Recurrent presence of the PLCG1 S345F mutation in nodal peripheral T-cell lymphomas

Peripheral T-cell lymphomas (PTCLs) are a group of non-Hodgkin lymphomas (NHLs) with heterogeneous clinical presentation, histology, response to treatment and outcome, whose genetic background is still poorly understood. Patients with PTCL are usually treated with CHOP or more intensive regimens, generally with minimal effectiveness, thus highlighting the need for new therapeutic strategies.[1]

Several findings suggest that the survival of normal and, frequently, neoplastic T cells depends upon T-cell receptor (TCR) signaling.[2] The t(5;9)(q33;q22), which results in an ITK-SYK fusion transcript, has been described in PTCL and angioimmunoblastic T-cell lymphoma (AITL). Moreover, transgenic mice with this translocation display chronic proximal TCR signaling, culminating in T-cell lymphomas that could be inhibited by treatment with SYK-inhibitors.[2,3] SYK is also over-expressed in almost 90% of nodal-PTCLs (n-PTCL) and mutated in other PTCL-specific subtypes.[4] In addition, Palomero *et al.*[5] have recently reported activating mutations in FYN tyrosine kinase, another SRC family kinase found in T lymphocytes that has an important role in T-cell activation upon TCR stimulation. Recently, the relevance of several mutated genes (*TET2, IDH2, DNMT3A, RHOA*) in T-cell lymphoma pathogenesis has become apparent.[5-7]

Nevertheless, as gene expression array studies have shown, not all PTCLs depend on TCR signaling.[8] De Leval *et al.* classified PTCL cases according to gene signatures associated with CD30 expression or T-cell activation/TCR-signaling.[8] Moreover, several authors have confirmed an inverse correlation between the levels of expression of CD30 and TCR genes.[9]

The TCR is a multimeric complex that is expressed on the cell surface in association with four CD3 molecules. Upon receptor ligation, two tyrosine residues are rapidly phosphorylated by a member of the src-family protein tyrosine kinase (PTK), transforming them into high-affinity ligands for Syk PTKs. The co-ordinated actions of Src and Syk PTK initiate a cascade of signals that ultimately leads to cell proliferation, cytokine secretion and effector functions. Nevertheless, the resulting increase in intracellular calcium concentration ($[Ca2^+]_i$), occurring partly as a result of phosphorylating and activating phospholipase C-γ1 (PLCG1),[10] is critical for TCR stimulation. Activated PLCG1 generates the second messenger inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) from the hydrolysis of phosphatidynositol-4,5-bisphosphate (PIP2). Whereas IP3 mediates the elevation of $[Ca2^+]_i$, which is essential for activating the nuclear factor of activated T cells (NFAT),[11] DAG activates the Ras-ERK pathway[12] and protein kinase C (PKC), which mediates the activation of NF-kB.[13]

A recurrent mutation in the *PLCG1* gene encoding a protein with p.Ser345Phe alteration (S345F) has recently been identified that affects the PLCx protein catalytic domain in approximately 20% of cutaneous T-cell lymphomas (CTCLs),[14] and a similar finding has been reported for one AITL case.[15] Functional studies showed that PLCG1 mutants could increase NFAT activity and were highly sensitive to calcineurin inhibitors.[14]

Due to the importance of the TCR pathway in PTCL, and because PLCG1 is a critical mediator of TCR signaling, we decided to explore the frequency and biological relevance of this PLCG1-S345F mutation in n-PTCL patients.

Table 1. Statistical analysis of the clinical and molecular parameters and the mutational status of the *PLCG1* gene in the cohort of 101 patients with PTCLs.

