virus (HCV), human hepatitis B virus (HBV), and human immunodeficiency virus (HIV) infections, to which only human and chimpanzee are susceptible. In each case, a complete understanding of these models requires knowledge of the MHC genes. There two major classes of MHC molecule, MHC class I molecule and MHC class II molecule. Especially, MHC class II molecules are heterodimeric cell surface glycoprotein that play a critical role in immune response by binding peptides and presenting them to T cell. In the present study, polymorphisms in the Patr MHC class II B1 allele were analyzed by sequencing of exon 2 region. For effectively identification of Patr MHC class II alleles, I developed an applied or modified typing

technique on each Patr-DPB1, -DQB1, and -DRB1 alleles. The present study was consisted of chapter 1, chapter 2 and chapter 3 as follows.

Chapter 1. Sequence analysis of MHC class II DPB1 gene in chimpanzees (*Pan troglodytes*)

In the Patr-DPB1 alleles, I applied to both cloning and direct sequencing. In order to design suitable primer pair which amplify exon 2 of Patr-DPB1 alleles, a fragment of approximately 8 kb from exon 1 to exon 3 was amplified from chimpanzee genomic DNA. After designing 500-bp primer pair at the 3' region of intron 1 and the 5' region of intron 2, the analysis of DPB1 exon 2 alleles of each chimpanzee was carried out. Particularly, the application of direct sequencing was useful to exclude a PCR error occurred during cloning and sequencing. I identified seven alleles on the Patr-DPB1 gene, including one new allele in the tested twenty-two chimpanzees. The results suggest that Patr-DPB1 alleles is characterized by genetic variations such as the exchange of sequence motifs and the accumulation of point mutations than other Patr MHC class II regions.

Chapter 2. Sequence analysis of MHC class II DQB1 (Patr-DQB1) alleles in chimpanzees by PCR-based methods

PCR-RFLP method and cloning and sequencing techniques were applied in this chapter. As a result, these methods were useful for the determination of Patr-DQB1 alleles and effectively detected mutations occurred in new alleles. Particularly, PCR-RFLP analysis using specific restriction enzymes for each Patr-DQB1 lineage was available for panel of Patr-DQB1 lineages involved individual chimpanzee. The six Patr-DQB1 alleles including two new alleles were identified in twenty-five chimpanzees.

And, the obtained nucleotide sequences and phylogenetic analysis indicated well the lineage-specific character of the Patr-DQB1 alleles.

Chapter 3. Identification and analysis of MHC class II DRB1 (Patr-DRB1) alleles in chimpanzees

In the Patr-DRB1 alleles, I attempted the selective identification using PCR technique applied with three steps: first, I performed Patr-DRB1*02 lineage-specific 8-kb PCR for *02 lineage detection in each chimpanzee; second, I performed 620-bp PCR for amplification of full-length exon 2; and finally, I carried out an insert check using the pattern of microsatellite repeat length variability. In the genomic DNA of twenty-three chimpanzees, nine Patr-DRB1 alleles containing two new alleles were detected. The sequence data and the phylogenetic analysis suggest that the β-pleated sheet contains most of the stable lineage-related sequence motifs and the α-helical portion appears to accumulate variations. The two new alleles appeared to be primarily generated by changes in the α-helical loop portion. In addition, the data of the microsatellite sequences of the chimpanzees analyzed in this chapter will provide a basis for examining the evolution of microsatellite sequences and for tracing the origin of individual Patr-DRB1 alleles.

In conclusion, the methods used the present study provided relatively effective identification of Patr MHC class II alleles in individual chimpanzee. Furthermore, it should facilitate colony management of chimpanzee groups in Japan and contribute to our understanding of the features of MHC molecules in non-human primates.