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FOXP1 molecular cytogenetics and protein expression analyses in primary cutaneous large B cell lymphoma, leg-type

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Summary. *FOXP1* protein is expressed in normal activated B cells and overexpressed in a subset of diffuse large B-cell lymphomas, including primary cutaneous large B-cell lymphomas (PCLBCL), leg type. High expression of FOXP1 has been associated to an unfavourable prognosis with independent survival significance. However, little is known regarding the mechanisms underlying the overexpression of FOXP1 in PCLBCL, leg type. Our aims were to analyze *FOXP1* cytogenetic status and protein expression in a series of PCLBCL, leg type. Finally, we compared the observed results with those obtained in a group of patients with primary cutaneous follicle centre lymphoma (PCFCL).

Fifteen patients with PCLBCL, leg type and nine patients with primary cutaneous follicle centre lymphoma (PCFCL) were included in the study. For each biopsy specimen, *FOXP1* translocation and copy number changes were evaluated by fluorescence in situ hybridization (FISH) and protein expression by immunohistochemistry (IHC).

Immunohistochemistry showed FOXP1 staining in 13 PCLBCL, leg type, whereas all PCFCL were negative. FISH analysis disclosed no translocations involving *FOXP1* gene in any of the cases. However, *FOXP1* gene gains (3 to 4 copies) were observed in 82% of samples of PCLBCL, leg type and in 37% of PCFCL. FOXP1 expression was independent from *FOXP1* translocation.

Our results confirm that overexpression of FOXP1 is present in a considerable proportion of PCLBCL, leg type and might indicate an unfavourable prognosis. Mechanisms not related to translocation seem to be responsible for this overexpression.

Key wods: Cutaneous lymphoma, Leg type, Large B cell, *FOXP1*, FISH

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Introduction

Primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, leg type) is a subgroup of primary cutaneous B-cell lymphomas, characterized clinically by red or violaceous plaques or nodules, often (but not exclusively) arising on one or both legs, and histologically by a neoplastic proliferation comprised of confluent sheets of centroblasts and immunoblasts (Willemze et al., 2005). The new WHO classification of tumors of hematopoietic and lymphoid tissues recognizes PCLBCL, leg type as a separate entity with an intermediate prognosis, frequent dissemination to extracutaneous sites and a short survival (Swerdlow et al., 2008).

Primary cutaneous follicle centre lymphoma (PCFCL) is another type of cutaneous B cell lymphoma with the presence of large neoplastic cells, particulary large centrocytes. PCFCL usually presents with skin lesions confined to a limited skin area on the head and trunk, rarely disseminates to extracutaneous localizations and usually has an excellent outcome (Senff et al.,

2007).

Recent studies have evaluated the genetic and gene expression profile, suggesting that PCFCL and PCLBCL, leg type show profiles similar to that of germinal center B-cell (GBC)-like and activated B-cell (ABC)-like diffuse large B-cell lymphomas (DLBCL), respectively (Hoefnagel et al., 2005).

Forkhead box-P1 (*FOXP1*) gene codifies for a transcription factor involved in the transcriptional regulatory network of B lymphopoiesis (Hu et al., 2006; Dengler et al., 2008), expressed in normal activated B cells (Shaffer et al., 2002) and overexpressed in a subset of systemic DLBCL with a predominantly ABC-like phenotype (Barrans et al., 2004; Banham et al., 2005). Among primary cutaneous lymphomas, FOXP1 protein expression has been detected in more than 70% of cases of PCLBCL, leg type, but it is observed in less than 10% of PCFCL (Kodama et al., 2005; Hoefnagel et al., 2006). A possible relationship between FOXP1 expression and the clinical and evolutive features has been described (Hallermann et al., 2007), and suggesting a role for *FOXP1* in the pathogenesis of PCLBCL, leg type.

Recurrent chromosomal translocations targeting the *FOXP1*, which commonly but not exclusively involve the *IGH* locus leading to the translocation t(3;14)(p14;q32), have been described in systemic DLBCL and marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT) (Streubel et al., 2005; Wlodarska et al., 2005; Fenton et al., 2006; Goatly et al., 2008). However, the mechanisms by which FOXP1 expression is deregulated in PCLBCL, leg type are largely unknown.

The aims of the present study were to analyze *FOXP1* cytogenetic status and protein expression in a series of PCLBCL, leg type. We designed a dual color, break-apart probes to study the genetic abnormalities of this locus, and correlations between FOXP1 protein expression and *FOXP1* gene status were also investigated. Finally, we compared the observed results with those obtained in a group of patients with primary cutaneous follicle centre lymphoma (PCFCL).

Materials and methods

Patient selection

Fifteen patients diagnosed with PCLBCL, leg type were retrieved retrospectively from the Catalonian Cutaneous Lymphoma Network database (Hospital de Bellvitge, Hospital del Mar and Hospital Clinic, Barcelona) and were included in the study. The diagnosis of primary cutaneous involvement was established on the basis of an exclusive skin involvement at diagnosis, and following a complete staging work-up. In addition, a group of nine patients diagnosed with PCFCL showing a prominent large cell population were included in the study. All cases fulfilled the standard classification criteria of the last World Health Organization (WHO) classification (Swerdlow et al., 2008).

