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IRF4 Expression without *IRF4* Rearrangement Is a General Feature of Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type

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TO THE EDITOR

The involvement of the interferon regulatory factor 4 gene (*IRF4*), also known as multiple myeloma antigen 1 (*MUM1*), in balanced rearrangement or translocation has been recently observed in a subset of cutaneous T-cell lymphomas (CTCLs), such as cutaneous anaplastic large-cell lymphoma (C-ALCL) and transformed mycosis fungoides (Feldman *et al.*, 2009; Pham-Ledard *et al.*, 2009). *IRF4* expression reaches a high level in differentiated plasma cells and is also detectable by immunostaining in some activated T cells and melanocytes, with the latter providing internal positive controls on skin sections (Falini *et al.*, 2000; Lu, 2008). Despite such a restricted immunostaining pattern, *IRF4* is an essential regulator at multiple steps of B-cell differentiation, such as pre-B-cell differentiation, germinal center formation, immunoglobulin class switch recombination, and terminal differentiation of B cells to plasma cells, as shown in *IRF4*-deficient mice (reviewed in Shaffer *et al.*, 2009). IRF4 is also essential for T-helper (Th) cell differentiation and is required for either Th2 or Th17 cell development (Brustle *et al.*, 2007; Zheng *et al.*, 2009). An oncogenic role of *IRF4* has first been supported by the identification of *IRF4* involvement in the t(6;14)(p25;q32) translocation in some cases of multiple myeloma (MM) (Iida *et al.*, 1997). In t(6;14)(p25;q32), IRF4 is juxtaposed with the immunoglobulin heavy-chain gene locus leading to *IRF4* deregulated expression (Iida *et al.*, 1997; Yoshida *et al.*, 1999; Shaffer *et al.*, 2008). Alternatively, *IRF4* rearrangements in peripheral T-cell lymphoma do not commonly involve either the *TCRB* or the *TCRA* gene locus, as shown in eight C-ALCL and two systemic T-cell lymphomas (Feldman *et al.*, 2009).

Among primary cutaneous B-cell lymphomas, primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, leg type) is an original entity with poor prognosis that was first reported in 1996 and that mostly affects the leg(s) in elderly but may also arise at other sites in approximately 10% of cases (Vermeer *et al.*, 1996; Willemze *et al.*, 2005; Meijer *et al.*, 2008). PCLBCL, leg type, differs from primary cutaneous follicle center lymphoma by the presence of confluent sheets of centroblasts and immunoblasts, many with a peculiar round cell morphology, which strongly express B-cell CLL/lymphoma 2 (BCL2), IRF4, and forkhead box P1 (FOXP1) (Willemze *et al.*, 2005; Meijer *et al.*, 2008). Interestingly, round cell morphology, strong BCL2

Abbreviations: FISH, fluorescence in situ *hybridization; IRF4, interferon regulatory factor 4; MM, multiple myeloma; MUM1, multiple myeloma antigen 1; PCLBCL, leg type, primary cutaneous diffuse large B-cell lymphoma, leg type*

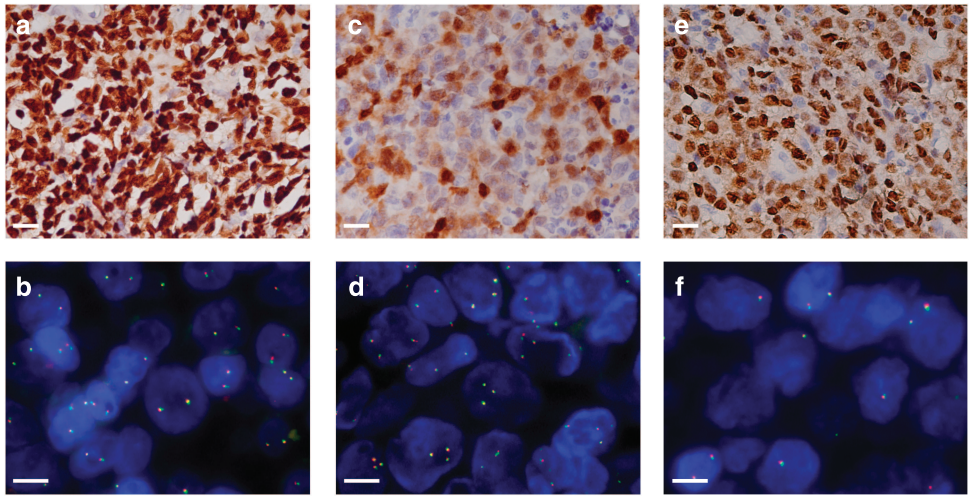

Figure 1. Interferon regulatory factor 4 (IRF4) expression according to *IRF4* fluorescence *in situ* hybridization (FISH) pattern in three cases of cutaneous large B-cell lymphoma, leg type (scale bar = 5 μm). (**a, b,** left panel) Skin sections of first case. (**a**) IRF4 immunostaining in most tumor cells. (**b**) Normal two fusion signals pattern showing *IRF4* normal status. (**c, d,** middle panel) Skin sections of second case. (**c**) IRF4 immunostaining in approximately 50% of tumor cells. (**d**) The three fusion signals pattern showing one extra copy of *IRF4* locus. (**e, f,** right panel) Skin sections of the third case. (**e**) IRF4 immunostaining in approximately 90% of tumor cells. (**f**) The one fusion signal pattern showing monoallelic deletion of *IRF4* locus.

