Genetics of Lymphocytes Influences the Emergence of Second Cancer in Chronic Lymphocytic Leukemia

MARTA BERNUÉS[1*], MARIA ANTÒNIA DURÁN[2], GUIOMAR PUGET[2], JULIO IGLESIAS[3], PILAR GALÁN[4], FRANCISCO JAVIER VERCHER[5], JOAN BESALDUCH[2] and JORDI ROSELL[1*]

Departments of [1]Genetics, [2]Hematology and [3]Immunology, Son Espases University Hospital, Palma de Mallorca, Balearic Islands, Spain; [4]Hematology Department, Mateu Orfila Hospital, Maó, Balearic Islands, Spain; [5]Hematology Department, Can Misses Hospital, Ibiza, Balearic Islands, Spain

Abstract. *Background: Patients affected by chronic lymphocytic leukemia (CLL) have an increased risk of developing a second cancer. There is not a definitive explanation for this phenomenon, although some hypotheses have been postulated. The aim of the present work was to assess the presence of second cancer in untreated patients with CLL who were cytogenetically characterized, and secondly to investigate if there is a correlation between the genetics of CLL and the emergence of second cancer. Patients and Methods: We performed conventional cytogenetics and Fluorescent in situ hybridization analyses in a series of 106 patients. Results: We observed that nearly 8% of cases developed second cancer, mostly epithelial tumors. The majority of them presented two common features, del(13)(q14.3) and the presence of at least two genetic alterations. Conclusion: We suggest that the genetic background of CLL, particularly the presence of several genetic alterations, influences the emergence of second cancer in patients affected by CLL.*

Chronic lymphocytic leukemia (CLL) is a common hematological malignancy that accounts for 25-30% of all leukemias in Western countries and patients have an increased risk of developing a second cancer (1-3). In these cases, dense peri-tumoral lymphocytic infiltrates composed of leukemia B-cells have been observed at the tumor sites (4-10).

*Marta Bernues and Jordi Rosell are members of CIBERER (Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain).

Correspondence to: Marta Bernués, Hospital Universitari Son Espases, Genetics Department, Cra. Valldemossa 79, 07120 Palma de Mallorca, Mallorca, Balearic islands, Spain. Tel: +34 871205191, e-mail: marta.bernues@ssib.es

Key Words: Chronic lymphocytic leukemia, CLL, genetics, second cancer

The biological basis of this phenomenon is not well-known, although some possible causes have been postulated to explain the relationship between the two diseases. Smoller and Warnke suggested that the infiltrates might result from a preferential recruitment of neoplastic cells at sites of immune reactions because of the predominant neoplastic composition of the lymphocytic pool in patients with CLL or because of a paracrine action on leukemia cells at the site of carcinoma-associated neo-vascularization (4). Dargent *et al*. proposed that the leukemic infiltrate was a consequence of the immune disorders occasionally seen in CLL patients (5). Cheung *et al*. suggested an underlying etiology that predisposed towards both tumor types (11). Catteau *et al*., in a report of CLL concomitant with breast cancer, also suggested a relationship between the two diseases (9).

We present here cytogenetic and Fluorescent in situ hybridization (FISH) results in a series of 106 untreated CLL to assess genetic risk markers of developing second cancer in these patients.

Patients and Methods

Patients. One hundred and six patients with CLL were enrolled between 2006 and 2012. Flow cytometric immunophenotyping on blood samples confirmed the clinical suspicion of CLL. None of the patients received previous chemotherapy or radiotherapy treatment. The median follow-up of patients was 9.5 (range=0-19) years. Eight patients developed second cancer.

Conventional cytogenetics. Cytogenetic analysis was performed following a standard methodology. For each patient, 0.5 ml of peripheral blood was cultured in RPMI-1640 medium with 10% fetal calf serum and phorbol myristate acetate (40 ng/ml) at 37˚C for 72 h. Colcemide was added at 0.3 μg/ml for 25 min to arrest cells in metaphase and chromosomal preparations were carried out according to standard procedures. G-Banding staining was performed to identify chromosome alterations, and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (12).