Clinical features

For each patient, the following clinical data were collected: age, sex, time period prior to diagnosis, primary anatomic site, number of lesions or disease extension, staging, type of treatment, response to treatment, evolution including cutaneous recurrence, time to first relapse, development of extracutaneous disease, current status and follow-up duration.

Histologic examination and immunohistochemistry

Formalin-fixed paraffin embedded tissues were retrieved from each case. Immunohistochemical studies for FOXP1 were performed in 4 μm tissue sections previously deparaffinized and rehydrated. Sections were heat-retrieved for antigen in a high pH Dako solution (pH 9.9) using a microwave pressure cooker system. Immunohistochemistry was performed with an automated immunostainer (Dako TechMate Immunostainer, Dako, Glostrup, Denmark) using a monoclonal anti-FOXP1 antibody (clone JC12, dilution 1:50) kindly provided by Dr. Alison H. Banham (Nuffield Department of Expression Clinical and Laboratory Sciences, University of Oxford, UK). FOXP1 expression by tumor cells was assessed at four levels of intensity (0=negative, 1=weak, 2=moderate, 3=strong) and four groups defining the percentage of positive cells (<25% negative, 25-50%, 50-75% and >75%). The rest of the antibodies used for the phenotypic characterization of these lesions were performed as previously described (Colomo et al., 2003).

Fluorescence in situ hybridization (FISH)

FISH analyses were performed in all samples on paraffin sections following standard procedures. A *FOXP1* (3p14.1) dual color break-apart non-commercial probe was designed using two bacterial artificial chromosomes (BACs) from the Children's Oakland Research Institute library: RP11-79P21 located at 3' of the gene labelled with SpectrumRed (Abbot Molecular, Abbott Park, IL) and RP11-713J7 located at 5' of the gene and labelled with SpectrumGreen (Abbot Molecular) (Fig. 1a) (Baró et al., 2009). FISH was analyzed in parallel sections from those studied for immunohistochemistry and morphology. Slides were evaluated in a fluorescence microscope (BX51, Olympus, Spain). The expected pattern in a nucleus harboring a *FOXP1* translocation was one fused red-green signal (1F), representing the normal chromosome 3, one red signal (1R) and one green signal (1G) representing the translocated chromosome 3 (1F1R1G pattern). A normal case should show two fused red-green signals (2F) and a case with trisomy or tetrasomy of *FOXP1* gene should show three or four fused red-green signals (3F or 4F) (Figure 1b). Moreover, in 3 cases (one PCFCL and two PCLBCL, leg type cases) FISH using the centromeric probe for chromosome 3 labelled in orange (CEP3, Abbot Molecular) and the

BAC RP11-713J7 located at 5'of the gene labelled in green was performed to test the presence of trisomy.

To assess the cut-off values of the tested FISH probes in healthy individuals, five paraffin-embedded tonsil tissue sections and ten peripheral blood samples from healthy donors were used as negative controls. The cut-off values were calculated as the mean of false-positive findings plus three times the standard deviation. Two hundred nuclei per case were scored by two different observers, taking into account the following abnormal patterns: 1F1R1G, 1F, 3F. The calculated values for these patterns in control tissues were 2%, 54% and 10%, respectively. In addition, three samples from patients affected with a DLBCL harbouring a *FOXP1* translocation previously detected by G-banding cytogenetics and/or FISH were included in the study as positive controls (kindly provided by Dr. Berthold Streubel from Department of Pathology, Medical University of Vienna, Vienna, Austia, and Dr. Iwona Wlodarska from Department of Haematology, University Hospital Gasthuisberg, Leuven, Belgium).

Finally, kappa/cross test was performed to assess the agreement between FOXP1 expression and FISH results.

Results

Clinical data

Patients with PCLBCL, leg type were seven men and eight women with a mean age of 68 years. The lesions corresponded to solitary nodules/tumors located on the leg (n=10) (Fig. 2a) and the trunk (n=2), or disseminated lesions (n=3). Patients with PCFCL were six men and three women (mean age 58 years). The lesions were present on the trunk (n=5) (Fig. 2c) or face (n=4). The clinical data are summarized in Table 1.

