

Characterization of c-Maf Transcription Factor in Normal and Neoplastic Hematolymphoid Tissue and Its Relevance in Plasma Cell Neoplasia

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Key Words: c-Maf; Plasma cell; Tissue microarray; Myeloma; Immunohistochemistry

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Upon completion of this activity you will be able to:

- understand the steps involved in characterization of protein expression using tissue microarrays and immunohistochemistry.
- describe the tissue expression pattern of c-Maf protein in normal hematopoietic tissues and hematopoietic neoplasms.
- discuss the relevance of c-Maf translocation and c-Maf protein expression in plasma cell neoplasms.

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Abstract

c-Maf, a leucine zipper-containing transcription factor, is involved in the t(14;16)(q32;q23) translocation found in 5% of myelomas. A causal role for c-Maf in myeloma pathogenesis has been proposed, but data on c-Maf protein expression are lacking. We therefore studied the expression of c-Maf protein by immunohistochemical analysis in myelomas and in a wide variety of hematopoietic tissue. c-Maf protein was detected in a small minority (4.3%) of myelomas, including a t(14;16)(q32;q22-23)/IgH-Maf+ case, suggesting that c-Maf protein is not expressed in the absence of c-Maf rearrangement. In contrast, c-Maf was strongly expressed in hairy cell leukemia (4/4) and in a significant proportion of T-cell (24/42 [57%]) and NK/T-cell (49/97 [51%]) lymphomas, which is in keeping with prior gene expression profiling and transgenic mouse studies. Up-regulation of c-Maf protein occurs in a small subset of myelomas, in hairy cell leukemia, and in T- and NK-cell neoplasms. Its detection may be of particular value in the differential diagnosis of small cell lymphomas.

Plasma cell myeloma (multiple myeloma) is an incurable neoplasm characterized by the accumulation of malignant plasma cells within the bone marrow.¹ Of the myelomas, 80% exhibit chromosomal translocations involving the immunoglobulin heavy chain (*IgH*) locus that cause deregulation of the following oncogenes: 4p16 (*MMSET* and *FGFR3*), 6p21 (*CCND3*), 11q13 (*CCND1*), 20q11 (*MafB*), and 16q23 (*c-Maf*).^{2,3} These translocations are also found in the pre-malignant condition known as monoclonal gammopathy of undetermined significance,⁴ suggesting their central role in the development of plasma cell neoplasia.

Translocations involving the *c-Maf* gene occur relatively rarely (5%) in myelomas.^{3,5} In addition, Hurt and colleagues⁶ reported that RNA encoding *c-Maf* was expressed in 50% (13/26) of myelomas and also in myeloma cell lines lacking the t(14;16) translocation. They suggested that expression of this transcription factor may have a direct causal role in myeloma by enhancing integrin $\beta 7$ adhesion to bone marrow stroma, vascular endothelial growth factor production, and cell cycle progression. The clinical implication is that *c-Maf* could be a target for therapeutic intervention. Despite many recent studies of *c-Maf* translocations and RNA expression that are potentially of major interest in terms of understanding the pathogenesis of plasma cell neoplasia, the distribution of the *c-Maf* protein has not been documented in human tissues.

In this study, we investigated by immunohistologic analysis the expression of *c-Maf* protein in myelomas and in normal tissues and B, T, NK, and Hodgkin lymphomas. We also compared *c-Maf* protein expression with that of other

plasma cell markers, CD138 (syndecan),⁷ IRF4 (MUM1),⁸ B lymphocyte-induced maturation protein-1 (BLIMP-1),^{9,10} ICSBP (IRF8),¹¹ and the transmembrane plasma cell-associated adaptor molecules, SIT and LIME.

Materials and Methods

Tissue Samples and Tissue Microarrays

Paraffin-embedded sections of normal lymphoid tissues (ie, lymph node, tonsil, spleen, and thymus); a total of 552 hematolymphoid tumors from the archives of the Departments of Pathology, Stanford University School of Medicine, Stanford, CA, and John Radcliffe Hospital, Oxford, England; and a bone marrow smear from a case of t(14;16)-positive myeloma from the files of the Institute of Human Genetics, Kiel, Germany, were used for immunocytochemical analysis of c-Maf protein. The hematolymphoid neoplasms were classified according to the World Health Organization classification scheme.¹ Tissue microarrays (TMAs) were prepared as previously described.¹² Approvals from the Stanford Institutional Review Board and the Oxford Radcliffe Clinical Research Ethics Committee were obtained for these studies.

Cell Lines

Cell lines derived from diffuse large B-cell lymphoma (SUDHL4, SUDHL6, SUDHL10, and OCI-LY3, courtesy of R.E. Davis, MD, National Cancer Institute, Bethesda, MD), Burkitt lymphoma (DAUDI and RAJI, obtained from the Sir William Dunn School of Pathology, University of Oxford, Oxford, England), myeloma (NCI-H929, RPMI 8226, and JLN3, courtesy of Claire Shipman, MD, Nuffield Department of Orthopaedic Surgery, and Thiel, courtesy of Karen Pulford, PhD, Nuffield Department of Clinical Laboratory Sciences, University of Oxford), T-cell lymphoma (CEM/CCRF and HUT78, courtesy of E. Macintyre, MD, PhD, Hôpital Necker-Enfants Malades, Paris, France, and Jurkat, obtained from the Sir William Dunn School of Pathology), classical Hodgkin lymphoma (L1236, L428, and KMH2, obtained from the DSMZ cell collection, Braunschweig, Germany), and myeloid leukemia (K562, obtained from the Sir William Dunn School of Pathology) were cultured and used for cytocentrifuged preparations and cell pellets as described elsewhere.¹³

Immunoblotting (Western)

Protein extracts from the RPMI 8226, NCI-H929, Thiel, SUDHL10, L1236, and K562 cell lines (cultured in RPMI 1640, supplemented with 10% fetal calf serum) were subjected to Western blotting with the anti-c-Maf antibody following

a conventional protocol described elsewhere.¹⁴ β -Actin was used as a protein loading control.