Table I. *Genetic patterns and second cancer in 106 patients with untreated Chronic Lymphocytic Leukemia (CLL).*

Genetic pattern	Number of cases (%)	Number of cases with second cancer (%)
Normal karyotype and normal FISH	16 (15%)	1 (0.9%)
Normal karyotype and altered FISH	35 (33%)	2 (1.9%)
Altered karyotype and normal FISH	8 (7.5%)	0 (0%)
Altered karyotype and altered FISH	46 (43.4%)	5 (4.7%)
Absent karyotype and altered FISH	1 (0.9%)	0 (0%)

FISH: Fluorescent *in situ* hybridization.

Table II. *Clinical data, cytogenetic and Fluorescent In Situ Hybridization (FISH) results in 106 untreated patients with Chronic Lymphocytic Leukemia (CLL).*

Case no.	Gender/ age (years)	Karyotype	FISH	Second cancer	Follow-up (years)
6	M/70	46,XY[20]	Normal	SCC of the skin	19
70	M/68	47,XY,+12[4]/47,idem,del(13)(q12q14)[5]/46,XY[13]	del(13)(q14.3), trisomy 12	BCC of the nose*, SCC of the skin*	0
73	F/78	47,XX,+12[3]/46,XX,der(12)t(12;12)(q13;q21)[2]/45,X,-X[3]/46,XX[21]	del(13)(q14.3), trisomy 12	Auricular BCC	4
83	F/76	45,X,-X[7]/47,XX,+12[13]/46,XX[19]	Trisomy 12	BCC of the forehead	3
107	M/81	46,XY[20]	del(13)(q14.3)x2	Lung carcinoma, cutaneous T-cell lymphoma	2
111	F/85	46,XX[16]	del(13)(q14.3), del(13)(q14.3)x2, del(17)(p13.1)	BCC of the skin*	0
116	M/59	45,X,-Y[4]/46,XY[40]	del(13)(q14.3)	Carcinoma of the prostate	2
67	M/90	46,XY,del(6)(q21)[6]/46,XY[1]	del(13)(q14.3)	BCC of the cheek	5

*Concomitant with CLL. BCC, Basal cell carcinoma; SCC: squamous cell carcinoma.

FISH. FISH was carried out following a standard protocol on fixed nuclei obtained after cytogenetic procedure with blood sample cultures. The following commercial probes (Abbott Molecular Inc., Max-Planck-Ring 2, Wiesbaden, Germany) were used according to the protocol provided by the manufacturer: Locus Specific Identifier Ataxia Telangiectasia Mutated (*LSIATM*)(11q23), Locus Specific Identifier D13S319 (LSI *D13S319*) (13q14), Chromosome Enumeration Probe 12 (*CEP12*) (12p11.1-q11) and *Locus Specific Identifier P53* (*LSIP53*) (17p13.1). We applied the following cut-off values on 200 interphase nuclei: 6% (*ATM*), 5.5% (D13S319 monosomy), 1.5% (*D13S319* nullisomy), 2.5% (*CEP12*) and 7% (P53).

Results

Detailed karyotypes and FISH results from all patients are available from the Authors. A summary of our findings is shown in Table I.

We observed an altered genetic pattern (at least one cytogenetic or FISH abnormality) in 90 out of 106 (84.9%) patients. Sixteen patients presented a normal karyotype and also a normal FISH pattern. Among the 90 abnormal cases, 34 (37.8%) exhibited only one genetic alteration and 56 (62.2%) had more than two alterations.

Among the 34 patients with a sole abnormality, the most frequent alterations were: del(13)(q14.3) (16/34, 47%), +12 (9/34, 26.5%), -X in female patients (5/34, 14.7%) and del(11)(q22.3) (2/34, 5.9%).