Patients with PCLBCL, leg type presenting early cutaneous stage of the disease were treated with

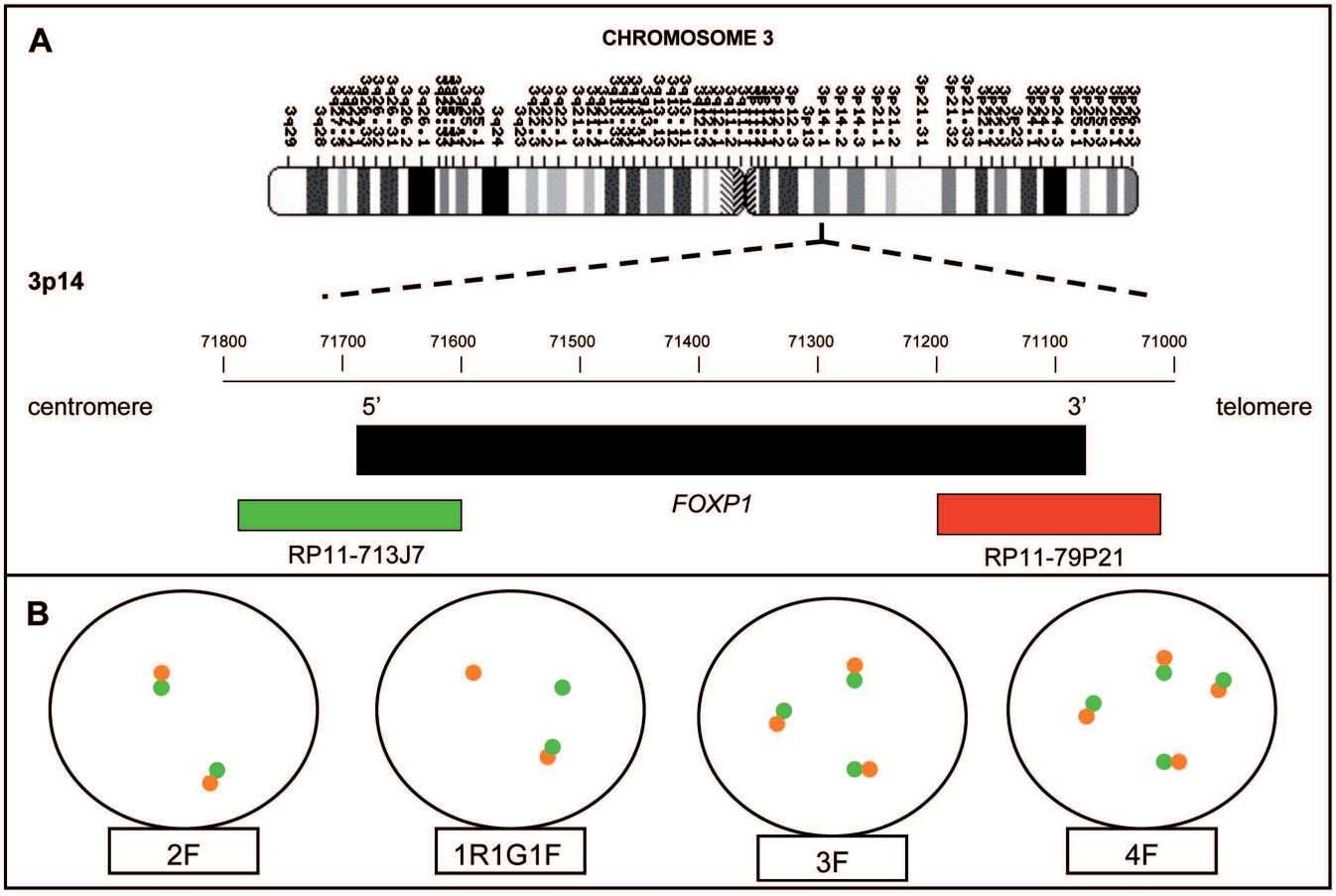

Fig. 1. A. Diagram of the *FOXP1* (3p14.1) dual color break-apart probe design, using two bacterial artificial chromosomes (BACs) from the Children's Oakland Research Institute library: RP11-79P21 located at 3' of the gene labelled with SpectrumRed (Abbott Molecular) and RP11-713J7 located at 5' of the gene and labelled with SpectrumGreen (Abbott Molecular). **B.** Schematic representation of different FISH patterns found in the present study using the *FOXP1* dual color break-apart non-commercial probe in interphase nuclei. The circles represent different nuclei and inside them there are different combinations of red and green dots representing 5'*FOXP1* and 3'*FOXP1* probe signals. Below each nucleus the nomenclature of the FISH patterns is indicated. Abbreviations: R: red; G: green; F: fusion

localized skin radiation therapy (RT) (n=8). For those patients with more advanced skin disease, chemotherapy alone (n=4) or in combination with RT (n=2) was prescribed. In one patient, no treatment was administered due to the poor performance status of the patient. After the initial treatment, 11 out of 14 patients achieved a complete response (78%). Seven patients experienced one or several relapses (cutaneous or extracutaneous). In total, nine out of 15 PCLBCL, leg type cases developed extracutaneous disease (lymph nodes [n=6], testis [n=2], central nervous system [n=1]) after a follow-up period ranging from 4-96 months (mean, 31 months). Six out of 15 patients died of lymphoma progression, five patients were alive (two of them presenting skin residual disease) and four died of unrelated causes.

In contrast, in all PCFCL cases a complete remission was obtained after an initial therapeutic approach (RT [n=6], surgery [n=2] or chemotherapy [n=1]). Two PCFCL presented limited skin relapses after the first treatment. The mean follow-up period for this group of patients was 55 months (range, 12-121 months). Clinical status at the end of the study was as follows: eight out of nine patients with PCFCL were in complete remission. and one patient died of an unrelated cause within this group.