Immunohistochemical Analysis

For immunohistochemical analysis, 2- μ m-thick paraffin-embedded tissue sections from conventional or TMA blocks cut onto poly-L-lysine-coated slides were deparaffinized in 2 changes of xylene and rehydrated in a series of graded alcohols. Pretreatment for heat-induced antigen retrieval consisted of heating the slides in tris(hydroxymethyl)aminomethane/EDTA buffer (50 mmol/L/2 mmol/L, pH 9.0) in a microwaveable pressure cooker at 700 W, timing 3 minutes when at pressure. Slides were cooled and transferred to deionized water, and sections were blocked for endogenous peroxidase in a 0.3% solution of hydrogen peroxide for 15 minutes. Following blocking, sections were incubated with the primary antibody for 30 minutes that had been diluted in 10% normal human serum to a concentration that gave background-free selective cellular labeling. All primary antibody reagents, dilutions used for immunohistochemical analysis, expected tissue reactivity, and sources are detailed in **Table 1**. Antibody binding was then detected with the peroxidase-based DAKO REAL EnVision kit (K5007, DAKO, Ely, England). Sections were counterstained with hematoxylin. Staining was optimized on normal paraffin-embedded tonsil sections. Double immunoenzymatic labeling of paraffin sections was performed by carrying out the protocol as for single immunoenzymatic staining above but eliminating the hematoxylin counterstain. c-Maf was always the first antibody to be detected.

In the second reaction, various antibodies were immunostained using the peroxidase-based DAKO REAL EnVision kit but replacing the substrate provided in the kit with the VECTOR SG Substrate Kit (Vector Laboratories, Peterborough, England) to give a contrasting blue-gray chromogen deposit. For both protocols, slides were washed in phosphate-buffered saline throughout all incubation stages except after the chromogen when deionized water was used.

Cytogenetic Analysis and Fluorescence In Situ Hybridization

Cytogenetic analysis of R-banded metaphases obtained from short-time cultures of bone marrow cells was performed as described recently.¹⁵ For the detection of aberrations affecting the *Maf* and *IgH* loci interphase, fluorescence in situ hybridization (FISH) was performed as previously described using the commercially available LSI IGH break-apart and LSI IgH/Maf double-color double-fusion assays (Abbott/Vysis, Downers Grove, IL) and a MAF break-apart probe prepared in house.¹⁵

Data Analysis

The stained TMA and whole section slides were scored using a standardized 4-tiered scoring scale as follows: staining

Table 1
Reagents, Conditions, and Sources of Antibodies Used for Immunohistochemical Analysis

Antibody	Clone	Dilution	Reactivity	Source
c-Maf	M-153	1:50	Reported in present article	Santa Cruz Biotechnology, Santa Cruz, CA
CD20	L26	1:1,000	Pan B cells	DAKO, Glostrup, Denmark
CD3	Rabbit polyclonal	1:100	Pan T cells	DAKO
CD138	M115	1:50	Plasma cells*	DAKO
MUM1/IRF4	Mum1p	1:200	B, T, and plasma cells ⁸	DAKO
CD68	KP1	1:1,000	Monocyte/macrophage	DAKO
ICSBP/IRF8	Rabbit polyclonal	1:50	B cells [†]	Santa Cruz Biotechnology
VS38c	P63	1:1	Plasma cells	DAKO
CD38	SPC32	1:50	Plasma cells, activated B, T, and myelomonocytic cells	Novocastra, Newcastle upon Tyne, England
BLIMP-1/PRDM1	ROS	1:1	Plasma cells, B- and T- cell lymphoma ¹⁰	G. Roncador, MD, Spanish National Cancer Centre, Madrid, Spain
SIT	SIT-02	1:20	T and plasma cells ¹¹	V. Horejsi, PhD, University of Prague, Prague, Czech Republic
LIME	LIME-10	1:20	T and plasma cells ¹¹	V. Horejsi, PhD
SAP	Rabbit polyclonal	1:20	Germinal center T cells [‡]	Santa Cruz Biotechnology

* Sebestyén A, Berczi L, Mihalik R, et al. Syndecan-1 (CD138) expression in human non-Hodgkin lymphomas. *Br J Haematol.* 1999;104:412-419.

† Martínez A, Pittaluga S, Rudelius M, et al. Expression of the interferon regulatory factor 8/ICSBP-1 in human reactive lymphoid tissues and B-cell lymphomas: a novel germinal center marker. *Am J Surg Pathol.* 2008;32:1190-1200.

‡ Roncador G, Garcia Verdes-Montenegro JF, Tedoldi S, et al. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. *Haematologica.* 2007;92:1059-1066.

in more than 30% of cells was scored positive and given the numeric score 3; staining in more than 5% but fewer than 30% of cells was given the numeric score 2; lack of staining or staining in fewer than 5% of cells was given the numeric score 0. Stains that were not interpretable owing to absence of diagnostic tissue in the core or to loss of the core during processing (no data) were given the numeric score 1. The cutoff for staining was based on the need for using a nonambiguous threshold for scoring TMAs and does not reflect differences in staining intensity between normal and neoplastic tissue or among different diagnoses. The cutoff was chosen before correlation with other immunohistologic markers. The cases were scored independently by 2 hematopathologists (Y.N. and T.M.), and discrepant cases were resolved by joint review on a double-headed microscope.

The “Deconvoluter” algorithm (custom WBS macro, Excel, Microsoft, Redmond, WA) with appropriate layout for use in the Cluster software was used for hierarchical clustering to integrate all immunohistologic staining results as previously described (<http://genome-www.stanford.edu/TMA/>).¹⁶ Positive staining is represented as red, lack of staining as green, and noninterpretable staining as white in the corresponding Treeview images.

