

### Translocation t(9;14)(p13;q32) in cases of splenic marginal zone lymphoma

**Translocation t(9;14)(p13;q32) involving PAX5 and IGH genes was first described in lymphoplasmacytic lymphoma. New data suggest that this translocation is not restricted to a specific morphologic subtype but occurs in other B-cell lymphomas. We present three cases with a diagnosis of splenic marginal zone lymphoma and t(9;14) confirmed by fluorescent *in situ* hybridization.**

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Translocation t(9;14)(p13;q32) involving *PAX5* and *IGH* genes was first described in lymphoplasmacytic lymphoma.<sup>1</sup> However, Tracy *et al.*<sup>2</sup> recently studied a series of 37 low-grade B-cell lymphomas: 13 lymphoplasmacytic lymphomas, 18 marginal zone lymphomas (8 with the splenic form) and 6 small lymphocytic lymphomas by fluorescence *in situ* hybridization (FISH) with a *PAX5* probe and did not detect any evidence of *PAX5* rearrangement, suggesting that t(9;14)(p13;q32) is not frequent in lymphoplasmacytic lymphomas and in other low-grade B-cell lymphomas. Poppe *et al.*<sup>3</sup> reported *PAX5/IGH* rearrangement in 10 B-cell lymphomas using FISH. This aberration was detected in four cases of histiocyte-rich, T-cell-rich B-cell lymphomas and in two cases of post-transplantation diffuse large B-cell lymphomas. They concluded that t(9;14)(p13;q32) was not restricted to a specific morphologic subtype and occurred in different clinical settings with advanced disease and adverse prognosis.

Given the heterogeneity of lymphomas associated with t(9;14)(p13;q32) illustrated by the previous reports,<sup>1-4</sup> our aim was to describe the involvement of this translocation in splenic marginal zone lymphoma (SMZL). The subjects were patients referred from different hospitals affiliated to the Spanish Cytogenetic Working Group (GCECGH, AEHH) and from *Red de Grupos de Linfomas* (G03/179)

between 2000 and 2005. Among our series of 160 SMZL (*unpublished data*), the t(9;14)(p13;q32) was detected by spectral karyotyping (SKY) in three cases with a complex karyotype (Figure 1A). The patients were diagnosed according to the criteria of Mollejo *et al.*<sup>5</sup> The diagnosis of case #1 was suggested by clinical, morphological, cytological, immunophenotypic, and cytogenetic studies from peripheral blood. Clinical, histological and cytogenetic data are summarized in Table 1.

FISH studies were performed with the aim of confirming the involvement of the *PAX5* and *IGH* genes. We used split probes with 5' sequences labeled in red and 3' sequences in green according to the Dako (*PAX5*, Dako, Denmark) and Vysis (*IGH*, Vysis, Downers Grove, IL, USA) data sheets. A minimum of 200 interphase nuclei and five metaphase cells were scored (Figure 1BC). To evaluate the cut-off of these locus-specific probes we analyzed five healthy donors.

In case #2, conventional cytogenetics revealed metaphases with t(14;19) as a sole translocation and SKY also revealed a t(9;14) in a low proportion of cells. One year after the diagnosis, G-banding was able to detect the two translocations in a large proportion of cells. According to FISH studies of paraffin-embedded spleen samples, this patient showed double translocation of the *IGH* gene in a low proportion of cells. A large population of cells presented only one rearrangement, suggesting that the t(9;14) was a secondary aberration. We performed FISH with a *BCL3* split probe (DAKO, Denmark) to check the involvement of this gene in t(14;19) (Figure 1D). The FISH results are summarised in Table 2.

Only a few series of SMZL patients have reported chromosomal data and these revealed a high incidence of deletions of 7q, gains of 3q and a few cases with translocations involving 14q32.<sup>6-7</sup> To our knowledge, only one case of SMZL with a complex translocation t(2;9;14)(p12;p13;q32) involving the *PAX5* gene has been previously reported.<sup>8</sup> Reviewing all reported cases with t(9;14), it is interesting to note that all of them had complex karyotypes and in some patients t(9;14) was detected after the use of multicolor FISH techniques.

Among additional anomalies in cases with t(9;14), involvement of chromosome 1 (usually duplications in 1q) and trisomy 3 have been found most frequently. Our three