Histopathological and immunohistochemical data

Histopathological and immunohistochemical

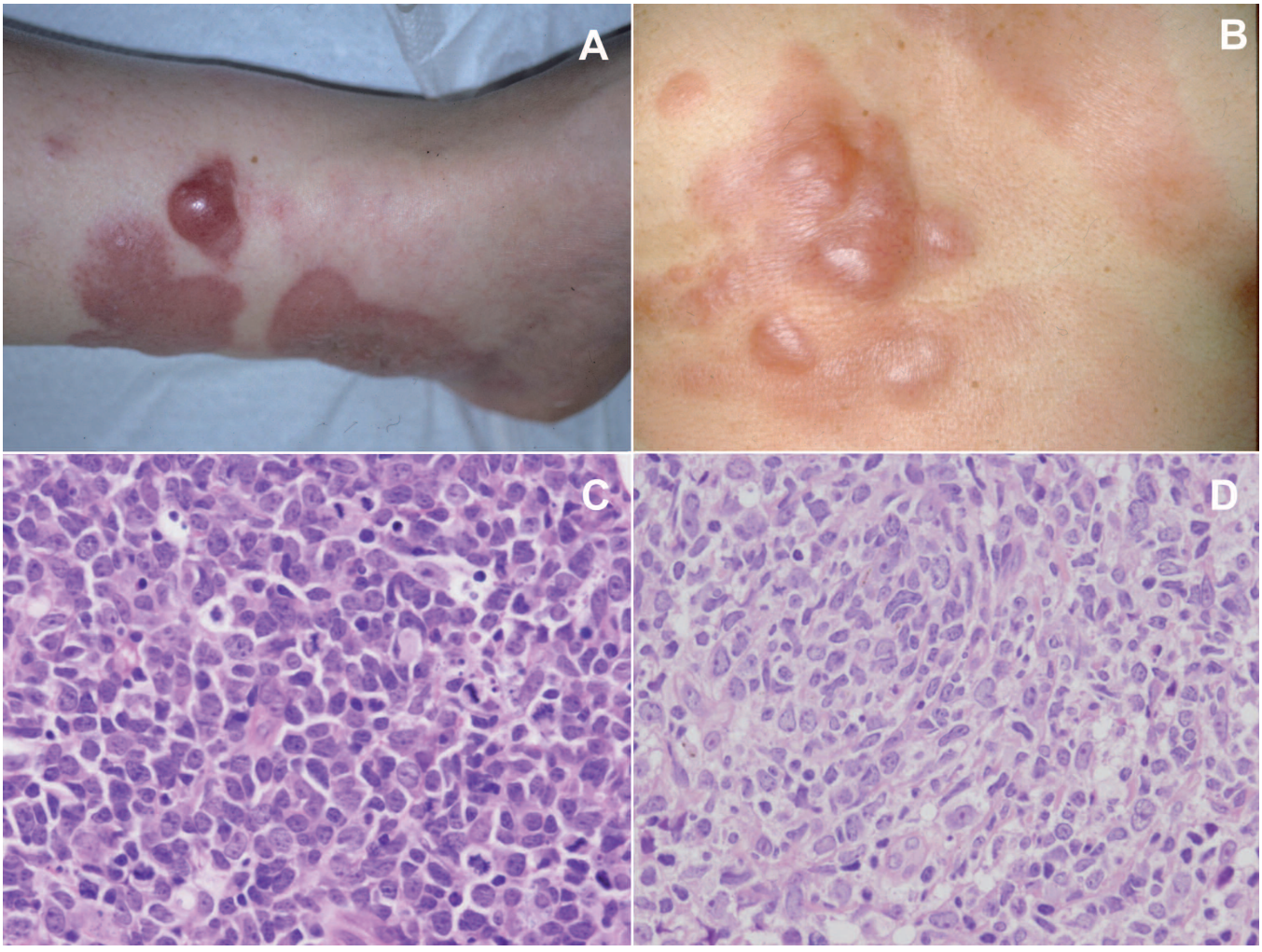

Fig. 2. Clinical presentation and histology pattern of PCLBCL. **A.** Clinical presentation of a primary cutaneous large B-cell lymphoma, leg type with a tumor on the left lower leg. **B.** Typical presentation of primary cutaneous follicle center lymphoma on the chest surrounded by less infiltrated erythematous skin areas. **C.** Diffuse infiltrate of centroblasts and immunoblasts. Hematoxilin-Eosin staining. x100. **D.** Diffuse dermal infiltrate with large centrocytes and multibobated cells. Hematoxilin-Eosin staining. x100

features of all included cases are described in Table 2. A diffuse, monomorphic dermal or subcutaneous infiltration by a proliferation of large B cells, predominantly centroblasts and immunoblasts with scattered centrocytes, was observed in PCLBCL, leg type cases (Fig. 2b). Thirteen (87%) PCLBCL, leg type cases were positive for the FOXP1 antigen. Among them, one case was weak, seven cases had moderate expression, and five showed strong FOXP1 positivity (Fig. 3).