	Clinical and molecular parameters			
	N. of cases (%)	WT	MUT	*P*
N	101	88	13	
DX	101	88	13	0.662
AITL	60 (59.4%)	53 (60.2%)	7 (53.8%)	
PTCL-NOS	41 (40.6%)	35 (39.8%)	6 (46.2%)	
Sex	98	85	13	0.525
Male	60 (61.2%)	51 (60%)	9 (69.2%)	
Female	38 (38.8%)	34 (40%)	4 (30.8%)	
Age at diagnosis	96	83	13	0.212
<60 years	30 (31.2%)	24 (28.9%)	6 (46.2%)	
≥60 years	66 (68.8%)	59 (71.1%)	7 (53.8%)	
IPI	87	74	13	0.829
Low risk	28 (32.2%)	23 (31.3%)	5 (38.5%)	
Low-intermediate risk	22 (25.3%)	19 (25.7%)	3 (23.1%)	
High-intermediate risk	21 (24.1%)	19 (25.7%)	2 (15.4%)	
High risk	16 (18.4%)	13 (17.6%)	3 (23.1%)	
PIT	76	64	12	0.504
Low risk	11 (14.5%)	10 (15.6%)	1 (8.3%)	
Low-intermediate risk	28 (36.8%)	25 (39.1%)	3 (25%)	
High-intermediate risk	22 (28.9%)	18 (28.1%)	4 (33.3%)	
High risk	15 (19.7%)	11 (17.2%)	4 (33.3%)	
ECOG	83	70	13	0.346
<1	60 (72.3%)	52 (74.3%)	8 (61.5%)	
≥1	23 (27.7%)	18 (25.7%)	5 (38.5%)	
Treatment	85	75	10	0.278
CHOP/CHOP-LIKE	63 (74.1%)	57 (76%)	6 (60%)	
Others	22 (25.9%)	18 (24%)	4 (40%)	
Response	81	69	12	0.280
CR	51 (63%)	45 (65.2%)	6 (50%)	
PR	16 (19.8%)	14 (20.3%)	2 (16.7%)	
No response	14 (17.3%)	10 (14.5%)	4 (33.3%)	
Recurrence	75	64	11	0.644
No	50 (66.7%)	42 (65.6%)	8 (72.7%)	
Yes	25 (33.3%)	22 (34.4%)	3 (27.3%)	
Patient status	93	80	13	0.212
Dead	57 (61.3%)	47 (58.8%)	10 (76.9%)	
Alive	36 (38.7%)	33 (41.2%)	3 (23.1%)	
NFATc1	95	83	12	0.754
Negative	28 (29.5%)	24 (28.9%)	4 (33.3%)	
Positive	67 (70.5%)	59 (71.1%)	8 (66.7%)	
P50	98	85	13	0.027
Negative	24 (24.5%)	24 (28.2%)	0 (0%)	
Positive	74 (75.5%)	61 (71.8%)	13 (100%)	
P52	97	84	13	0.294
Negative	35 (36.1%)	32 (38.1%)	3 (23.1%)	
Positive	62 (63.9%)	52 (61.9%)	10 (76.9%)	
P-ERK	102	89	13	0.975
Negative	71 (69.6%)	62 (69.7%)	9 (69.2%)	
Positive	31 (30.4%)	27 (30.3%)	4 (30.8%)	
CD30	94	81	13	<0.001
Negative	77 (81.9%)	71 (87.7%)	6 (46.2%)	
Positive	17 (18.1%)	10 (12.3%)	7 (53.8%)	
Ki67	100	87	13	0.148
Negative	78 (78%)	70 (80.5%)	8 (61.5%)	
Positive	22 (22%)	17 (19.5%)	5 (38.5%)	
CD3	94	81	13	0.148
Negative	6 (6.4%)	4 (4.9%)	2 (15.4%)	
Positive	88 (93.6%)	77 (95.1%)	11 (84.6%)	

DX: diagnosis; AITL: angioimmunoblastic T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma not specified; WT: wild type; MUT: mutated; IPI: International Prognostic Index; PIT: Prognostic Index for PTCL; ECOG: Eastern Cooperative Oncology Group; CHOP: cyclophosphamide, vincristine, doxorubicin, prednisone; CR: total response; PR: partial response.

