

Development of an RNA *In Situ* Hybridization Method for the Detection of CXCL13 mRNA in AITL and Other Lymphomas

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Background

Angioimmunoblastic T-cell lymphoma (AITL) is a subtype of peripheral T cell lymphoma (PTCL) within the subcategory of nodal T cell lymphomas with T follicular helper (TFH) phenotype. The differential diagnosis of AITL is complex owing to its polymorphous composition and includes benign entities as well as malignancies. CXCL13 is a chemokine that controls the organization of B cells within follicles; when expressed in T lymphocytes, the biomarker reflects a germinal center origin of the T cell (TFH phenotype). CXCL13 is one of the most useful markers in the diagnosis of AITL and, when used as part of a panel, can help differentiate AITL from other lymphomas and reactive lymphoid conditions. However, IHC antibodies for CXCL13 are not widely available, leading to challenges in the use of this biomarker for routine diagnosis. In the current study, we have developed a highly sensitive and specific RNA *in situ* hybridization (RISH) approach using the RNAscope technology to examine CXCL13 mRNA expression in a panel of B and T cells lymphomas, including several confirmed cases of AITL.

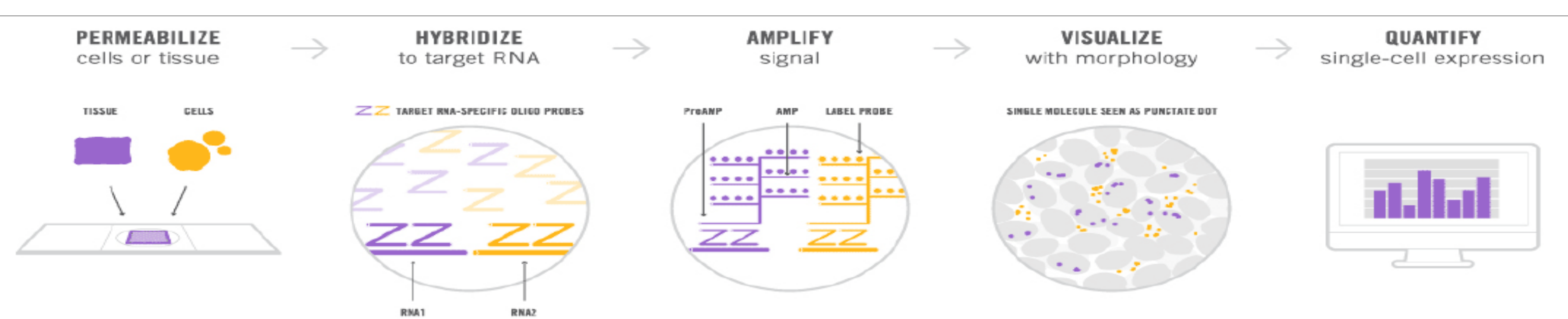
Design

Samples: A total of 43 FFPE lymphoma samples were studied, including 16 T cell lymphomas and 27 non-T cell lymphomas, as summarized in Table 1 (annotated, deidentified tissues provided courtesy of Beijing Shijitan Hospital and reviewed for accuracy of diagnosis by an independent pathologist at ACD).

RNAscope[®] ISH assay for RNA detection: For each case, separate serial sections were stained for *PPIB* (positive RNA control), *DapB* (negative control) and *CXCL13* expression by using the RNAscope 2.5 HD Manual Brown assay (Figure 1).

Imaging and scores: Images were acquired using a Leica Biosystems Aperio AT2 Digital Pathology Slide Scanner. Semi-quantitative scoring was performed according to ACD's recommended RNAscope scoring guidelines (0, no staining or <1 dot for every 10 cells; 1, 1-3 dots/cell; 2, 4-10 dots/cell, very few dot clusters; 3, >10 dots/cell, less than 10% positive cells have dot clusters; 4, >10 dots/cell, more than 10% positive cells have dot clusters).

Figure 1. RNAscope ISH assay overview



Results

43/43 FFPE samples passed RISH quality control (*PPIB* score 2 or higher, *DapB* score 0). While some degree of *CXCL13* mRNA staining was observed in all lymphoma cases, reflective of the presence of scattered T_{FH} cells, 7 AITL cases exhibited strong, diffuse expression of *CXCL13* in large numbers of cells across the tissue. 9 non-AITL T cell lymphomas and 27 B cell lymphomas showed only scattered *CXCL13*⁺ cells in significantly reduced numbers relative to the AITL cases (Table 1 and Figure 2).