Expression of BCL2 in more than 50% of the neoplastic cells was observed in all the cases of PCLBCL, leg type cases, whereas a positive staining for MUM1 protein was present in 10 of 14 available tumors. Bcl-6 was expressed in six of 14 (43%) tumors and CD10 was weakly expressed in two out of 15 (13%) cases.

Neoplastic cells of PCFCL corresponded to large cleaved cells and showed a rather polymorphous appearance (Fig. 2d). Immunohistochemistry for FOXP1 was successfully performed in six out of nine cases and all evaluated samples were negative for FOXP1 expression (Fig. 3). Three cases expressed BCL2 but with less intensity than admixed T cells. MUM1 was negative in all nine cases examined and expression of CD10 was identified in four (44%) cases and Bcl6 was

Table 1. Clinical characteristics of a series of 24 PCLCL.

CBCL subtype	Age	Sex	Site	Clinical features	Initial therapy and Response	First recurrence (months)	Nº of recurrences	Extracutaneous involvement (months)	Follow-up (months)	Status
PCLBCL, leg-1	64	F	LEG	grouped tumors	PD after CHOP	skin (same site)	progression	LN (8)	23	Death of disease
PCLBCL, leg-2	77	M	LEG	grouped tumors	CR after CHOP+ RDT	testis	NO	TESTIS (6)	17	Death of unrelated causes
PCLBCL, leg-3	69	M	TRUNK	grouped tumors	CR after local RDT	NO	NO	NO	13	Alive with no disease
PCLBCL, leg-4	81	F	LEG	grouped tumors	CR after local RDT	skin, local lymph nodes	1	LN (9)	19	Death of disease
PCLBCL, leg-5	83	F	LEG	solitary nodule	none	NO	NA	NA	4	Death of unrelated causes
PCLBCL, leg-6	64	F	LEG	multiple infiltrated plaques and nodules	CR after local RDT	UK	UK	NO	12	Alive with no disease
PCLBCL, leg-7	80	F	DISSEMINATED	multiple infiltrated plaques and nodules	CR after RDT	NO	NO	NO	14	Alive with no disease
PCLBCL, leg-8	61	F	LEG	multiple infiltrated plaques and nodules	CR after local RDT	skin (24)	progression	LN (24)	72	Death of disease
PCLBCL, leg-9	88	F	DISSEMINATED	multiple infiltrated plaques and nodules	PR after cyclofosphamide and prednisone+RDT	progressive disease, lymph nodes	progression	LN (6)	7	Death of disease
PCLBCL, leg-10	49	M	DISSEMINATED	multiple infiltrated plaques and nodules	CR after CHOP	skin (48)	progression	LN (48)	96	Death of lung adenocarcinoma
PCLBCL, leg-11	65	M	LEG	multiple infiltrated plaques and nodules	PD after CHOP	progressive disease, testis	progression	TESTIS (6)	32	Death of disease
PCLBCL, leg-12	86	M	TRUNK	solitary nodule	CR after local RDT	lymph nodes	1	LN (6)	26	Death of disease
PCLBCL, leg-13	76	M	LEG	grouped tumors	CR after local RDT	skin (12)	2	NO	51	Alive with no disease
PCLBCL, leg-14	89	F	LEG	solitary nodule	CR after local RDT	NO	NO	NO	40	Death of unrelated causes
PCLBCL, leg-15	67	M	LEG	grouped tumors	CR after R-EPOCH	skin (15), progressive disease CNS	2	CNS (40)	44	Alive with disease
PCFCL-1	60	M	TRUNK	multiple nodules	CR after local RDT	NO	NO	NO	14	Alive with no disease
PCFCL-2	37	F	TRUNK	multiple infiltrated plaques and nodules	CR after local RDT	NO	NO	NO	12	Alive with no disease
PCFCL-3	51	M	TRUNK	multiple infiltrated plaques and nodules	CR after local RDT	skin (40)	4	NO	121	Alive with disease
PCFCL-4	82	M	HEAD AND NECK	grouped tumors	CR after local RDT	skin (10)	3	NO	61	Death of unrelated causes
PCFCL-5	75	M	TRUNK	multiple infiltrated plaques and nodules	CR after local RDT	NO	NO	NO	43	Alive with no disease
PCFCL-6	71	F	HEAD AND NECK	solitary nodule	CR after surgery	NO	NO	NO	28	Alive with no disease
PCFCL-7	37	F	HEAD AND NECK	solitary nodule	CR after surgery	NO	NO	NO	34	Alive with no disease
PCFCL-8	65	M	HEAD AND NECK	grouped tumors	CR after CHOP	NO	NO	NO	36	Alive with no disease
PCFCL-9	46	M	TRUNK	grouped tumors	CR after local RDT	NO	NO	NO	96	Alive with no disease

PCFCL: primary cutaneous follicle center lymphoma; PCLBCL, leg type: primary cutaneous large B-cell lymphoma, leg type; M: male; F: female; PD: progressive disease; PR: partial remission; CR: complete remission; RDT: radiotherapy; CHOP: Ciclophosphamide, Doxorubicin, Vincristine and Prednisolone; R-EPOCH: Rituximab, Etoposide, Ciclophosphamide, Doxorubicin, Vincristine and Prednisolone; NA: not available; UK: unknown.