**Table 1.** Clinical data in three patients with splenic marginal zone lymphoma (SMZL) and t(9;14)(p13;q32).

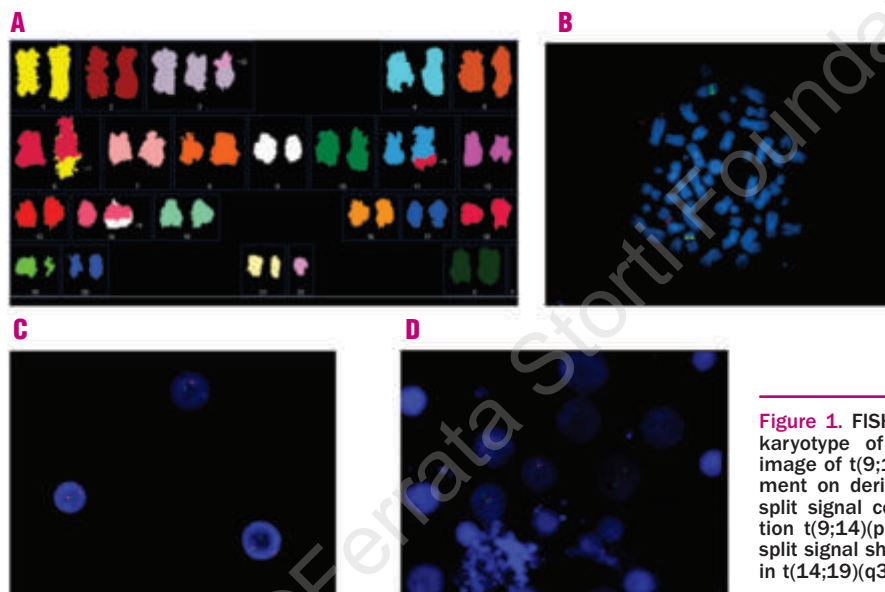
Case	Age/Sex	Splenomegaly	Bone marrow involvement	Immunophenotype (tissue)	Histology	Karyotype/SKY (tissue)
1 <sup>†</sup>	69/F	Yes	No	CD10-, CD20+, CD79b-, CD5-, CD23+, CyclinD1- (PB)	Not done	46-49,XX,+3, der(3)t(3;22)(p11;q11), der(6)t(1;6)(q10;q27), del(7)(q32), der(11)t(6;11)(?;q23), t(9;14)(p13;q32), -22, +mar(2), +mar(9), +mar(11) [cp5] (PB)
2	73/F	Yes 500 g	Yes	CD10-, CD20+, CD79b+, IgD+, CD5-, P53-, Cyclin D1- (BM)	SMZL diffuse pattern	47-49,X,-X,+3, der(3)t(3;8)(pter?8p11::3p11?3qter), +7, der(8)t(3;8)(3?q::8p11?8q22::3?q), t(9;14)(p13;q32), t(14;19)(q32;q13), -21, +mar(11), +r(1)[cp4] (BM)
3	73/F	Yes 2279 g	Yes	CD10-, CD20+, CD79a+, CD43-, IgD+, IgM+, CD5-, CD23-, P53-, Cyclin D1- (PB)	SMZL diffuse pattern	46-49,XX, dup(1)(q22q32), +4, del(4)(p11), del(4)(p11), +7, del(9)(p21), der(9)t(9;14)(9pter?9p13::14q32?14q11::9p13?9qter), t(2;16)(?;q22), +mar (?4) [cp3] (PB)

†: died; PB: peripheral blood; BM: bone marrow.

**Table 2.** FISH studies of the three cases of SMZL and *PAX5/IGH* rearrangement.

Case	Aberration	Tissue Sample	Probe	Conclusions and comments
1	t(9;14)(p13;q32)	PB	<i>PAX5</i> split <i>IGH</i> split	reciprocal translocation <i>PAX5/IGH</i>
2	t(9;14)(p13;q32)	BM	<i>PAX5</i> split <i>IGH</i> split	reciprocal translocation <i>PAX5/IGH</i>
	t(14;19)(q32;q13)	BM	<i>BCL3</i> split <i>IGH</i> split	reciprocal translocation <i>BCL3/IGH</i>
		Spleen	<i>PAX5</i> split <i>IGH</i> split	double split signal reciprocal translocations <i>PAX5/IGH</i> <i>BCL3/IGH</i>
3	t(9;14)(p13;q32)	PB	<i>PAX5</i> split <i>IGH</i> split	reciprocal translocation <i>PAX5/IGH</i>
		Spleen	<i>PAX5</i> split <i>IGH</i> split	

PB: peripheral blood; BM: bone marrow.



**Figure 1.** FISH results. **A.** SKY of the complex karyotype of case #1 as a representative image of t(9;14)(p13;q32); **B.** *PAX5* rearrangement on derivative 14 (case #2); **C.** The *IGH* split signal confirms the reciprocal translocation t(9;14)(p13;q32) (case #3); **D.** The *BCL3* split signal shows the involvement of this gene in t(14;19)(q32;q13) (case #2).

patients with SMZL also had complex karyotypes with partial gains of 1q (3/3 cases) and trisomies 3 and 7 (2/3 cases). In two of them, t(9;14) was detected by SKY. Poppe *et al.* reported that t(9;14) was associated with an adverse prognosis but it could be argued that the poor prognosis of t(9;14) is due to the complexity of the karyotypes rather than to the t(9;14). In our series, one patient died 4 years after being diagnosed and the follow-up of the other two cases is too short to determine the prognostic significance of this aberration in SMZL.

A histological evaluation of the spleen was available for two of our three patients. Interestingly, both these cases had a diffuse splenic pattern uncommon in this type of lymphoma. In one of them, the infiltration was just detected after the molecular study of *IGH* rearrangement (Genescan). Mollejo *et al.*<sup>8</sup> considered cases with a diffuse pattern of infiltration a putative variant of SMZL, with some distinctive features, such as lack of micronodules, marginal morphology with abundant cytoplasm, p53 inactivation and cutaneous involvement. Our two patients did

not, however, have these features.

To conclude, our findings confirm the low incidence of t(9;14)(p13;q32) in SMZL and that the histology of the spleen of this entity could be atypical. In the light of both the complexity of the karyotypes and the results obtained by G-banding and FISH analysis in case #2, we hypothesize that t(9;14) could be a secondary event. Further cases and follow-up of patients with this anomaly are necessary for a better understanding of the role of *PAX5* in SMZL.

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