論　　文　　概　　要

論文題目　Genetic analysis to evaluate disease specific mutations in non-Hodgkin's lymphoma.

(非ホジキンリンパ腫における疾患特異的遺伝子変異の解析)

指導教員　Kato Mitsuyasu

人間総合科学研究科

Graduate School of Comprehensive Human Sciences

Doctoral Program in Biomedical Science

（所　　属）　筑波大学大学院人間総合科学研究科　専攻

(Graduate School of Comprehensive Human Sciences in University Doctoral Program in Biomedical Science)

（氏　　名）　TANZIMA NUHAT SHARNA

Research 1: Droplet digital PCR assay for RHOA mutation detection in angioimmunoblastic T-cell lymphoma

目　　的：Angioimmunoblastic T-cell lymphoma (AITL) is a subtype of nodal peripheral T-cell lymphoma (PTCL). Besides, a part of peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) which is referred to nodal PTCL with T_{FH} phenotype share similar genetic features of AITL. The hotspot mutation is the G17V *RHOA* mutations which was observed approximately 60% of the cases. Routine detection of the G17V *RHOA* mutation is troublesome as tumor contents are low in clinical specimen. In addition, quality of genomic DNAs derived from biopsy specimens are relatively poor. Therefore, my purpose was to establish a sensitive method to detect G17V *RHOA* mutation in clinical samples.

対象と方法：I analyzed 67 PTCL (40 AITL and 27 PTCL-NOS) patient samples by droplet digital PCR (ddPCR), and compared the results with PNA-LNA clamp method and next generation sequencing.

結　　果：The G17V *RHOA* mutation was identified in 28 of 67 (41.8%) PTCL samples. ddPCR assay and PNA-LNA clamp method both detected the mutations in 32 of 67 (47.8%) samples. Three other *RHOA* mutations (p.Gly17Val, p.Gly17Leu, and p.Gly17Glu) which were detected by NGS, could not be detected by ddPCR and PNA-LNA clamp method. Variant allele frequencies by ddPCR and those by NGS showed high concordance ($p<0.001$).

考　　察：The ddPCR and PNA-LNA clamp method were more sensitive for detection of low frequency mutations than NGS, although NGS was convenient for detection of exact mutational types.

結　　論：A combination of ddPCR or PNA-LNA clamp method and NGS was required for appropriate diagnosis of AITL.

Research 2: Analysis of control region of mitochondrial DNA in samples of diffuse large B-cell lymphoma.

目　　的：Diffuse large B-cell lymphoma (DLBCL, incidence 37%) is the most prevalent entities of mature B-cell neoplasms in adulthood. Tumor-related somatic mutations of DNA in cancer patients is being used thoroughly to acquire genetic information. Not only genomic DNA but also tumor derived mutant mitochondrial DNA (mtDNA) can serve as a predictive biomarker, because, mitochondrial D-loop region is known as a hotspot for mutations and control region (CR) controls mitochondrial transcription and replication process. The purpose of this study was to evaluate the mutation frequency in control region of mitochondrial DNA in DLBCL patients.

対象と方法：I collected tumor and paired bone marrow from DLBCL patients and detected somatic mutations by NGS.

結　　果：I found somatic mutations in 2 out of 14 (14%) tumor samples in control region (CR) of mtDNA. I also found 4 presumably heteroplasmic mutations, among these mutations m.16093T>C was known site of tissue specific heteroplasmy which was found in two patients.

考　　察：Disease-specific somatic mutations and recurrent heteroplasmic mutations were present in mtDNA of DLBCL patients.

結　　論：For studying the molecular mechanism of DLBCL, mtDNA can be considered as potential target to detect mutations other than genomic DNA.