Images of immunohistologic staining were acquired using a Nikon Eclipse E400 microscope (Nikon, Tokyo, Japan) and a Nikon digital camera (DS-L1, Nikon), using a 10×/0.30 Plan Fluor or a 20×/0.50, 40×/0.75, or 60×/0.85 Plan Fluor objective lens (Nikon). Adobe Photoshop 7 image processing and manipulation software (Adobe, San Jose, CA) was used.

Results

Expression of c-Maf Protein in Normal Hematolymphoid Tissue

c-Maf protein was expressed in the nuclei of many cells in germinal centers and in the interfollicular area in normal tonsils; however, plasma cells, particularly evident in the proximity of tonsillar epithelium, were largely negative with rare cells with plasmacytoid morphologic features showing weak expression of c-Maf. This latter finding was further addressed by double immunohistologic labeling for c-Maf and plasma cell-associated markers, VS38c, CD38, and CD138. These studies showed that c-Maf+ cells did not coexpress other plasma cell markers tested in this study

Image 1. Staining was also seen in a minority of germinal center cells, often localized to the outer rim of the light zone, and was only very rarely present in mantle zone cells. Double labeling showed that approximately equal numbers of c-Maf+ germinal center cells coexpressed CD20 and CD3. c-Maf was also expressed in scattered cells within the interfollicular T zones, and double labeling showed that these were commonly CD3+ T cells.

In other lymphoid tissues, c-Maf staining was largely absent, being seen in the thymus frequently in histiocytes and in scattered lymphoid cells surrounding Hassall corpuscles and at the edges of the cortical lobules. In the spleen, c-Maf staining was seen in rare cells in the white pulp and in scattered plasma cells, lymphocytes, and histiocytes within the red pulp. Similarly, in bone marrow sections, c-Maf staining was

restricted to occasional plasma cells (usually weak staining), lymphocytes, and histiocytes and was absent in myeloid and erythroid precursors and in megakaryocytes.

Expression of c-Maf Protein in Hematolymphoid Neoplasia

The results of immunohistologic staining of hematolymphoid neoplasms are summarized in **Table 2**, and specific examples are illustrated in **Image 2**. Among the 164 plasma cell neoplasms tested, c-Maf staining was present in 6 of 140 myelomas and 4 of 15 plasma cell leukemias, but in none of the 9 cases of monoclonal gammopathy

of undetermined significance. All positive myelomas and plasma cell leukemias showed strong nuclear reactivity of comparable intensity. The difference between c-Maf staining in myelomas and plasma cell leukemias was statistically significant ($P = .0086$; Fisher exact test).

When other B-cell lymphomas were studied, a striking finding was that intense nuclear staining was seen in all 4 cases of hairy cell leukemias studied (Image 2). In contrast, c-Maf immunoreactivity among other B-cell lymphomas was usually present in only a minority of cases (Table 2), including 6 of 97 follicular lymphomas of all 3 grades, 3 of