Table 2. Histological, immunophenotypical and molecular cytogenetics characteristics of a series of 24 PCLCL.

CBCL subtype	Morphology, pattern	Predominant cell morphology	BCL2	MUM1	BCL6	CD10	IHC FOXP1	% FOXP1 positive cells	FOXP1 intensity	FISH FOXP1
PCLBCL, leg-1	Diffuse, dermis	Centroblasts	POS	POS	NEG	NEG	NEG	NEG	NEG	3-4 COPIES
PCLBCL, leg-2	Diffuse, dermis + subcutis	Centroblasts	POS	POS	POS	NEG	POS	>75	2	3-4 COPIES
PCLBCL, leg-3	Diffuse, dermis	Centroblasts	POS	POS	POS	NEG	POS	50-75	2	3-4 COPIES
PCLBCL, leg-4	Diffuse, dermis + subcutis	Centroblasts	POS	POS	NEG	NEG	POS	>75	2	3-4 COPIES
PCLBCL, leg-5	Diffuse, dermis + subcutis	Centroblasts	POS	POS	NEG	NEG	POS	25-50	2	NORMAL
PCLBCL, leg-6	Diffuse, dermis	Centroblasts	POS	POS	POS	NEG	POS	>75	3	3-4 COPIES
PCLBCL, leg-7	Diffuse, dermis	Centroblasts	POS	POS	NEG	NEG	NEG	NEG	NEG	NA
PCLBCL, leg-8	Diffuse, dermis	Centroblasts / large centrocytes	POS	NEG	NEG	POS	POS	>75	3	NA
PCLBCL, leg-9	Diffuse, dermis	Centroblasts	POS	NEG	NEG	NEG	POS	>75	3	NA
PCLBCL, leg-10	Diffuse, dermis	Centroblasts / large centrocytes	POS	NEG	POS	NEG	POS	25-50	2	NA
PCLBCL, leg-11	Diffuse, dermis	Centroblasts / immunoblasts	POS	POS	NEG	NEG	POS	>75	1	3-4 COPIES
PCLBCL, leg-12	Diffuse, dermis	Centroblasts	POS	POS	POS	NEG	POS	>75	3	3-4 COPIES
PCLBCL, leg-13	Diffuse, dermis + subcutis	Centroblasts / immunoblasts	POS	NEG	POS	NEG	POS	>75	3	3-4 COPIES
PCLBCL, leg-14	Diffuse, dermis + subcutis	Immunoblasts	POS	NA	NA	NEG	POS	50-75	2	NORMAL
PCLBCL, leg-15	Diffuse, dermis + subcutis	Centroblasts / large centrocytes	POS	POS	NEG	POS	POS	>75	2	3-4 COPIES
PCFCL-1	Diffuse, dermis	Large centrocytes / centroblasts	NEG	NEG	POS	NEG	NA	NA	NA	NORMAL
PCFCL-2	Diffuse, dermis	Large centrocytes / centroblasts	NEG	NEG	POS	NEG	NA	NA	NA	3-4 COPIES
PCFCL-3	Diffuse, dermis + subcutis	Large centrocytes / centroblasts	NEG	NEG	POS	NEG	NEG	NEG	NEG	3-4 COPIES
PCFCL-4	Diffuse, dermis	Large centrocytes / centroblasts	POS	NEG	POS	POS	NA	NA	NA	NORMAL
PCFCL-5	Diffuse, dermis	Large centrocytes / centroblasts	POS	NEG	POS	NEG	NEG	NEG	NEG	3-4 COPIES
PCFCL-6	Diffuse, dermis + subcutis	Large centrocytes / centroblasts	NEG	NEG	POS	POS	NEG	NEG	NEG	NORMAL
PCFCL-7	Nodular, dermis	Large centrocytes / centroblasts	POS	NEG	POS	POS	NEG	NEG	NEG	NORMAL
PCFCL-8	Nodular, dermis + subcutis	Centroblasts / large centrocytes	NEG	NEG	POS	NEG	NEG	NEG	NEG	NA
PCFCL-9	Diffuse, dermis + subcutis	Centroblasts / large centrocytes	NEG	NEG	POS	POS	NEG	NEG	NEG	NORMAL

PCFCL: primary cutaneous follicle center lymphoma; PCLBCL, leg type: primary cutaneous large B-cell lymphoma, leg type; POS: positive; NEG: negative; NA: not available.

positive in all PCFCL studied.