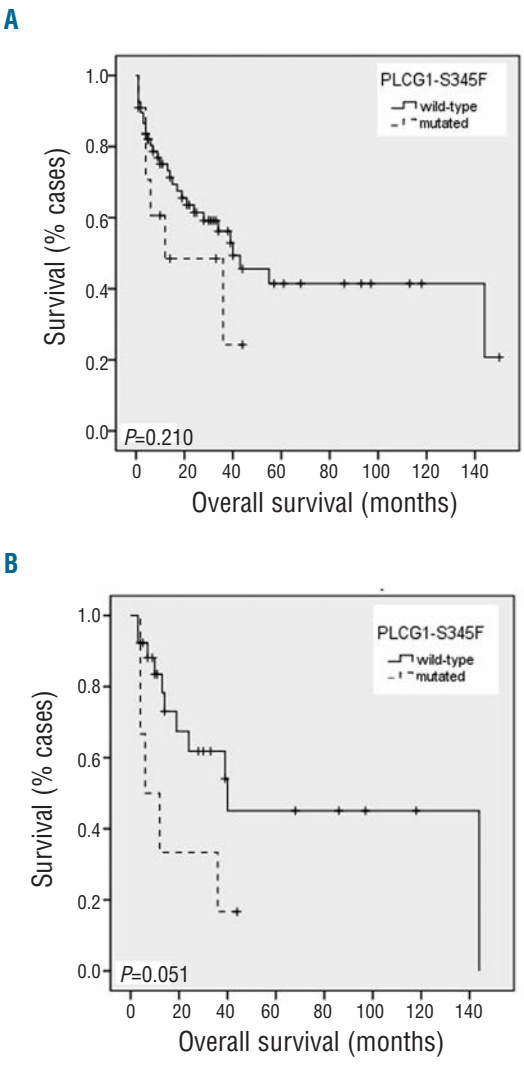

Figure 1. Kaplan-Meier survival curves for PLCG1 mutated and wild-type patients. (A) Whole cohort of nodal-PTCL. (B) PTCL-NOS subgroup.

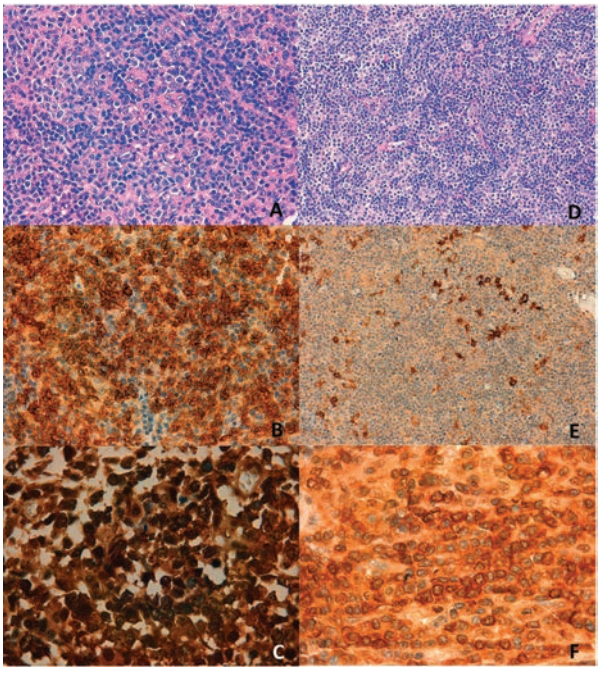

Figure 2. Representative images corresponding to a PLCG1-mutated (A-C) and a non-mutated (D-F) case, respectively. (A and D) H&E staining. (B and E) CD30 IHC detection showing positivity in the mutated case (B) *versus* the lack of expression in the tumoral cells in the *PLCG1*-wt case. Scarce CD30-positive blasts could be seen (E). (C and F) p50 immunoreactivity. p50 shows a clear nuclear expression in the mutated case (C) whereas its expression is restricted to the cytoplasm in the non-mutated case (F).