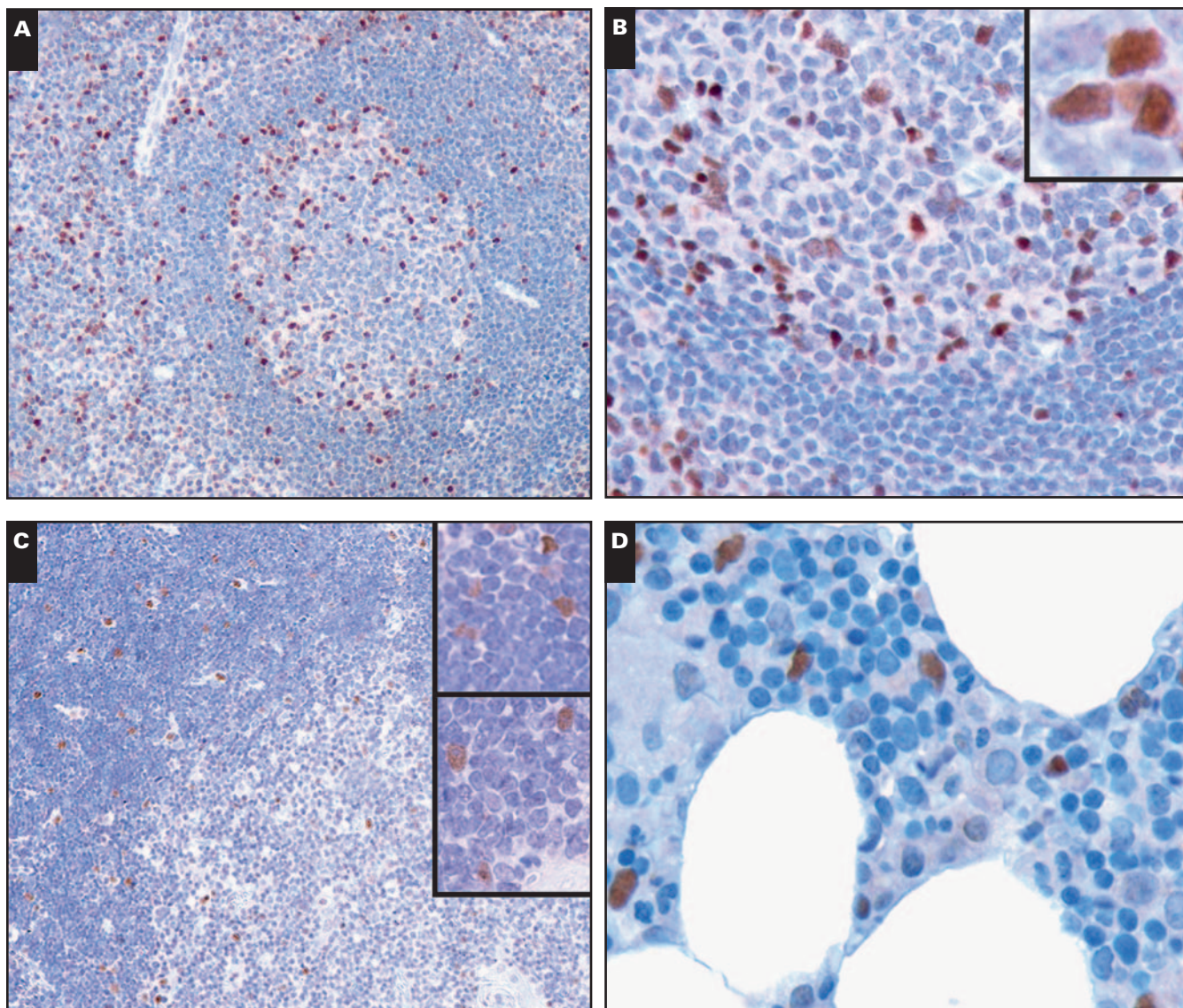


Image 1 Immunohistochemical staining for c-Maf in normal hematopoietic tissue. **A** and **B**, Low- and high-magnification images of normal tonsil sections with c-Maf-specific staining within the nuclei of many cells in germinal centers and interfollicular areas; however, plasma cells were negative (**A**, $\times 10$; **B**, $\times 40$). **C** and **D**, In the thymus (**C**, $\times 10$) and bone marrow (**D**, $\times 60$), c-Maf staining was largely absent and was restricted to occasional histiocytes, plasma cells, and lymphocytes.

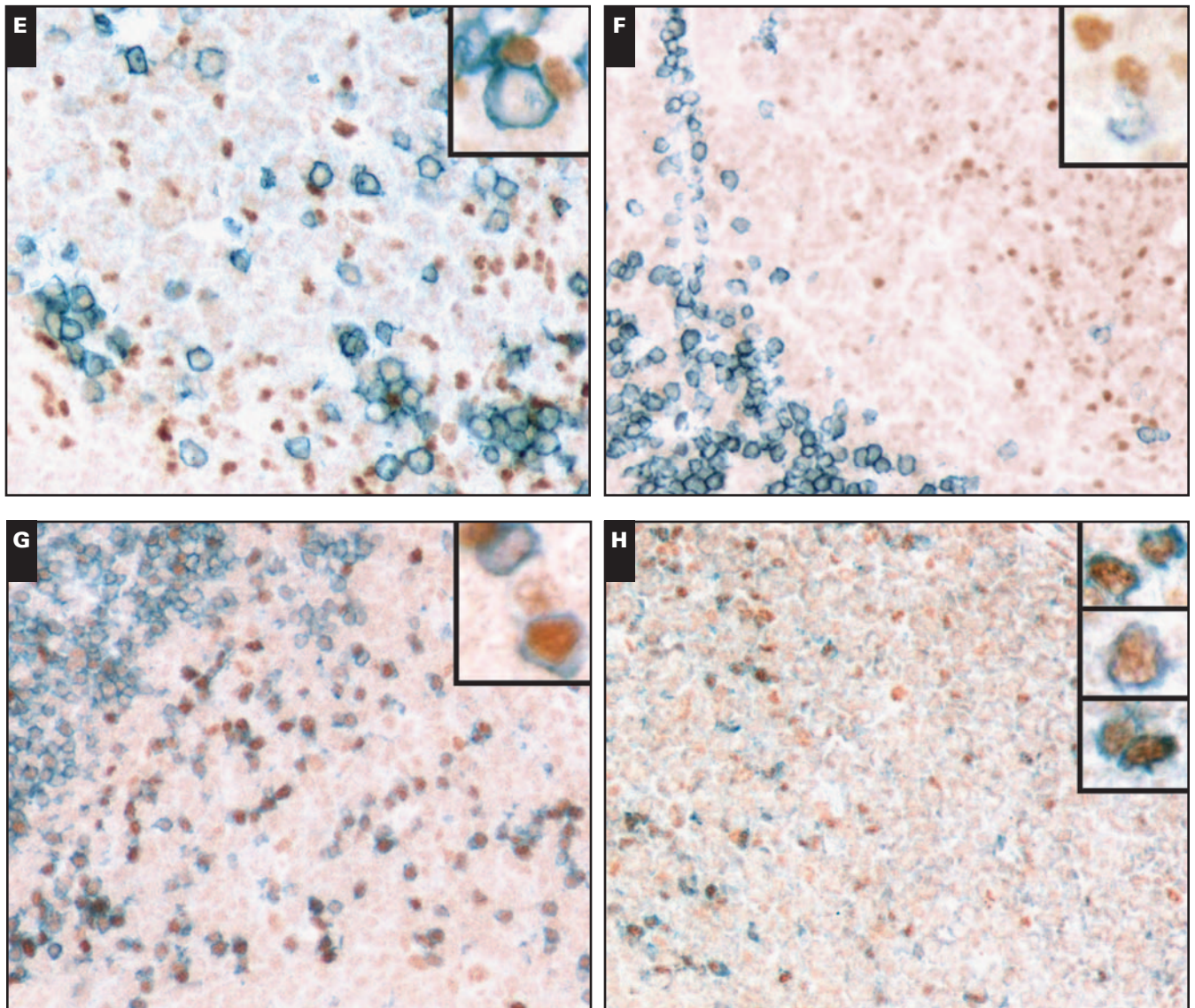
11 extranodal marginal zone B-cell lymphomas, and 1 of 7 mantle cell lymphomas. Furthermore, in 11 of the 17 c-Maf+ B-cell lymphomas (including 2 of the 3 lymphoplasmacytic lymphomas), staining was weak in intensity and clearly not at the same level as that seen in hairy cell leukemia. In a minority of cases of classical (5/17) and lymphocyte predominant (2/14) Hodgkin lymphomas, c-Maf was expressed by the neoplastic cells, usually only weakly.

