Clinical Laboratory of the Detection of Genomic Aberrations in Formalin-fixed, Paraffin-Embedded Small Lymphocytic Lymphoma Specimens by aCGH

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Introduction
Chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are the same clonal B-cell disorder diagnostically distinguished based on disease distribution. Risk stratification for patients diagnosed with PLL/SLL is essential due to the highly variable clinical course of the disease. Current prognostication in CLL encompasses clinical features such as disease stage together with various molecular biomarkers including the presence of specific chromosomal aberrations. In SLL, implementation of routine detection of the same biomarkers in a clinical diagnostic setting is hampered by the solid tissue type which often requires working with thin sections taken from formalin-fixed, paraffin-embedded (FFPE) specimens with highly inconsistent sample qualities. Here, we established appropriate laboratory procedures for the clinical implementation of array comparative genomic hybridization (aCGH) using SLL FFPE samples.

Materials and Methods
FFPE specimen and DNA extraction
Six FFPE tonsl samples and ten FFPE SLL specimens were analyzed for the study. SLL samples contain greater than 90% tumor cells except for SLLS which contains approximately 70% based on HAE staining. Genomic DNA was extracted using a modified DNA extraction procedure based on the Agilent aCGH Sample Preparation kit protocol (L. Duan et al., in preparation). QC criteria: OD260/280 between 1.6 and 2.0 and OD260/230>1.9.

Targeted aCGH
Array processing procedures were performed essentially based on Agilent Oligonucleotide Array-Base CGH for Genomic DNA Analysis protocol except genomic DNA was first heat treated at 450 °C in to length and 1µg of each DNA sample was then labeled using Enzo CGH Labeling Kit for Oligo Arrays. An equal mixture of normal male/female DNAs from Promega was used as reference. For analysis, the log2-ratios for duplicate probes were first combined then the Aberration Detection Method 2 (ADM2) algorithm at thresholds of 0.6 with a filter against aberrations that contain fewer than 8 probes and a log2 ratio average less than ±0.4 was used to identify genomic aberrations.

Quantitative PCR (QPCR)
Tagman-based QPCR was performed with copy-number assay primers/probes selected from the Applied Biosystems Inc. (ABI) copy-number assay library. Samples with a relative ratio lower than 0.75 (deletion) or higher than 1.25 (amplification) when compared to the average of two reference genes were determined to contain authentic copy number change as calculated based on the <u>ΔCt</u> method. When there was potential copy number change in one of the two reference genes, then one reference gene was used and the experiment was repeated using different primer sets.

Fluorescence in situ hybridization (FISH)
The procedure for processing FFPE samples for FISH analysis was carried out essentially as described (H.C. Stirling Methods, Dako North America, 2009, pp. 67-73). The three color probe panel for Oligo was purchased from Abbott Laboratories while for TP53, home-brew, BAC-based probes were used. For analysis, the ratio of R/A or R/G signals per 100 cells were calculated for each FFPE sample using 400 cells (TP53) from different regions in each sample. Ratio decreases greater than 2 standard deviations from controls were taken as positive.

Genomic Aberrations Detected by aCGH
Using aCGH, we detected aberrations commonly found in CLL in 5/10 SLL FFPE samples. In addition, two large aberrations were observed. These were evaluated by repeat hybridization of all samples used in the study on different days with slightly different slides at twice. Same aberrations were observed for each sample after independent runs.

Confirmation by FISH
A selected set of genomic aberrations detected in the 10 SLL FFPE samples were subjected to independent confirmation by FISH. For the three cases studied, we observed concordance between aCGH data and FISH results.

Conclusion
Using DNA extracted from FFPE samples, aCGH was carried out to detect aberrations in SLL. In our genetic testing, the detected genomic aberrations are commonly found in CLL patients and they could be independently validated by QPCR and FISH. Commonly detected genomic aberrations in CLL/SLL have significant prognostic values and have been used together with other biomarkers such as TP53 mutation status to predict patient overall survival and time to first treatment length. Taken together, our data extended the usage of aCGH to the detection of genomic aberrations in SLL patient samples and suggested that aCGH can be used as a clinical diagnostic tool in SLL patient populations. We have implemented this new diagnostic technology in our laboratory.

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References