C-MYC is related to GATA3 expression and associated with poor prognosis in nodal peripheral T-cell lymphomas

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Material and methods

Patient samples

The series comprised 128 formalin-fixed, paraffin-embedded (FFPE) n-PTCLs samples (54 peripheral T-cell lymphomas not specified (PTCL-NOSs) and 74 angioimmunoblastic T-cell lymphomas (AITLs)). These were used for immunohistochemical studies. Diagnostic criteria were based on the WHO classification[1]. All samples were reviewed by two pathologists (SMR-P and MAP) to confirm the diagnoses. Patients' clinical data have been reported in previous publications[2-4]. Samples and clinical data of patients included in the study were provided by several Spanish Biobanks. The project was supervised by the Ethical Committees of the Spanish institutions: Hospital Carlos III (Madrid), Hospital Universitario Marqués de Valdecilla (Santander), and Fundación Jiménez Díaz (Madrid).

Tissue microarray construction

Representative areas from FFPE lymphomas were carefully selected from H&E-stained sections. Three tissue cores of 1 mm diameter were obtained from each specimen. The cores were precisely arrayed into a new paraffin block using a tissue microarray (TMA) workstation (Beecher Instruments, Silver Spring, MD).

Immunohistochemical studies

TMA sections were stained by the Endvision method with a heat-induced antigen-retrieval step for C-MYC, GATA3 and Ki-67. When quantifying C-MYC, two groups were established: 0, 0-20% positive cells; 1, more than 20% positive cells[5]. Expression levels of GATA3 were established in two groups according to previous studies[6, 7]: 0, 0-10% positive cells; 1, more than 10% positive cells. According to a previous study[3], cases with a high proliferation index (>80% tumoral cells) were considered to be positive for Ki-67.

Reactive tonsil tissue was included as a control. The primary antibodies were omitted to provide negative controls (Supplementary Table 1).

Statistical analysis

To assess associations between categorical variables, we used the X^2 contingency test with Yates' correction, or Fisher's exact test, as appropriate. Overall survival (OS) was taken to be the period between the date of diagnosis and the date of death from any cause, or of last contact for living patients. Disease-specific OS was calculated as the period from date of diagnosis until death from the tumor. Kaplan–Meier survival analyses were carried out for OS and lymphoma-specific survival, using the log-rank test to examine differences between groups. A multivariate Cox regression model was also derived. Estimates were considered statistically significant for two-tailed values of $p<0.05$. All analyses were carried out with SPSS v.12.0 (SPSS Inc., Chicago, IL)[3].

Supplementary information about GATA3 expression

In this study, GATA3 expression was observed in 21.9% (28/128) of the nPTCL cases 20.3% (15/74) of the AITLs and 24.1% of the (13/54) PTCL-NOS patients were positive. Previous studies had shown GATA3 expression in 33%[6] and 45%[7] of the PTCL-NOS patients. Differences in the percentage positivity may have been due to the antibody used (ABCAM (ours) *vs.* Santa Cruz Biotechnology (previous papers)) or to the clinical series analyzed.

Wang et al.[7] showed that the cut-off used to define GATA3 expression was highly variable, due to both the percentage of positive cells and the intensity of staining. They found that more than 85% of the cases positive for GATA3 showed reactivity in more than 30% of the tumoral cells. However, not all of them had the same staining intensity. Therefore, they concluded that cases were GATA3-positive if nuclear staining was observed in more than 10% of tumor cells. On the other hand, Iqbal et al.[6] established expression levels of GATA3 in three groups: 0, 0-40% positive cells; 1, 40-80% positive cells; 2, more than 80% positive cells.

We also observed these differences in staining intensity (Supplementary Figure 1A-C). In the present series, most cases (23 out of 28, 82%) had more than 30% of tumoral cells positive for GATA3 with either moderate or intense intensity of staining. GATA3 expression was intense and presence in almost 100% of tumoral cells in five cases in our series. Only other five cases showed positivity for GATA3 in fewer than 40% of tumoral cells (Supplementary Table 2).

Supplementary References

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Supplementary Tables

Supplementary Table 1. Panel of antibodies used in this series.

Antibody	Clone	Source	Cut-off value for positivity
C-MYC	Rabbit monoclonal (Y69)	ABCAM	>20%
GATA3	Rabbit polyclonal (32858)	ABCAM	>10%
Ki-67 FLEX	Mouse monoclonal (MIB-1)	DAKO	>80%

Supplementary Table 2. GATA3 positive n-PTCL cases of our series.

