Lack of activation-induced cytidine deaminase expression in *in situ* follicular neoplasia

Tanu Goyal, Sarah L. Ondrejka , Juraj Bodo, Lisa Durkin and Eric D. Hsi
Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute, Cleveland, OH, USA
Correspondence: ERIC D. HSI - hsie@ccf.org
doi:10.3324/haematol.2020.249342

Supplemental Data

Supplemental Methods:

Immunohistochemistry:

The antibody panel used to assess ISFN cases was as follows: CD3 (2GV6, Ventana), CD20 (L26, Dako), BCL6 (PG-B6p, Dako), CD10 (56C6, Leica), BCL2 (124, Cell Marque, Rocklin, CA), AID (ZA001, ThermoFisher, Waltham, MA). For all markers except AID, formalin-fixed, paraffin-embedded (FFPE) tissue sections were stained using an automated IHC instrument (Benchmark Ultra, Ventana Medical Systems, Tucson, AZ). For AID, the tissue sections were subjected to heat-induced epitope retrieval (pH 9.0, 20 minutes) and incubated with anti-AID mouse monoclonal antibody (ZA001, 1:200) for 15 minutes at room temperature. Visualization was achieved with Bond Polymer Refine detection system in Bond Max instrument (Leica Microsystems, Buffalo Grove, IL).

Immunofluorescent AID/BCL-2 double staining:

Deparaffinization and heat-induced epitope retrieval of FFPE samples was performed using the Bond Max instrument (Leica Biosystems, Buffalo Grove, IL). Avidin and biotin was blocked with block A and block B reagents (ThermoFisher, Waltham, MA), respectively. Before adding antibody against AID (1:100) for 30 minutes, samples were incubated in 10% normal goat serum (Vector; Burlingame, CA), 5% BSA/PBS (Sigma-Aldrich; St. Louis, MO). AID was visualized with biotinylated anti-mouse antibody (Vector) followed by streptavidin conjugated QD605 (ThermoFisher). Subsequent staining with BCL-2 (clone: EP36, 1:200, CellMarque, Rocklin, CA) was preceded by avidin and biotin blocking. BCL-2 was visualized with biotinylated anti-rabbit antibody followed by streptavidin conjugated QD655 (ThermoFisher). Hoechst 33342 (ThermoFisher) was used as a nuclear counterstain.

Quantitation of Double AID/BCL2 staining double staining:

Sections labeled with AID – QD 605 and BCL2 – QD 655 were viewed with emission filters of 605/20 and 655/20 nm. Image files were analyzed using the Image-Pro Plus software (Media Cybernetics; Silver Spring, MD). First, the image of Hoechst 42333 counterstain, together with images of QD staining, was loaded into the composite preview. Using the "count/size" function and "Watershed" split, individual cells were segmented based on Hoechst 42333 counterstain. Subsequently, the composite preview was switched to the AID – QD 605 or the BCL2 – QD 655 image, and the data were analyzed to determine the percentage of double positive cells. Samples with ≥10% AID/BCL2 co-expressing cells within follicles were considered as AID positive regardless of the nuclear or cytoplasmic localization of the molecule.

RNAscope

For mRNA AID detection, FFPE tissue sections were stained using an automated IHC instrument (Discovery Ultra, Ventana Medical Systems, Tucson, AZ). Briefly, tissue sections were subjected to heat-induced epitope retrieval, pH 9.0 for 24 minutes at 97°C, followed by 16 minutes protease treatment at 37°C and 2 hour hybridization with Hs-AICDA probe (Advanced Cell Diagnostics, Newark, CA) at 43°C. Visualization was achieved with mRNA DAB Detection Kit (Roche, Basel, Switzerland). For readers not familiar with RNAscope assays, a positive signal is a single dot or multiple dots within a cell. Per manufacturer, positivity for AID in a sample is defined as at least 1 dot in every ten cells visible at 20-40X magnification. For our purposes of illustrating colocalization of AID with BCL2, we considered individual cells with at least one dot as positive for AID.

Statistics:

Results were compared using Fisher Exact test performed at a significance level of $P<0.05$, using statistical analysis software (StatSoft, Inc., Tulsa, OK)

Supplementary table1: Clinicopathological features of patients with in situ follicular neoplasia.

n	Site	Indication for LN removal	M/F	Age (years)	Previous lymphoma	Concurrent lymphoma	New lymphoma on follow up	Other malignancy	Other benign change in same specimen
1	Left tonsil	n/a	M	80	n/a	DLBCL, GC in same lymph node	n/a	Squamous cell ca (conjunctiva)	None
2	Right inguinal LN	LAD concerning for lymphoma recurrence	F	83	FL-G2 (diagnosed 20 years before and then relapsed 8 years before ISFN)	-	-	Breast Ca, Basal cell Ca	None
3	R4 LN	Mediastinal LAD	M	61	-	-	-	Smoldering multiple myeloma (IgA kappa)	EBV+ mild polytypic plasmacytosis
4	Right breast	Axillary LAD	F	58	-	-	-	Breast Ca	Folllicular / paracortical hyperplasia
5	Mediastinal and periortic LN	LAD, lung nodules and thrombocytopenia, lymphopenia	F	65	-	-	-	-	Non necrotizing granulomas /sarcoidosis
6	Level 3, 4 LN	Fever of unknown origin, thoracic and abdominal mass and LAD	M	60	-	MGUS/concurrent B cell lymphoma, not biopsy proven	-	-	None
7	Left nasopaharynx	Nasopharyngeal mass	F	76	-	FL-G1 of another site	-	-	None

8	Left inguinal LN	n/a	M	67	-	-	-	BCC and melanoma of skin	Dermatopathic changes and sinus histiocytosis
9	Periduodenal LN	1.4cm enhancing mass involving the proximal small bowel	F	50	-	-	-	-	None
10	Neck LN	Neck mass	F	72	-	-	EBV + DLBCL, *IGH-BCL2* negative (7 years after ISFN)	Breast Ca	None
11	Level 4 LN	Fever of unknown origin, splenomegaly and neck LAD	M	66	-	DLBCL with underlying NLPHL in same LN		-	None
12	Spleen (290 g)	Splenic mass	F	80	FL-G1 (4.5 years before ISFN)	-	-	Papillary serous borderline ovarian cancer	Sclerosing angiomatoid nodular transformation of the spleen
13	Left inguinal LN	n/a	F	53	n/a	n/a	n/a	n/a	None
14	Sigmoid colon polyp	Colon polyps	F	59	-	-	-	-	None
15	Spleen (425 g)	Pancytopenia and splenomegaly	M	66	-	-	-	CMML	None
16	Spleen	n/a	F	73	n/a	n/a	n/a	n/a	Follicular hyperplasia, focal EMH

Legends: LN- Lymph node, M- Male, F- Female, n/a- Not available, LAD-Lymphadenopathy, FL-Follicular lymphoma, DLBCL, GC- Diffuse large B cell lymphoma of germinal center phenotype, MGUS- Monoclonal gammopathy of unknown significance, NLPHL- Nodular lymphocytic predominant Hodgkin lymphoma, BCL- B cell lymphoma, BCC- Basal cell carcinoma, CMML- Chronic myelomonocytic leukemia, EMH- Extramedullary hematopoiesis