FISH results

Eleven cases of PCLBCL, leg type were examined for translocations using FISH analysis with the *FOXP1* (3p14.1) dual color break-apart non-commercial probe. As summarized in Table 2, none of the analyzed cases displayed translocations involving the *FOXP1* gene. Two cases of PCLBCL, leg type presented a normal FISH pattern and nine cases showed a 3F/4F pattern (Fig. 3). Two cases with extra copies for *FOXP1* gene that had sufficient material for additional FISH analysis had displayed extra copies of the chromosome 3. All cases with gains of *FOXP1* gene except one, presented overexpression of the FOXP1 protein in more than 50% of tumoral cells. However, FOXP1 was also expressed in two cases without extra copies for *FOXP1*.

Available FISH results were obtained in 8 out of 9 cases of PCFCL. No translocations of the *FOXP1* gene were detected. Among PCFCL, five cases showed a normal 2F pattern (Fig. 3) and three cases displayed extra copies of *FOXP1* gene (3 to 4 copies) with a 3F/4F pattern, in absence of protein expression. Poliploidy of chromosome 3 using the CEP3 probe was detected in one patient with extra copies for *FOXP1* gene. However, no statistical relationship could be assessed between FOXP1 expression and *FOXP1* gene status.

Discussion

PCLBCL, leg type is an aggressive tumor that may occur in locations other than the leg, and overlapping clinical and morphological features with PCFCL may exist, particularly in cases of PCFCL mainly composed by large cells. FOXP1 protein expression has been postulated as a useful tool to distinguish both entities. However, there is no information regarding *FOXP1* gene alterations in primary cutaneous lymphomas. The current study was designed to investigate *FOXP1* gene status (numerical and structural aberrations) in a series of PCLBCL, leg type and PCFCL patients using paraffin section interphase FISH analysis. A screening approach using a dual color break-apart probe for *FOXP1* locus was used because this technique allows the identification of rearrangements at the *FOXP1* gene region, regardless of what other chromosomal regions are involved.

In our series, we observed a high expression of FOXP1 protein (87%) in PCLBCL, leg type. In contrast, PCFCL were all negative for FOXP1 in the six cases with available material. Similar results have been reported in the literature. Kodama et al. (2005), Hoefnagel et al. (2006) and more recently Hallermann et al. (2007) reported that 23/29, 11/11 and 19/21 of PCLBCL, leg type expressed FOXP1, respectively.

FISH studies have not shown the presence of rearrangements involving the *FOXP1* gene among our

PCLBCL, leg type or PCFCL. As all samples included in this study consistently contained more than 50% of tumor cells, it is highly unlikely that the lack of rearrangements detectable by FISH was caused by technical limitations. Although *FOXP1* rearrangements have been demonstrated at a higher frequency in extranodal DLBCL with gastric presentation (Haralambieva et al., 2006), our results are in