Case	Diagnosis	GATA3 (%)	GATA3 (IS)
1	PTCL-NOS	80	I
2	PTCL-NOS	100	I
3	AITL	20	M
4	PTCL-NOS	90	L
5	AITL	40	I
6	AITL	50	M
7	PTCL-NOS	80	M
8	AITL	20	L
9	AITL	30	M
10	PTCL-NOS	20	I
11	AITL	100	I
12	AITL	100	I
13	PTCL-NOS	50	L
14	AITL	80	M
15	PTCL-NOS	100	I
16	PTCL-NOS	100	I
17	AITL	40	I
18	AITL	65	M
19	AITL	70	M
20	PTCL-NOS	40	I
21	PTCL-NOS	80	M
22	PTCL-NOS	65	M
23	PTCL-NOS	40	M
24	AITL	70	M
25	AITL	75	M
26	AITL	60	I
27	PTCL-NOS	80	M
28	AITL	20	M

AITL: angioimmunoblastic T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma not specified; Percentage of positive neoplastic cells: %; Intensity of staining: IS; Intense: I; Moderate: M; Low: L; Diagnostic according to the 2008 WHO classification.

Supplementary Table 3. Univariate analysis of the clinical and molecular characteristics of 128 patients with n-PTCLs, with respect to C-MYC expression.

Clinical and molecular characteristics				
	Total cases	**Negative**	**Positive**	**p**
Diagnosis	128/128			0.537
AITL		63/74 (85.1%)	11/74 (14.9%)	
PTCL-NOS		48/54 (88.9%)	6/54 (11.1%)	
Sex	128/128			0.611
Male		66/75 (88.0%)	9/75 (12.0%)	
Female		45/53 (84.9%)	8/53 (15.1%)	
Age at diagnosis	128/128			0.091
<60 years		43/46 (93.5%)	3/46 (6.5%)	
≥60 years		68/82 (82.9%)	14/82 (17.1%)	
IPI	113/128			0.053
Low risk		31/33 (93.9%)	2/33 (6.1%)	
Low-intermediate risk		27/31 (87.1%)	4/31 (12.9%)	
High-intermediate risk		24/28 (85.7%)	4/28 (14.3%)	
High risk		14/21 (66.7%)	7/21 (33.3%)	
PIT	101/128			0.001
Low risk		13/13 (100%)	0/13 (0%)	
Low-intermediate risk		34/40 (85.0%)	6/40 (15.0%)	
High-intermediate risk		23/24 (95.8%)	1/24 (4.2%)	
High risk		14/24 (58.3%)	10/24 (41.7%)	
ECOG	109/128			0.008
<1		71/78 (91.0%)	7/78 (9.0%)	
≥1		22/31 (71.0%)	9/31 (29.0%)	
Treatment	109/128			0.489
CHOP or CHOP-like		72/84 (85.7%)	12/84 (14.3%)	
Others		20/25 (80.0%)	5/25 (20.0%)	
Response	103/128			0.296
CR		53/61 (86.9%)	8/61 (13.1%)	
PR		18/19 (94.7%)	1/19 (5.3%)	
No response		18/23 (78.3%)	5/23 (21.7%)	
Recurrence	98/128			0.928
No		59/69 (85.5%)	10/69 (14.5%)	
Yes		25/29 (86.2%)	4/29 (13.8%)	
State of the patient	119/128			0.100
Dead		63/77 (81.8%)	14/77 (18.2%)	
Alive		39/42 (92.9%)	3/42 (7.1%)	

AITL: angioimmunoblastic T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma not specified; IPI: International Prognostic Index; PIT: Prognostic Index for T-cell lymphomas; ECOG: performance status (Eastern Cooperative Oncology Group); CHOP: cyclophosphamide, vincristine, doxorubicin, prednisone; CR: complete response; PR: partial response.

Supplementary Table 4. Univariate analysis of the clinical and molecular characteristics of 128 patients with n-PTCLs, with respect to Ki-67 expression.

