

Optimising Technical Protocols to Detect AID Expression in Archived FFPE Samples of Patients with Follicular Lymphoma

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The B-cell mutator, activation induced cytidine deaminase (AID), induces genomic alterations required for immunoglobulin SHM and CSR in normal B cells. The mutagenic effects of AID may also contribute to genomic instability in B-cell malignancies. Measuring AID in B-cell malignancies is hampered by technical difficulties in obtaining good quantity and quality of RNA and protein in FFPE tissues. The aim of our study was to optimize test conditions for the detection of AID mRNA and protein levels in FFPE lymph nodes from 87 patients with FL.

By comparing different RNA extraction kits, we found that the Qiagen RNeasy FFPE kit produced the highest yield of RNA using the smallest amount of FFPE tissue. RNAs prepared from 60 of the 87 FFPE samples that had RNA integrity number (RIN) ≥ 2.1 , were successfully used to quantify AID expression in RT-qPCR with primers targeting a 90-bp sequence.

We used IHC and a standard scoring system to quantify AID protein expression in FFPE tissue sections from all of the 87 patients. Staining was scored by two independent observers. A significantly positive correlation between the mRNA and protein expression was observed, ($r=0.36$, $p=0.005$; Pearson correlation).

To investigate AID subcellular localization, we found that the Hoechst stain with IF produced the best images with confocal microscopy. We used Image J software, and improved the program's ability to make a clear nuclear-cytoplasmic distinction and automatically measure subcellular AID in a large number of cells.

We demonstrated a significantly higher proportion of nuclear AID in 20 patients with high total AID protein by IHC ($r=0.54$, $p=0.013$; Pearson correlation).

In summary, we have successfully optimized methods for quantification of AID mRNA, as well as total and nuclear proportion of AID protein in archived FFPE samples from patients with FL. These protocols are applicable to measure expression of other genes from FFPE tissues in any disease.