In contrast with the rarity of c-Maf expression among most B-cell lymphoma categories, approximately half of all T- and NK-cell lymphomas (73 of 139) stained for c-Maf,

including T-cell acute lymphoblastic lymphomas, peripheral T-cell lymphomas (not otherwise specified), anaplastic large cell lymphomas, and T/NK-cell lymphomas, nasal type (Table 2 and Image 2). Furthermore, again in contrast with findings in B-cell lymphomas, the majority of positive cases showed strong staining.

Correlation of c-Maf Protein Expression and FISH

A bone marrow smear from a case of multiple myeloma that carried a t(14;16)(q32;q22-23), shown by R-banding cytogenetic analysis, and in which FISH confirmed breakpoints in



E and **F**, Double immunohistologic labeling of c-Maf and plasma cell-associated markers CD38 (**E**, $\times 10$) and CD138 (**F**, $\times 10$) shows that the vast majority of c-Maf+ cells did not coexpress the other plasma cell markers tested. **G** and **H**, c-Maf was also expressed in scattered cells within the interfollicular T zones, and double labeling showed that these were commonly CD3+ (**G**, $\times 10$) and germinal center T cell-associated marker SAP-positive (**H**, $\times 10$) T cells (high-magnification insets, $\times 100$ - $\times 300$).

the *IgH* and *Maf* loci and an *IgH-Maf* fusion (data not shown), was studied for Maf protein expression. Strong c-Maf protein expression was observed in the tumor cells. In addition, we performed FISH analysis on a TMA containing a variety of lymphomas previously screened by immunohistochemical

analysis for c-Maf protein expression as shown in Table 2. Of 114 informative cases that included 94 B-cell lymphomas, 13 T- and NK-cell lymphomas, and 1 myeloma (lacking c-Maf staining), none showed an *IgH-Maf* fusion indicating the t(14;16) translocation. We also attempted to perform FISH

Table 2
Expression of c-Maf in Hematolymphoid Neoplasms

Lymphoma Subtype	Score 2*	Score 3*	No. Positive/Total	Percentage Positive
B-cell lymphoma (n = 244)				
Follicular lymphoma	5	1	6/97	6
Grade 1	1	0	1/25	4
Grade 2	3	1	4/22	18
Grade 3	1	0	1/50	2
Diffuse large B-cell lymphoma	0	0	0/51	0
Primary mediastinal B-cell lymphoma	0	0	0/9	0
T cell-rich B-cell lymphoma	0	0	0/5	0
Burkitt lymphoma	0	0	0/8	0
Extranodal marginal zone lymphoma	3	0	3/11	27
Splenic marginal zone lymphoma	0	0	0/19	0
Nodal marginal zone lymphoma	0	0	0/16	0
Mantle cell lymphoma	1	0	1/7	14
Small lymphocytic lymphoma/chronic lymphocytic leukemia	0	0	0/7	0
Lymphoplasmacytic lymphoma	2	1	3/3	100
Hairy cell leukemia	0	4	4/4	100
Precursor B-lymphoblastic lymphoma	0	0	0/7	0
Hodgkin lymphoma (n = 31)				
Lymphocyte predominance	2	0	2/14	14
Classical	4	1	5/17	29
Plasma cell neoplasms (n = 164)				
Multiple myeloma	0	6	6/140	4.3
Plasma cell leukemia	0	4	4/15	27
Monoclonal gammopathy of uncertain significance	0	0	0/9	0
T-cell lymphoma (n = 139)				
Precursor T-lymphoblastic lymphoma	1	6	7/11	64
Peripheral T-cell lymphoma	6	8	14/19	74
Anaplastic large cell lymphoma, anaplastic lymphoma kinase+	1	2	3/12	25
T/NK-cell lymphoma, nasal type	2	47	49/97	51

* Score 2, reactivity in fewer than 30% of lymphoma cells; score 3, reactivity in ≥30% of lymphoma cells.

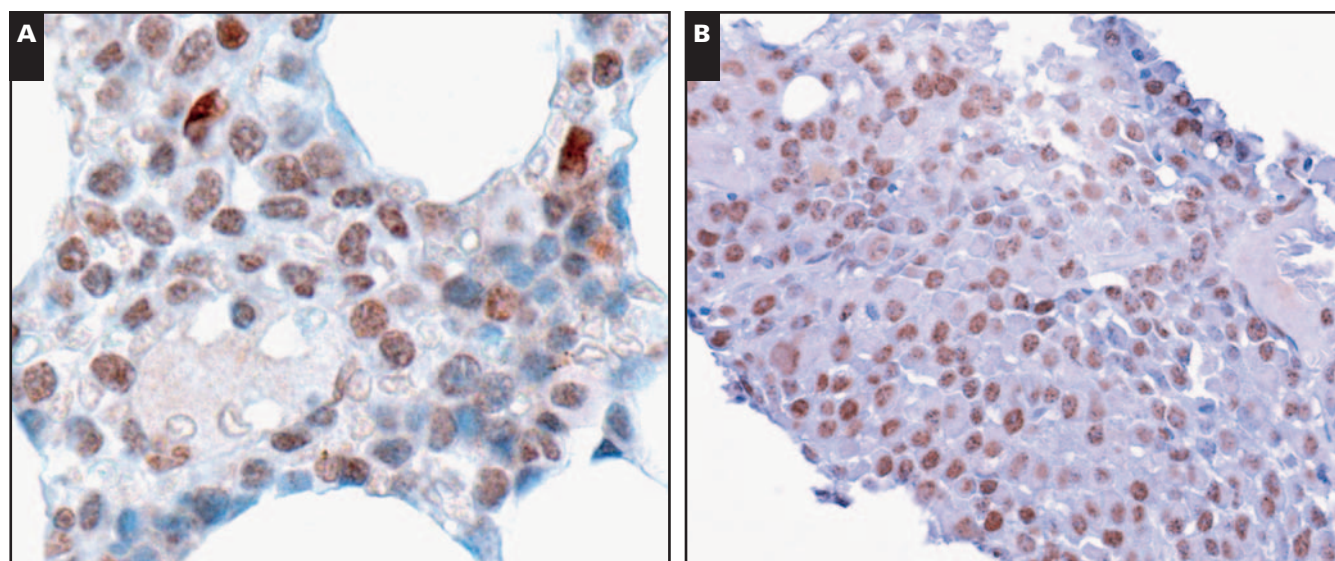


Image 2 Immunohistologic staining for c-Maf in hematolymphoid neoplasia. Representative examples of c-Maf immunostaining in hairy cell leukemia (A, ×60), myeloma (B, ×40), splenic marginal zone lymphoma (C, ×20), T-lymphoblastic lymphoma (D, ×40), NK-cell lymphoma (E, ×40), and peripheral T-cell lymphoma (F, ×40) are shown.