Clinical and molecular characteristics				
	Total cases	**Negative**	**Positive**	**p**
Diagnosis	127/128			0.656
AITL		57/74 (77.0%)	17/74 (23.0%)	
PTCL-NOS		39/53 (73.6%)	14/53 (26.4%)	
Sex	127/128			0.583
Male		58/75 (77.3%)	17/75 (22.7%)	
Female		38/52 (73.1%)	14/52 (26.9%)	
Age at diagnosis	127/128			0.165
<60 years		38/46 (82.6%)	8/46 (17.4%)	
≥60 years		58/81 (71.6%)	23/81 (28.4%)	
IPI	112/128			0.054
Low risk		25/33 (75.8%)	8/33 (24.2%)	
Low-intermediate risk		27/31 (87.1%)	4/31 (12.9%)	
High-intermediate risk		23/27 (85.2%)	4/27 (14.8%)	
High risk		12/21 (57.1%)	9/21 (42.9%)	
PIT	101/128			0.123
Low risk		10/13 (76.9%)	3/13 (23.1%)	
Low-intermediate risk		33/40 (82.5%)	7/40 (17.5%)	
High-intermediate risk		20/24 (83.3%)	4/24 (16.7%)	
High risk		14/24 (58.3%)	10/24 (41.7%)	
ECOG	108/128			0.213
<1		63/77 (81.8%)	14/77 (18.2%)	
≥1		22/31 (71.0%)	9/31 (29.0%)	
Treatment	108/128			0.992
CHOP or CHOP-like		63/83 (75.9%)	20/83 (24.1%)	
Others		19/25 (76.0%)	6/25 (24.0%)	
Response	102/128			0.490
CR		49/61 (80.3%)	12/61 (19.7%)	
PR		14/19 (73.7%)	5/19 (26.3%)	
No response		15/22 (68.2%)	7/22 (31.8%)	
Recurrence	97/128			0.558
No		53/68 (77.9%)	15/68 (22.1%)	
Yes		21/29 (72.4%)	8/29 (27.6%)	
State of the patient	118/128			0.180
Dead		55/76 (72.4%)	21/76 (27.6%)	
Alive		35/42 (83.3%)	7/42 (16.7%)	

AITL: angioimmunoblastic T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma not specified; IPI: International Prognostic Index; PIT: Prognostic Index for T-cell lymphomas; ECOG: performance status (Eastern Cooperative Oncology Group); CHOP: cyclophosphamide, vincristine, doxorubicin, prednisone; CR: complete response; PR: partial response.

Supplementary Table 5. Univariate analysis of the clinical and molecular characteristics of 128 patients with n-PTCLs, with respect to GATA3 expression.

Clinical and molecular characteristics				
	Total cases	**Negative**	**Positive**	**p**
Diagnosis	115/128			0.717
AITL		50/65 (76.9%)	15/65 (23.1%)	
PTCL-NOS		37/50 (74.0%)	13/50 (26.0%)	
Sex	115/128			0.890
Male		51/67 (76.1%)	16/67 (23.9%)	
Female		36/48 (75.0%)	12/48 (25.0%)	
Age at diagnosis	115/128			0.656
<60 years		32/41 (78.0%)	9/41 (22.0%)	
≥60 years		55/74 (25.7%)	19/74 (25.7%)	
IPI	100/128			0.988
Low risk		22/29 (75.9%)	7/29 (24.1%)	
Low-intermediate risk		19/26 (73.1%)	7/26 (26.9%)	
High-intermediate risk		20/26 (76.9%)	6/26 (23.1%)	
High risk		14/19 (73.7%)	5/19 (26.3%)	
PIT	89/128			0.941
Low risk		8/11 (72.7%)	3/11 (27.3%)	
Low-intermediate risk		28/37 (75.7%)	9/37 (24.3%)	
High-intermediate risk		14/19 (73.7%)	5/19 (26.3%)	
High risk		15/22 (68.2%)	7/22 (31.8%)	
ECOG	96/128			0.120
<1		54/68 (79.4%)	14/68 (20.6%)	
≥1		18/28 (64.3%)	10/28 (35.7%)	
Treatment	97/128			0.623
CHOP or CHOP-like		54/75 (72.0%)	21/75 (28.0%)	
Others		17/22 (77.3%)	5/22 (22.7%)	
Response	92/128			0.708
CR		37/51 (72.5%)	14/51 (27.5%)	
PR		12/18 (66.7%)	6/18 (33.3%)	
No response		18/23 (78.3%)	5/23 (21.7%)	
Recurrence	88/128			0.187
No		49/64 (76.6%)	15/64 (23.4%)	
Yes		15/24 (62.5%)	9/24 (37.5%)	
State of the patient	106/128			0.275
Dead		49/68 (72.1%)	19/68 (27.9%)	
Alive		31/38 (81.6%)	7/38 (18.4%)	

AITL: angioimmunoblastic T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma not specified; IPI: International Prognostic Index; PIT: Prognostic Index for T-cell lymphomas; ECOG: performance status (Eastern Cooperative Oncology Group); CHOP: cyclophosphamide, vincristine, doxorubicin, prednisone; CR: complete response; PR: partial response.

Supplementary Figure.

Supplementary Figure 1. Nodal PTCL cases negative for GATA3 (**A**) and positive for GATA3 showing either moderate (**B**) or high (**C**) intensity staining. Nodal PTCL cases negative and positive for MYC (**D and E**) expression, respectively.