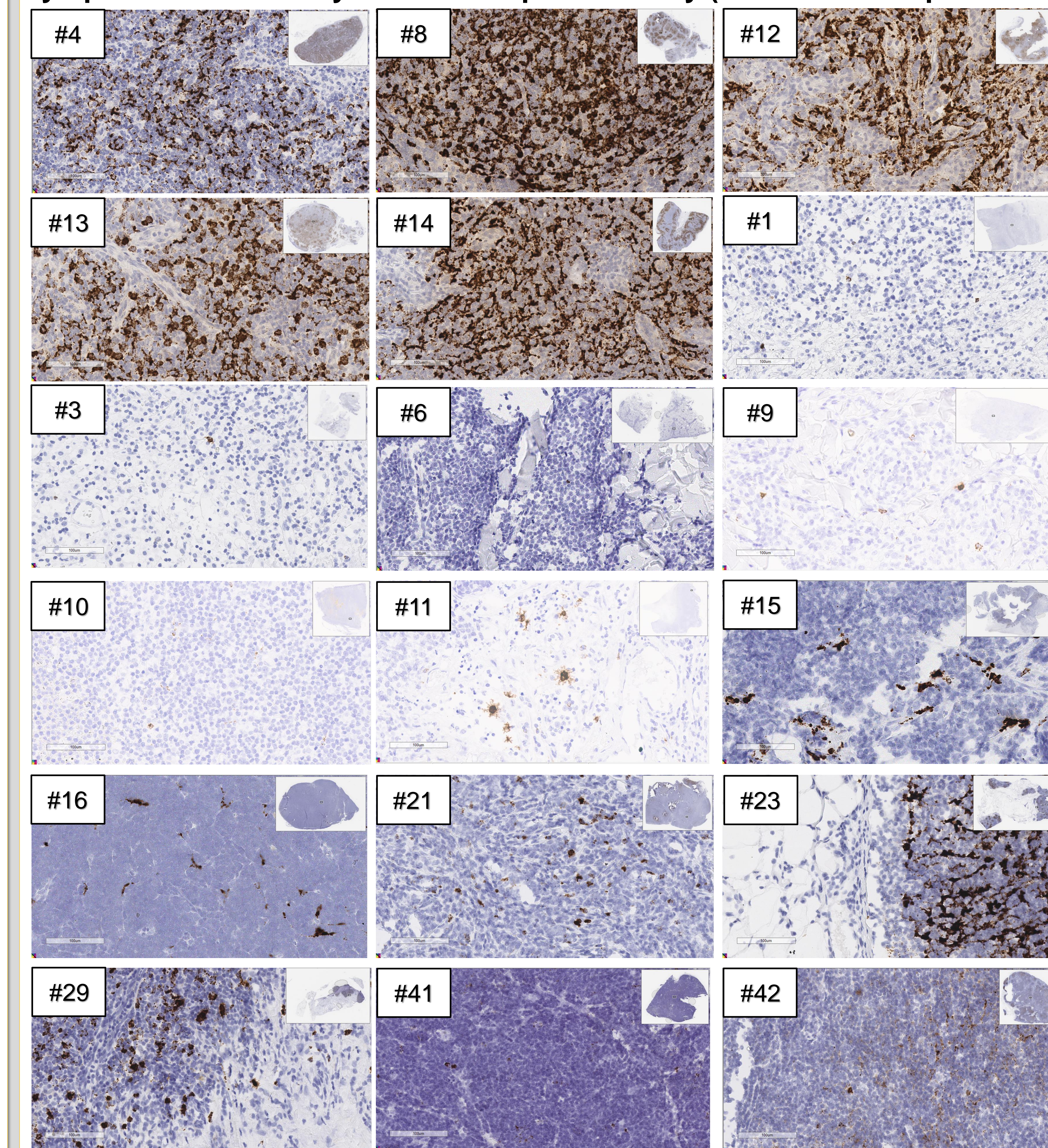
Results

Table 1. *CXCL13* RNA ISH in 43 lymphomas

Sample Number	CXCL13 staining pattern	Pathology diagnosis	Anatomical Site	Age	Gender (M/F)
1	scattered	Extranodal NK/T cell lymphoma (nasal type)	Right hemicolon	56	Male
2	widespread	Angioimmunoblastic T cell lymphoma	Neck lymph node	54	Female
3	scattered	NK/T cell lymphoma	Nasal cavity tumor (Left)	66	Male
4	widespread	Angioimmunoblastic T cell lymphoma	Left supraclavicular lymph node	51	Female
5	scattered	NK/T cell lymphoma	Right inferior turbinate tumor	29	Male
6	scattered	Extranodal nasal type NK/T cell lymphoma	Back tumor	39	Female
7	widespread	Angioimmunoblastic T cell lymphoma	Left inguinal lymph node	68	Male
8	widespread	Angioimmunoblastic T cell lymphoma	Neck lymph node	76	Female
9	scattered	Subcutaneous panniculitis like T cell lymphoma	Chest wall tumor	53	Male
10	scattered	Peripheral T cell lymphoma	Left inguinal tumor	63	Female
11	scattered	Enteropathy associated T cell lymphoma	Partial small intestine	75	Male
12	widespread	Angioimmunoblastic T cell lymphoma	Left supraclavicular lymph node	65	Female
13	widespread	Angioimmunoblastic T cell lymphoma	Right submandibular tumor	84	Female
14	widespread	Angioimmunoblastic T cell lymphoma	Left inguinal lymph node	72	Male
15	scattered	Enteropathy associated T cell lymphoma	Small intestine	75	Male
16	scattered	T cell lymphoblastic lymphoma	Left neck	28	Female
17	scattered	Follicular lymphoma	Right subaxillary and anterolateral chest	51	Male
18	scattered	Classical Hodgkin's lymphoma mixed cellularity, MCH	Left neck	32	Male
19	scattered	Diffuse large B-cell lymphoma	Right submandibular	24	Male
20	scattered	B-cell non Hodgkin's lymphoma	Back	66	Male
21	scattered	Diffuse large B-cell lymphoma (non-GCB)	Mesenteric root	72	Female
22	scattered	Non Hodgkin's B-cell lymphoma, lymphoplasmacytic lymphoma	Small intestine	66	Male
23	scattered	Castleman Disease, Plasma cell type	Right axillary lymph nodes	40	Female
24	scattered	Diffuse large B-cell lymphoma (non-GCB)	Right tonsil	58	Male
25	scattered	Adenocarcinoma	Lymph node	52	Male