analysis of trephine biopsy specimens from Maf+ myelomas, but this was unsuccessful, presumably because of the known difficulty of performing FISH analysis on fixed and decalcified bone marrow trephine biopsy specimens.

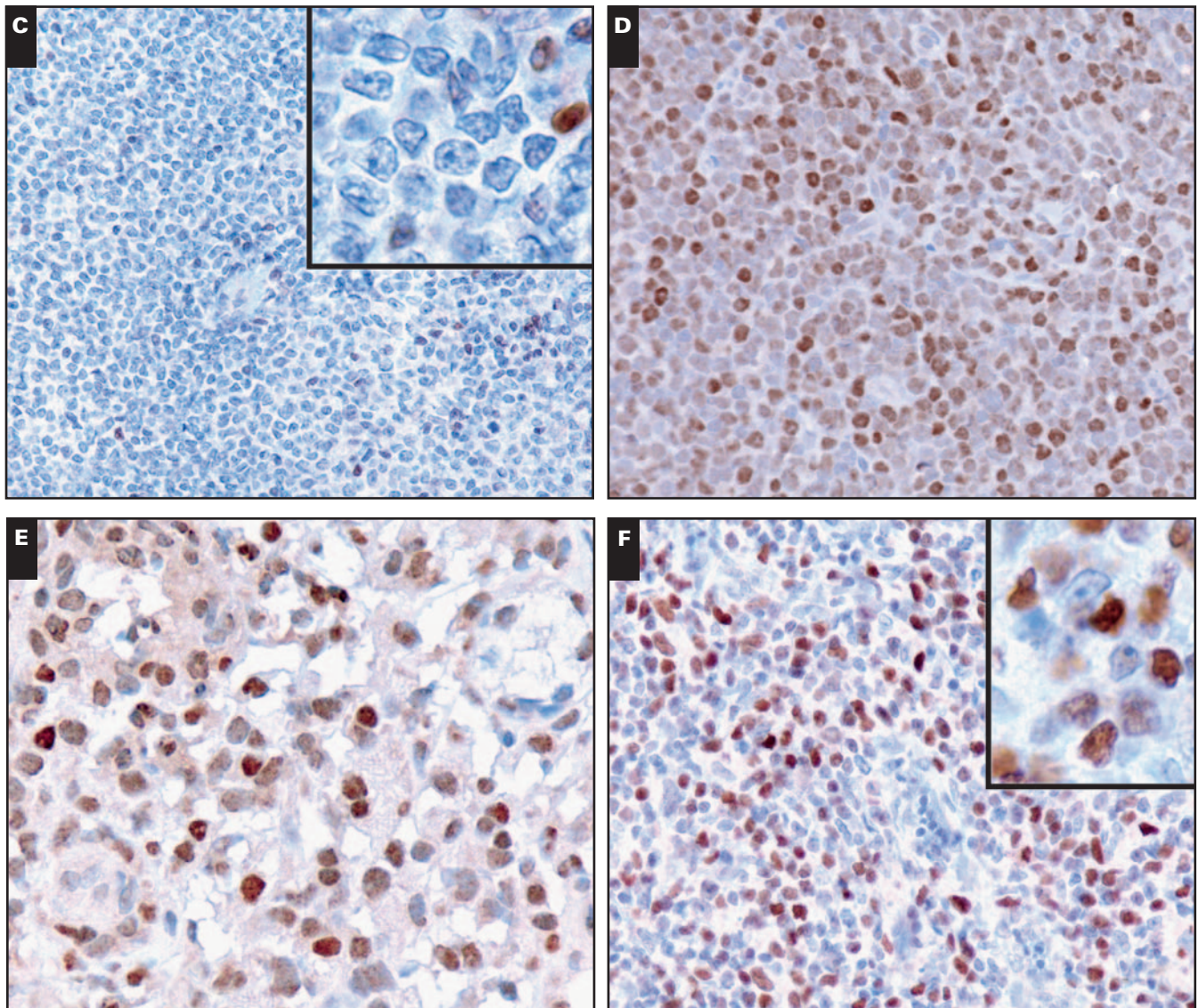
Expression of c-Maf Protein in Cell Lines

Immunoblotting of cellular lysates demonstrated the presence of a single band corresponding to endogenous c-Maf protein (48 kDa) in cell lines derived from myeloma [RPMI 8226, NCI-H929, and Thiel, which harbor the t(14;16) translocation], diffuse large B-cell lymphoma (SUDHL10), classical Hodgkin lymphoma (L1236), and myeloid leukemia (K562) (data not shown). Immunostained cytocentrifuged

preparations corroborated that 4 of 4 cell lines derived from myeloma (RPMI 8226, NCI H929, Thiel, and JLN3), 3 of 4 derived from diffuse large B-cell lymphoma (SUDHL6, SUDHL10, and OCI-LY3), 2 of 3 derived from classical Hodgkin lymphoma (L1236 and L428), and 1 derived from T-cell lymphoma (Jurkat) were c-Maf+ (Table 3).