Among the 56 patients with two or more alterations, we found that 29 (51.8%) presented bi-allelic del(13)(q14.3) alone or concomitant with other alterations, 15 (26.8%) had mono-allelic del(13)(q14.3) concomitant with other alterations, and 12 (21.4%) had other altered genetic patterns.

Among patients with a second cancer (Table II), seven out of eight had two or more alterations and only one patient (case 6) presented a normal genetic pattern.

Six out of the seven genetically altered cases shared del(13q). This genetic alteration was found together with loss of chromosome X in women (cases 73 and 83) and loss of Y chromosome in a man (case 116), +12 (cases 70 and 73), del(6q) (case 67) and del(17p) (case 111).

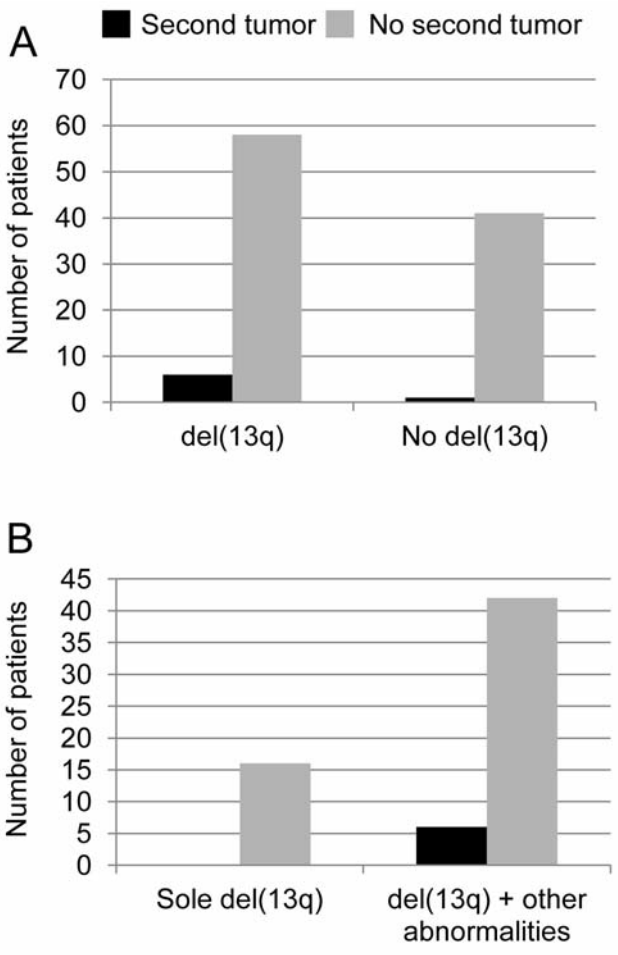

Figure 1. *Del(13)(q14.3) and second cancer in 106 patients with untreated chronic lymphocytic leukemia. A: Frequency of second tumors related to del(13q). B. Frequency of second tumors in patients with del(13q) according to the genetic background.*

Discussion

We selected a series of 106 patients with CLL that did not receive any treatment during disease and we assessed the presence of second cancer in those cases. Eight patients (7.5%) developed second tumors.

The most frequent alteration in this series was del(13)(q14.3), as a sole genetic event or concomitant with other alterations (64/106 cases, 60.4%). Mono-allelic and bi-allelic deletions were observed in 34 and 30 cases, respectively. Interestingly, del(13)(q14.3) was the common genetic trait in seven out of the eight cases that developed second tumors. Only one out of the 42 cases that did not have del(13)(q14.3) presented a second cancer (Figure 1A). This patient (case 6), with a quiescent CLL (19 years of disease), had a normal karyotype and also a normal FISH pattern.

There are only three reported cases of CLL where both the presence of second tumors and the genetic data of CLL were considered: a basal cell carcinoma (BCC) of the skin (13), a breast cancer (9) and a lung cancer (14). The latter presented del(13)(q34) and monosomy 12, while the other two cases presented solely del(13q). Our results and those from the literature suggest that del(13q) could be involved in CLL with second cancer.