expression (>50% of cells), IRF4 expression (>30% of cells), or FOXP1 expression have been used to differentiate PCLBCL, leg type, from primary cutaneous follicle center lymphoma with a diffuse pattern, although 10% of the latter may express BCL2, IRF4, or less frequently FOXP1 (Kodama *et al.*, 2005; Senff *et al.*, 2007). Strong BCL2 expression has been found in 85–100% of PCLBCL, leg type, whereas IRF4 expression was found in 68–90% of cases that may also depend on differences in fixation and antigen retrieval procedures or positivity threshold between series (Kodama *et al.*, 2005; Grange *et al.*, 2007; Senff *et al.*, 2007). With some differences between series, each of these histological criteria has been reported as an independent adverse prognostic factor together with clinical parameters such as location on the leg or multiple skin lesions (Kodama *et al.*, 2005; Grange *et al.*, 2007; Senff *et al.*, 2007). Conversely, IRF4 expression is a rare finding in other primary cutaneous B-cell lymphomas (Kodama *et al.*, 2005; Willemze *et al.*, 2005; Senff *et al.*, 2007).

Owing to the oncogenic role of *IRF4* translocation in MM and the detection of IRF4 rearrangements in a subset of CTCL with IRF4 expression (Pham-Ledard *et al.*, 2009), we decided to analyze *IRF4* expression and rearrangements in 29 cases of PCLBCL, leg type. Inclusion criteria were a complete clinical staging and follow-up to exclude lymphoma with secondary skin involvement. Formalin-fixed paraffin-embedded sections were used for immunostaining with an anti-MUM-1 antibody and for fluorescence *in situ* hybridization (FISH) analysis of *IRF4* status with break-apart probes, as reported recently (Pham-Ledard *et al.*, 2009). The 29 patients (mean age 82 years) had a male:female ratio of 10:19 and presented typical lesions of PCLBCL, leg type, located on the leg ($n=21$), upper limb ($n=4$), trunk ($n=1$), or head and neck ($n=3$) (see Supplementary Table S1 online). Immunostaining was scored positive in 25 out of 29 analyzed cases (86%) with >50% of tumor cells expressing IRF4 (Figure 1a,c). It was scored negative in 4 out of 29 cases (14%) with <30% positive tumor cells. BCL2 immunostaining was scored positive in 26 out of 29 cases (90%) and negative in 3 out of 29 cases (10%) with no overlap with negative IRF4 immunostaining. No case showed a break-apart or split signal. A normal FISH pattern with two fusion signals was observed in 26 out of 29 cases (90%; Figure 1b). In two cases, one extra copy of *IRF4* locus with three fusion signals was observed in 64 and 75% of tumor cells, respectively (Figure 1d). A single case showed a deletion of one *IRF4* allele in 67% of tumor cells (Figure 1f), although this case showed a strong IRF4 immunoreactivity (Figure 1e).

Our study clearly shows that the typical IRF4 expression by PCLBCL, leg type, is not associated with *IRF4* gene rearrangement or amplification. Moreover, extra copy of *IRF4* allele was not associated with an increase in IRF4 immunostaining pattern, as previously reported in CTCL (Pham-Ledard *et al.*, 2009). Alternatively, the four PCLBCL, leg type, cases with negative IRF4 immunostaining showed a normal FISH pattern and the single case with *IRF4* monoallelic deletion was strongly IRF4 positive. Therefore, *IRF4* expression in PCLBCL, leg type, is likely to be the result of several mechanisms, including differentiation stage, epigenetic regulation, or other oncogenes deregulation. Recent data have shown that constitutive activation of the intrinsic-mediated apoptosis pathway with concomitant downstream inhibition of this pathway may support the cellular resistance of PCLBCL, leg type, to chemotherapy (van Galen *et al.*, 2008). Moreover, RNA interference with *IRF4* expression is lethal in MM cell lines, irrespective of *IRF4* genetic status (Shaffer *et al.*, 2009). Whether PCLBCL, leg type, is addicted to the presence of IRF4 and dependent

upon its functions for tumor cell survival or proliferation has to be further analyzed.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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