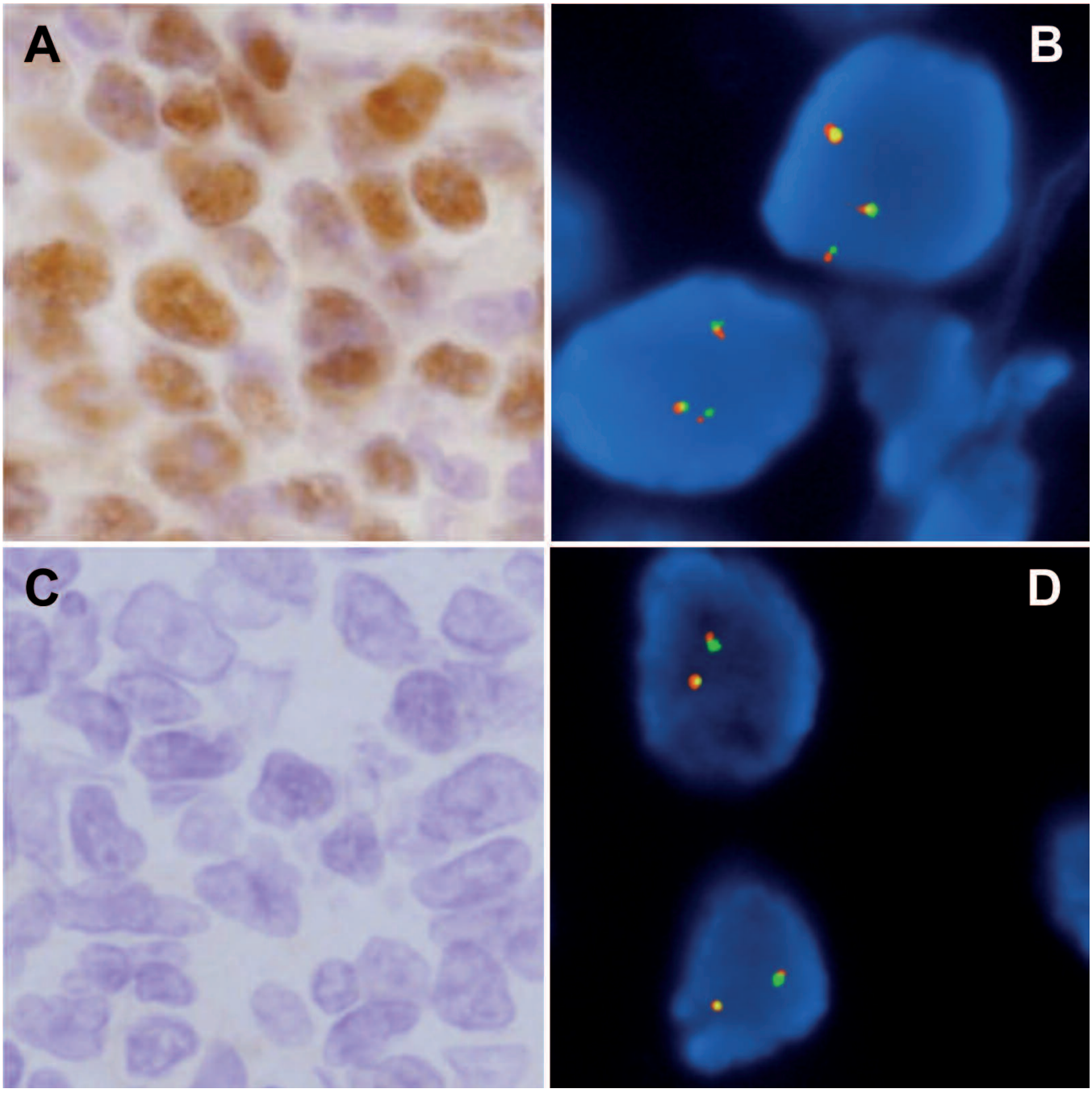

Fig. 3. Representative images of FOXP1 immunostaining and fluorescence in situ hybridization (FISH) in PCLBCL. **A, B.** Primary cutaneous large B-cell lymphoma, leg type (PCLBCL, leg type) showing FOXP1 high protein expression **(A)** and 3F *FOXP1* dual color break-apart FISH pattern **(B)** indicating the presence of an extra copy of the *FOXP1* gene. **C, D.** Primary cutaneous follicle center lymphoma (PCFCL) showing negativity for FOXP1 protein expression **(C)** and 2F *FOXP1* dual color break-apart FISH pattern **(D)** indicating the absence of translocations or gains of this gene. A, C, x 600; B, D, x 1000

accordance with results of a study by Barrans et al involving a large series of nodal and extranodal systemic DLBCL (Barrans et al., 2007). Recently, Goatly et al. studied a large series of 595 lymphomas by interphase FISH with 10 BAC clones covering the whole *FOXP1* gene, in order to map *FOXP1* breakpoints. In this series, there was only one case of cutaneous DLBCL, which did not present *FOXP1* translocation (Goatly et al., 2008). Regarding *FOXP1* translocations, the break-apart probe used in the present study includes only two BAC clones, RP11-79P21 and RP11-713J7, located at 5' and 3' ends of the gene, and allows the detection of all the possible breakpoints described by Goatly et al. (2008).

Extra copies of the *FOXP1* gene were observed in 82% of cases of PCLBCL, leg type investigated, which supports this alteration as a mechanism of over-expression of the protein. Interestingly, gains of genomic material at the *FOXP1* locus have also been associated to the ABC-like DLBCL subtype, and therefore with an adverse prognosis (Wlodarska et al., 2005; Fenton et al., 2006; Haralambieva et al., 2006; Barrans et al., 2007). Other mechanisms linked to increased FOXP1 expression levels are gains of the entire chromosome 3, suggested by the presence of additional FISH signals at the *FOXP1* locus, and confirmed by conventional GTG banding cytogenetics and FISH with a chromosome 3 alpha-satellite pericentromeric probe (Haralambieva et al., 2006). In this way, three cases of our series with extra copies of chromosome 3 were observed. These cases were previously studied by conventional comparative genomic hybridization (CGH) in a series of 18 primary and secondary cutaneous LBCL (Giménez et al., 2005). In five cases, a gain of the centromere of chromosome 3 was observed, and gains on 3p and/or 3q were found in approximately 30% of cases. However, these findings have not been confirmed in a study using BAC-arrayCGH (Dijkman et al., 2006).

The presence of low-level gene copy number (three to four copies) have also been described in solid tumors, such as low-level *EGFR* (7p12) copy number increase in prostate and lung cancers. In these cases, a simultaneous increase of centromere 7 copy number in virtually all of these cases suggests that a gain of the entire chromosome 7 (polysomy 7) caused most *EGFR* copy number gains. The clear-cut association of such low-level gene copy number gains with EGFR protein expression emphasizes the strong effect of minimal DNA copy number changes on *EGFR* gene expression (Schlomm et al. 2007). In contrast, in our series, two cases of PCLBCL, leg type had strong expression of the protein in absence of any increase in copy number and two cases of PCFCL had gains of *FOXP1* in absence of protein expression.

In conclusion, our observations indicate that Aalternative mechanisms other than genetic alterations, B-cell activation among them, are capable of regulatating *FOXP1*. These results provide give additional information regarding genetic features in pathogenesis of PCLBCL, leg type.

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