Out of the patients presented here, however, seven out of the eight with second cancer presented del(13)(q14.3) together with other alterations (Figure 1B, Table II). None of the 16 patients with sole del(13)(q14.3), some of them with more than three years of follow-up, developed a second cancer. As a consequence, it is reasonable to believe that del(13)(q14.3) could be a risky genetic alteration but not enough to trigger a second tumor. The acquisition of further genetic alterations must be necessary as a second step in tumorigenesis. Although the number of patients is scarce, our results support the concept that as the number of genetic alterations increases, including del(13)(q14.3), the risk of developing second cancer in patients with CLL also increases.

All cases of second cancer but one (case 107) were epithelial tumors, especially squamous cell carcinomas (SCC) and BCC of the skin. Our findings are in agreement with previous reports where skin tumors are described as the most frequent second tumors in patients with CLL (1, 2). Among the eight patients with second cancer, only two (cases 70 and 111) presented a concomitant second cancer at the time of diagnosis of CLL. In these cases, CLL might have been masked before the emergence of epithelial tumors. The other six patients were followed-up for a range of 2-19 years. This supports the nation that the risk of emergence of second tumors in patients with CLL might be related not only to the genetic background of lymphocytes in CLL but also to the duration of CLL.

It is also interesting to note that none of the patients we present here received any chemotherapy, consequently second tumors cannot be related to treatment. Literature concerning the association of CLL and carcinomas has varying interpretations. Immunological disorders and an underlying predisposing etiology are mostly postulated to explain this phenomenon (4-5, 9).

It is well-known that solid tumors emerge as a consequence of several genetic abnormalities that are progressively accumulated in cells. Therefore, a pre-neoplastic lesion is required before a tumor emerges. Interestingly, CLL lymphocyte infiltrates are not exclusively found at tumor sites but also in other diseases (actinic keratoses, benign prostate hypertrophy) (4, 16). This implies that in patients with CLL, a pre-neoplastic lesion (induced by chemicals, infections, ultraviolet radiation *etc*.), in an immunologically-impaired environment, could be the first

step towards solid tumorigenesis. In agreement with Smoller and Warnke (4), the second step would be a paracrine action of leukemic cell infiltrates at the altered site. This would result in a somewhat altered cell environment at the infiltrate site, depending on the molecular trafficking, and this could be important for initiating an epithelial tumor. Based on our analyses, we suggest that del(13)(q14.3) in addition to other genetic alterations of lymphocytes in CLL is a risk factor for developing second cancer. In cases with only one alteration, or none, molecular trafficking was less altered and this would explain why these patients rarely developed second cancers.

All chromosomes altered in CLL carry several genes that are involved in epithelial tumors: absent in melanoma-1 (AIM1) (6q21), tumor suppressor TSG1 (TSG1) (6q16), squamous cell carcinoma antigen recognized by T-cells-3 (SART3) (12q24.1), fibroblast growth factors (12p13, 13q11-q12, 13q34, 17p13), epidermal growth factor receptor pathway substrate 8 (EPS8) (12p12), several keratins (12q13), P53 (17p13), regulator of cell cycle (RGCC) (13q14), epithelial stromal interaction 1 (EPSTI1) (13q13) and retinoblastoma 1 (RB1) (13q14), among others. In addition, deletions of chromosomes 6q, 13q and 17p; losses of X (in females) and Y (in males) chromosomes; and re-arrangements of chromosome 12 have been observed not only in CLL but also in skin, prostate and lung carcinomas (15).

In conclusion, the presence of second cancer in untreated patients with CLL cannot be attributed to chance. There is a genetic association between both diseases. The genetic background of CLL, particularly the presence of del(13)(q14.3) together with other genetic alterations in lymphocytes, increases the risk of developing second cancer in these patients.

Acknowledgements

We thank the technical staff from Genetics, Immunology and Hematology Departments for technical assistance. We thank Lynne Dorrien for the English revision of the manuscript.