Comparative Immunohistologic Studies With Other Plasma Cell-Associated Markers

Hierarchical cluster analysis of immunostaining results of plasma cell-associated markers showed that c-Maf expression correlated most closely with that of ICSBP (not expressed or expressed in a minority of cases), followed by LIME and



All 4 cases of hairy cell leukemia showed strong nuclear reactivity for c-Maf, whereas none of the splenic marginal zone lymphomas were positive. A significant proportion of precursor and mature T-cell and NK-cell lymphomas showed c-Maf protein expression (high-magnification insets, $\times 300$).

Table 3
Immunostaining Results of c-Maf Protein in Cell Lines

Lymphoma Subtype/Cell Line (n = 16)	Positive/Negative
Diffuse large B-cell lymphoma	
SUDHL4	Negative
SUDHL6	Positive (weak)
SUDHL10	Positive (weak)
OCI-LY3	Positive (weak)
Burkitt lymphoma	
DAUDI	Negative
RAJI	Negative
Myeloma	
NCI-H929	Positive (strong)
RPMI 8226	Positive (strong)
Thiel	Positive (weak)
JJN3	Positive (strong)
T-cell lymphoma	
CEM/CCRF	Negative
HUT78	Negative
Jurkat	Positive (weak)
Classical Hodgkin lymphoma	
L1236	Positive (weak)
L428	Positive (weak)
KMH2	Negative

Table 4
Comparison of Immunohistologic Markers in Plasma Cell Neoplasms

	Weak Positive, Score 2*	Strong Positive, Score 3*	Total Positive (%)
Myeloma			
c-Maf	0/140	6/140	4.3
CD138	0/148	142/148	95.9
MUM1/IRF4	44/134	68/134	83.6
BLIMP-1	34/139	91/139	89.9
SIT	34/140	38/140	51.4
LIME	23/142	32/142	38.7
ICSBP	0/140	0/140	0.0
Plasma cell leukemia			
c-Maf	0/15	4/15	27
CD138	0/15	15/15	100
MUM1/IRF4	2/13	9/13	85
BLIMP-1	1/11	8/11	82
SIT	2/15	4/15	40
LIME	5/15	4/15	60
ICSBP	0/15	0/15	0

* Score 2, reactivity in fewer than 30% of lymphoma cells; score 3, reactivity in ≥30% of lymphoma cells.

SIT (expressed in approximately 40%-60% of cases). c-Maf expression was most unlike that of MUM1, CD138, and BLIMP-1, which were expressed in more than 80% of the myelomas studied (Table 4) and (Image 3).

Discussion

The *Maf* family of proto-oncogenes, the first of which was initially identified in avian musculoaponeurotic fibrosarcoma,¹⁷ comprises transcription factors containing the basic leucine-zipper motif. c-Maf has a role in diverse physiologic settings, including the regulation of interleukin-4 and interleukin-10 expression by T-helper-2 cells, monocyte differentiation, and formation of the lens.¹⁸⁻²²

The oncogenic potential of c-Maf in animals was confirmed by the observation that transgenic mice overexpressing c-Maf develop T-cell lymphomas,^{2,23} although it may be added that Pouponnot and colleagues²⁴ have shown that c-Maf is capable of functioning as an activator or as a tumor suppressor, depending on its cellular environment. In humans, the only genetic alterations involving c-Maf comprise the translocations involving 16q23 found in myeloma. These appear to be primary events and are assumed to contribute directly to neoplastic cell growth in these tumors.

Among other human tumors, high levels of c-Maf expression were detected by Morito et al²³ in a proportion of T-cell neoplasms. Furthermore, a microarray-based gene expression profiling study showed, unexpectedly, that *c-Maf*

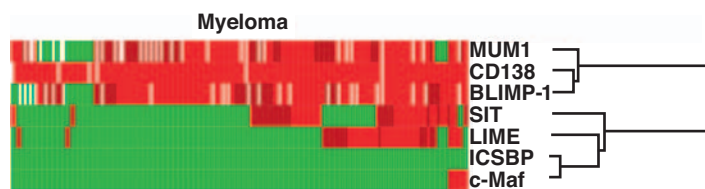


Image 3 Hierarchical cluster analysis of immunohistologic data. The expression patterns of 7 proteins, c-Maf, ICSBP, LIME, SIT, BLIMP-1, CD138, and MUM1/IRF4 (MUM1), in 143 cases of myeloma are shown. Strong positive staining (score 3) is indicated in bright red, weak positive staining (score 2) in dark red, lack of staining (score 0) in green, and uninformative data (score 1) in white. c-Maf protein expression is most closely clustered with that of ICSBP (not expressed or expressed in a minority of cases), followed by LIME and SIT (expressed in approximately 40%-60% of cases). c-Maf expression was most unlike that of IRF4/MUM1, CD138, and BLIMP-1 (clustered on a separate branch on the dendrogram), which were expressed in more than 80% of the myelomas studied.

messenger RNA (mRNA) is expressed in about 50% of myelomas (ie, many more than harbor the translocation) and in myeloma cell lines, suggesting a possible direct causal role for c-Maf in many cases of this disease.⁶ Three putative c-Maf target genes were identified in that study, integrin β 7, cyclin D2, and C-C chemokine receptor-1 (*CCR1*), and it was hypothesized, on the basis of quantitative reverse transcriptase-polymerase chain reaction and short interfering RNA studies, that these target genes are up-regulated by c-Maf and have a direct role in myeloma cell proliferation and adhesion to bone marrow stroma.⁶

