A multi-parameter assay for the detection of rare malignant cells in classic Hodgkin lymphoma

Lance O’Reni,1, David WJ,2 Jackie Stilwell,1 Jonathan R. Fromm,1,2 Eric Kaldjian1,3
1RareCyte, Inc., Seattle, WA, 2University of Washington, Seattle, WA, 3Co-senior authors

Background
Classic Hodgkin Lymphoma (CHL) ranks among the most frequently occurring lymphomas and is marked by Hodgkin and Reed-Sternberg (HRS) cells, frequently multicentric neoplastic cells that are derived from germinal center B cells. The genetic alterations involved in the pathogenesis of this lymphoma have been difficult to study because HRS cells are rare and are surrounded by robust, non-neoplastic infiltrate, therefore they frequently represent less than 0.1% of cells within involved lymph node. Consequently, methods that isolate these rare cells are required to understand pathobiology of this lymphoma. We used the CyteFinder® system (RareCyte) to develop a method for detection and isolation of HRS cells.

Methods
A 6-parameter immunofluorescence assay was designed to identify HRS cells that included HRS markers (CD40, CD20, CD15), CD3, CD14, and CD8, together with CD20, CD15, and CD8, and a cell membrane permeabil stabilizer dye. The assay was tested by staining ~200K BMO Hodgkin lymphoma cell clusters with 5 x 10^5 PMACs and transfected to well slides for imaging by CyteFinder. This instrument utilizes 6-color scanning microscopy coupled with predefined algorithms to identify rare-cell populations. The assay was then applied to frozen fine needle aspirate (FNA) samples taken from the lymph node of six patients with histologically confirmed CHL. The FNA samples were thawed, prepared into a suspension, stained and analyzed as above. Cells identified as HRS phenotype were individually retrieved using the integrated CyteFinder module and the isolated single cell DNA subjected to whole genome amplification (WGA). PCR of framework regions of the immunoglobulin heavy chain gene (IGH) was performed on single HRS cells (in situ) to assess clonality. Samples of four reactive lymph nodes from patients without CHL were processed as above and used as negative controls.

Results
Of ~200K HRS 2 cells spiked into 5 x 10^5, the assay identified 180 (90%) of the correct HRS phenotype. HRS phenotype cells were identified in CHL patient lymph node FNA samples but from reactive lymph node samples patients without CHL. In 3 of the 4 CHL patients studied we were able to demonstrate that HRS cells picked from individual patients were clonal as determined by PCR product sizes against the lightweight framework.

Hodgkin’s disease model
KMO2 cells spiked into blood-99% recovery using 6-parameter analysis

Laboratory workflow

Conclusions
A multi-parameter assay was successfully developed for identifying rare HRS cells with the CyteFinder system. We were able to find cells with HRS phenotype from patients with confirmed CHL, but not from reactive lymph nodes of patients without CHL. Utilizing the CyteFinder module we retrieved individual HRS cells for downstream molecular analysis. Cells with HRS phenotype in a lymph node FNA sample were demonstrated to have clonal light rearrangements, confirming the identity of these putative HRS cells. We also demonstrate that this method can be utilized to determine the expression level of FOL1 on HRS cells.

Significance
The identification of rare HRS cells from FNA specimens may be a useful tool for diagnosis and the investigation of the molecular genetics of CHL. Identifying and retrieving HRS cells from FNA samples is a relatively non-invasive approach to follow CHL patients over time without obtaining repeated biopsies. The ability to follow FOL1 expression or the expression of other checkpoint molecules, over time could be a valuable tool to help select therapies for CHL patients. However, further work and correlative studies need to be performed.

Acknowledgments
We thank the staff of the UW Molecular Hematology Laboratory for help with performing the kH1SGK clonality analysis and Breeman Enright for carrying out the WGA reactions.