References

1 Travis LB, Curtis RE, Hankey BF and Fraumeni JF Jr.: Second cancers in patients with chronic lymphocytic leukemia. J Natl Cancer Inst *84*: 1422-1427, 1992.

2 Gu Y, Strom SS, Lerner S and Keating MJ: Second cancers after CLL diagnosis. Blood (ASH Annual Meeting Abstracts) *104*: Abstract 18, 2004

3 Schöllkopf C, Rosendahl D, Rostgaard K, Pipper C and Hjalgrim H: Risk of second cancer after chronic lymphocitic leukemia. Int J Cancer *121*: 151-156, 2007.

4 Smoller BR and Warnke RA: Cutaneous infiltrate of chronic lymphocytic leukemia and relationship to primary cutaneous epithelial neoplasms. J Cutan Pathol *25(3)*: 160-164, 1998

5 Dargent JL, Kornreich A, André L and Lespagnard L: Cutaneous infiltrate of chronic lymphocytic leukemia surrounding a primary squamous cell carcinoma of the skin. Report of an additional case and reflection of its pathogenesis. J Cutan Pathol *25*: 479-480, 1998.

6 Mehrany K, Byrd DR, Roenigk RK, Weenig RH, Phillips PK, Nguyen TH and Otley CC: Lymphocytic infiltrates and subclinical epithelial tumor extension in patients with chronic leukemia and solid-organ transplantation. Dermatol Surg *29(2)*: 129-134, 2003.

7 Agnew KL, Ruchlemer R, Catovsky D, Matutes E and Bunker CB: Cutaneous findings in chronic lymphocytic leukemia. Br J Dermatol *150*: 1129-1135, 2004.

8 Wilson ML, Elston DM, Tyler WB, Marks VJ and Ferringer T: Dense lymphocytic infiltrates associated with non-melanoma skin cancer in patients with chronic lymphocytic leukemia. Dermatol Online J 16(3), 2010.

9 Catteau X, Dehou MF, Dargent JL, Hackx M and Noël JC: Chronic lymphocytic leukemia mimicking recurrent carcinoma of the breast: case report and review of the literature. Pathol Res Prac *207*: 514-517, 2011.

10 Hammami B, Mnejja M, Achour I, Chakroun A, Khabir A, Chakroun A, Boudawara T, Charfeddine I and Ghorbel A: Association of squamous cell carcinoma of the larynx and chronic lymphoid leukemia. Eur Ann Otorhinolayngol Head Neck Dis *127*: 153-155, 2010.

11 Cheung KJ, Tam W, Chuang E and Osborne MP: Concurrent invasive ductal carcinoma and chronic lymphocytic leukemia manifesting as a collision tumour in breast. Breast J *13*: 413-420, 2007.

12 ISCN (2013): An International System for Human Cytogenetic Nomenclature. Shaffer LG, McGowan-Jordan J and Schmid M (eds.). S. Karger, Basel, 2013.

13 Khandelwal A, Seilstad KH and Magro CM: Subclinical chronic lymphocytic leukaemia associated with a 13q deletion presenting initially in the skin: *Apropos* of a case. J Cutan Pathol *33(3)*: 256-259, 2006.

14 Antic D, Dencic-Fekete M, Tomin D and Djunic I: Monosomy 12 and deletion of 13q34 in a case of chronic lymphocytic leukemia with concomitant lung cancer. Vojnosanit Pregl *67(10)*: 864-866, 2010.

15 Mitelman F: Mitelman database of chromosome aberrations in cancer. [http://cgap.nci.nih.gov/Chromosomes/Mitelman].

16 Cachia PG, McIntyre MA, Dewar AE and Stockdill G: Prostatic infiltration in chronic lymphatic leukaemia. J Clin Pathol *40*: 342-345, 1987.

Received January 10, 2014
Revised February 24, 2014
Accepted February 25, 2014