The optimization of copy number analysis of FFPE tumor DNA by array-CGH for implementation into a clinical diagnostic laboratory

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Introduction

Array comparative genomic hybridization (aCGH) has evolved as a valuable tool for the identification of genomic copy number aberrations (gain/loss) in tumors. These studies have mostly used fresh frozen specimens for genomic DNA (gDNA) extraction. For retrospective and diagnostic purposes, often formalin-fixed paraffin-embedded (FFPE) tissue is the only available sample or biopsy for study. Such specimens also permit identification of the tumor burden, which is an important issue considering the overall sensitivity of aCGH is 20-30%. However, gDNA extracted from FFPE specimens is of poorer quality and presents a challenge for aCGH using current protocols. The goal of this study was to optimize the procedure for the isolation and labeling of gDNA from FFPE specimens for aCGH and establish quality control parameters to implement it into a clinical diagnostic laboratory.

Materials and Methods

aCGH for Fresh Frozen Tissue Specimens

Genomic DNA was isolated from a 500 µm thick slice of fresh tissue (test). Only FFPE tumor DNA (Agilent, Santa Clara, CA). The quantity and quality were measured by spectrophotometry and gel electrophoresis. The composition and normal DNA (Promega) served as the reference DNA (RM). Test and reference (RM) DNAs were labeled with equimolar Cy5 and Cy3 dUTP, respectively, essentially as described in the Affymetrix protocol. fluorescently labeled DNAs were mixed and hybridized for 24 hrs to Affymetrix™ essentially as described by the manufacturer (Agilent Technologies). After washing, the slides were scanned using the Workstation (Agilent Technologies). The quality of aCGH was assessed using the ADM2 algorithm (threshold 4, at least 8 contiguous pixels with an average log value of 4.1 after combining intra-array replicates).

aCGH for FFPE Specimens

In the case of the FFPE specimens, gDNA was isolated from either five 10 or 20 micron sections of one binocular (five normal or five dysplastic DNA sample sets) prior to hybridization. The quality and quantity were measured by spectrophotometry. DNA not meeting quality criteria were re-isolated using Amicon Ultra-0.5, Ultrafree-0.5 Membranes (Millipore, Billerica MA) and the quality checked prior to further procedures. The integrity of the DNA was assessed by agarose gel electrophoresis and gDNA (Rm) was size fractionated for the time required for the bulk of the DNA to range from 4-kbp to 300-bp in size. Reference (Rm) DNA was also heat fractionated accordingly, and both test and reference DNA fragments were then labeled either using the Cy3 and Cy5 Labeling Kit for Agilent Arrays (Eppendorf Life Sciences) or non-enzymatically using the Genomic DNA Labeling Kit (Agilent Technologies). Arrays were hybridized for 48 hrs to Affymetrix™ essentially as described by the manufacturer (Agilent Technologies) and analyzed as described for fresh frozen tissue samples.

Non-Enzymatic VS. Enzymatic Labeling of FFPE Specimen DNAs

Six pairs of matched FFPE and fresh frozen tumor tissue specimens (four lymphoma tumors [FL], one diffuse large B-cell lymphoma [DLBCL], four multiple myelomas (MM)) were used to assess differences between enzymatic and non-enzymatic labeling of FFPE samples. Enzymatically labeled DNAs were mixed and hybridized for 24 hrs to Affymetrix™ essentially as described by the manufacturer (Agilent Technologies). After washing, the slides were scanned using the Workstation (Agilent Technologies). The quality of aCGH was assessed using the ADM2 algorithm (threshold 4, at least 8 contiguous pixels with an average log value of 4.1 after combining intra-array replicates).

DNA Quality and Quantity Parameters

For both of the four Hodgkin’s lymphomas no aberrations were detected in fresh frozen tissue or the matched FFPE specimen at the same settings. This is not surprising given the 5-10% tumor burden for these specimens. For one of the remaining two Hodgkin’s lymphomas some aberrations were detected in the fresh frozen tissue sample but not the matched FFPE specimen again, not surprising given the low tumor burden of the FFPE specimen (10%). For the last case, aberrations were detected in the FFPE specimen (70% tumor cell content) but not the fresh frozen tissue. Unfortunately, the tumor burden of the frozen tissue specimen was not known.

For the FL specimen where the tumor burden was known to be 40-60% and the tCGH specimen with 100% tumor burden were present. No aberrations were detected in the FFPE specimen but not in the matched tissue when arrays were analyzed at the same settings. Again, these results are most likely not surprising given the tumor burden, or a direct comparison of results could not be made.

Conclusions

With tumor enrichment, the overall log ratio of aberrations was higher and many aberrations previously reported to occur in these tumors were frequently identified in the current study group.

Clinical Implementation

Clinical implementation of MabTAI™ aCGH for small lymphocytic lymphomas (SLL), considered the same disease as CLL but presenting as a more serious condition. Genomic data have been established for extracted DNA (wax below) and for hybridization quality and aberration detection. Aberrations are confirmed by QPCR. FFPE sections of SLL with greater than 70% tumor burden are requested. Analytical sensitivity and specificity, reproducibility, and accuracy were assessed.

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