We examined in detail c-Maf protein expression in 155 plasma cell neoplasms and found that it was expressed in only about 6% of cases, which corresponds to the reported frequency of t(14;16)/Maf translocations in myelomas.^{3,5} This result is in keeping with the report of Fabris and colleagues²⁵ who found, in a series of 39 myeloma samples, high levels of c-Maf mRNA in only 3 cases with the t(14;16) translocation. An additional 4 translocation-negative cases showed an increase in mRNA levels above the cutoff level for positivity, but these were well below the levels seen in translocation-positive cases.²⁵ Furthermore, Rasmussen et al²⁶ reported that c-Maf mRNA was increased to levels comparable to those seen in a cell line carrying a rearranged c-Maf gene in only 6 (4.4%) of 136 myeloma cases, again a frequency consistent with that of the t(14;16) translocation. It may also be noted that CCND2 protein, encoded by a target gene for c-Maf, was found to be absent from myelomas lacking Maf/MafB translocation (but present in translocation-positive cases).²⁷ It may also be relevant that gene expression profiling of plasma cell dyscrasias has identified a gene expression pattern that correlates closely with the t(14;16) translocation, and this does not appear in translocation-negative samples (as might occur if c-Maf were commonly overexpressed in such cases).²⁷

Among the plasma cell dyscrasia cases studied, we found c-Maf protein expression in 4 (27%) of 15 plasma cell leukemias, a frequency greater than seen in myelomas (4.3%). This difference was statistically significant. Although myelomas and plasma cell leukemias are considered a continuous spectrum of the same disease, a number of markers that correlate with dissemination and increased disease aggressiveness have been described,^{28,29} and our observation suggests that c-Maf expression may function as a marker of high risk in this context. It may be noted that Chang et al,³⁰ in an interphase FISH study of 14 patients with plasma cell leukemia, observed a t(14;16) translocation in 1 case. It may also be added that there is evidence that the t(14;16) translocation is associated with poorer survival.³¹

In hematopoietic neoplasms, a striking finding was the strong c-Maf protein expression observed in each of the 4 hairy cell leukemia samples tested. This finding is in keeping with microarray-based gene expression profiling of 14 cases

of this disease, which identified c-Maf as 1 of 82 genes that were selectively up-regulated (compared with other normal and malignant B cells).^{32,33} Many of these genes are likely to be up-regulated secondarily because Maf is known to induce expression of a number of other molecules, including those associated with myelomonocytic differentiation,¹⁸ and Maf was one of the few genes with transcriptional regulatory activity. It is therefore possible that c-Maf is an aberrantly expressed transcription factor with a primary role in the pathogenesis of this neoplasm.

The expression of c-Maf protein in hairy cell leukemia may also be of diagnostic interest because the distinction of hairy cell leukemias from splenic marginal zone lymphomas can be challenging in the clinical setting: patient symptomatology, clinical laboratory parameters, morphologic findings in peripheral blood and bone marrow, and immunophenotypic workup by flow cytometric or immunohistochemical analysis often overlap between these lymphomas. Hairy cell leukemia does not respond to conventional chemotherapy, but long-term remission can be induced by purine analogues and interferon alfa,^{34,35} so that its distinction from marginal zone lymphomas and other chronic lymphoproliferative disorders is clinically essential.

Immunophenotypic markers such as DBA.44, tartrate-resistant acid phosphatase, CD25, and CD103 are used in the differentiation of these lymphomas but remain problematic owing to expression of these markers in a subset of both types of lymphomas. The combined expression of DBA.44 and tartrate-resistant acid phosphatase has been found to be of some usefulness in separating hairy cell leukemia from splenic marginal zone and other low-grade B-cell lymphomas in its differential diagnosis³⁶; however, specific markers that provide better discrimination among these entities are desirable. Annexin A1, a highly sensitive marker for hairy cell leukemia, has been described³⁷; however, annexin A1 also stains myeloid lineage cells and poses difficulties in interpretation of the stain, particularly in the context of a subtle interstitial infiltrate of hairy cell leukemia involving the bone marrow. If further studies confirm our preliminary observation that c-Maf protein is expressed in all cases of hairy cell leukemias but not in splenic marginal zone lymphomas, it may represent a valuable marker for the diagnosis of hairy cell leukemia in routine hematopathology practice.

In contrast with the rarity of c-Maf expression in B-cell lymphomas, more than half of the T- and NK-cell lymphomas and the T-cell line, Jurkat, were c-Maf+. This is consistent with the only previous study of c-Maf expression, in which 13 of 32 T-cell lymphoma cases were c-Maf+.²³ It is also in keeping with the observation that enforced expression of c-Maf in transgenic mice leads to the development of T-cell neoplasms. Our FISH analysis on TMAs also showed that the *IgH-Maf* fusion indicating the t(14;16) translocation was

absent in all 13 informative cases of T- and NK-cell lymphomas. This result suggests that up-regulation of the c-Maf protein can occur independently of the t(14;16) chromosomal translocation. However, we also found c-Maf in a minority of normal T cells, so that it is possible that its expression in T-cell neoplasms reflects their cellular origin. We and others have previously shown that another plasma cell-associated marker, IRF4/MUM1, is frequently expressed in T- and NK-cell lymphomas.^{8,38} Similarly, BLIMP-1 has also been shown to have a role in T-cell homeostasis and function.^{39,40} These findings suggest that c-Maf may represent a potentially useful marker for further study in T- and NK-cell lymphomas.

In normal hematopoietic tissues, we found that c-Maf was expressed in scattered B and T cells, in some histiocytes, and in a very small subset of plasma cells in which weak positivity was detected, although double-staining experiments showed no coexpression with other plasma cell-associated markers. This latter finding is in keeping with the reported absence of c-Maf mRNA in normal plasma cells purified from bone marrow aspirate samples.⁶ It was also found in rare T cells. The limited expression of c-Maf in normal lymphoid cells, therefore, implies that its presence in lymphomas may indicate a causal role in these tumors.

Our studies suggest that the up-regulation of c-Maf protein in myeloma is likely related to, but may not be limited to, the translocated c-Maf gene. In addition, c-Maf may be implicated in hairy cell leukemia and possibly in some T- and NK-cell lymphomas. Its restricted expression pattern is suggestive of a causal role in the pathogenesis of these neoplasms and warrants further study. Moreover, the immunohistologic detection of c-Maf protein may be of value in the differential diagnosis of small cell lymphomas.

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