26	scattered	Non Hodgkin's lymphoma, Diffuse large B-cell lymphoma	Right hemicolon	67	Male
27	scattered	B-cell lymphoma	Thigh	80	Male
28	scattered	Chronic lymphocytic leukemia/ Small B Lymphoid Lymphoma	Lymph node	77	Female
29	scattered	Small B Lymphoid Lymphoma	Right submandibular gland	42	Male
30	scattered	Lymphoma	Right neck+Right tonsil	54	Female
31	scattered	Diffuse large B-cell lymphoma	Right submandibular	66	Female
32	scattered	Mantle cell lymphoma	Right cheek	83	Female
33	scattered	Non Hodgkin's lymphoma, high grade B-cell lymphoma	Right tonsil	69	Male
34	scattered	Non Hodgkin's lymphoma, high grade B-cell lymphoma	Right tonsil	69	Male
35	scattered	Diffuse large B-cell lymphoma (non-GCB)	Right hemicolon	77	Male
36	scattered	Follicular Lymphoma (3A)	Left armpit	64	Male
37	scattered	Submental lymph node metastatic squamous cell carcinoma	Submental lymph node	50	Male
38	scattered	Lymph node metastatic renal clear cell carcinoma	Cervical/neck lymph nodes	72	Male
39	scattered	Lymph node metastatic breast invasive ductal carcinoma, Histological grade Stage 2	Left axillary lymph node	53	Female
40	scattered	Borderline B-cell lymphoma	Right neck+Right jaw	71	Male
41	scattered	Mantle Cell Lymphoma	Right parotid gland+Left jaw	67	Male
42	scattered	Follicular lymphoma grade 3 with diffuse large B-cell lymphoma	Left inguinal lymph nodes	73	Female
43	scattered	Follicular Lymphoma (IIB type)	Left axillary	59	Female

Results

Figure 2. Representative images of *CXCL13* in AITL and non-AITL lymphoma detected by the RNAscope ISH assay (# refers to sample)



Conclusions

In the current study, we have developed a novel RISH method for the detection of *CXCL13* mRNA in FFPE tissues from a variety of B and T cell lymphomas. Although *CXCL13*⁺ cells were present in all lymphomas, only AITL exhibited a strong, diffuse, widespread mRNA staining pattern, reflective of the T_{FH} phenotype of AITL. Overall, our results demonstrate that RNAscope RISH is a reliable and straightforward methodology for the assessment of *CXCL13* expression in lymphomas. RNAscope for *CXCL13* provides a sensitive and specific alternative to IHC for the assessment and diagnosis of AITL.