We first examined the presence of the PLCG1-S345F mutation in a series of 101 formalin-fixed, paraffin-embedded (FFPE) PTCLs samples including 60 AITL and 41 peripheral T-cell lymphoma not otherwise specified (PTCL-NOS). Clinical data for the patients and some mutational and GEP data have been reported in two previous studies[6,9] (see *Online Supplementary Appendix for further information*).

First, DNA was extracted from tumor from the FFPE samples of this group of patients. The PLCG1-S345F mutation was analyzed using two independent techniques, as previously reported[14] (*Online Supplementary Appendix*). Only those cases found to be positive by both techniques were considered as mutated. All experiments were carried out blinded with respect to the clinical data.

The PLCG1-S345F mutation was found in 12.9% of the patients (13 of 101 PTCLs), comprising 11.7% (7 of 60) of AITL and 14.6% (6 of 41) of PTCL-NOS patients (Table 1), of whom 1 of 6 showed AITL-features. Interestingly, no correlation was found between the presence of *RHOA* and *PLCG1* mutation in this series (*data not shown*).

We analyzed the association of *PLCG1* mutation with clinical data, and found no clear association between the *PLCG1* mutation and overall survival (OS) or other prognostic factors in the whole series; however, we found that PLCG-mutated PTCL-NOSs showed a lower OS (log rank χ^2=3.81; *P*=0.05) (Figure 1). There was also an association with response to treatment (*P*=0.08) that narrowly failed to reach statistical significance, probably as a consequence of the small sample (*Online Supplementary Tables S1 and S2*).

Tissue micro arrays (TMA) were also constructed from FFPE samples and TMA sections were stained by the Endvision method with a heat-induced antigen-retrieval step for CD3, CD30, NFATc1, Ki67, p-ERK antibodies and NF-KB subunits for the classic and alternative NF-KB pathways, p50 and p52, respectively. Cases were considered positive for each marker following previously reported cut-off values for each.[6,9,14] Reactive tonsil tissue was included as a control. The primary antibodies were omitted to provide negative controls (*Online Supplementary Appendix and Online Supplementary Table S3*).

Immunohistochemical studies revealed positivity for CD3 in 87.1% (88 of 101), CD30 in 16.8% (17 of 101) and Ki67 in 21.8% (22 of 101) of the cases. Nuclear immunostaining for NFATc1, p-ERK, p50 and p52 was found in 66.3% (67 of 101), 29.7% (30 of 101), 73.3% (74 of 101) and 61.4% (62 of 101) of the cases, respectively.

No significant correlation was found between the presence of the PLCG1-S345F mutation and NFATc1 expression, although 66.7% of the mutated cases (8 of 12) were positive. This figure is lower than the 81.8% previously reported for CTCLs. Overexpression of NFAT in non-mutated cases could be explained by the presence of a preserved TCR/CD3 pathway in this subgroup of tumors. On the other hand, a direct statistically significant relationship was found between the presence of the PLCG1-S345F

mutation and both CD30 [7 out of 13 mutated cases (53.8%) expressed CD30 *vs.* 10 out of 81 [(12.3%) of the non-mutated cases; $P<0.001$)] and p50 [(13 out of 13 mutated cases (100%)] showed p50 nuclear expression; $P=0.027$)] (Table 1 and Figure 2). Analyzing this in greater depth, with respect to the histological type diagnosed, we found that the statistical relationship with CD30 was maintained in the PTCL-NOS and AITL groups (*Online Supplementary Tables S1, S2 and S4*). Increased signaling from mutated PLCG1, associated with increased NF-kB activity and CD30 expression, could theoretically replace the survival signaling from T-cell receptor. These data are consistent with previously published data showing that CD30 and TCR signaling are mutually exclusive in PTCL.[8,9]

These findings are of potential therapeutic relevance, since PLC and NF-kB inhibition and CD30-targeted treatments could be explored for *PLCG1*-mutated cases, thereby contributing to the selection of targeted treatment based on the molecular features of the tumors.

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