A der(11)t(4;11)(q21;p15) as part of a very complex karyotype in T-cell acute lymphoblastic leukemia/lymphoma

Sandra Colli[1], Lilian Furforo[2], Eduardo Rojo Pisarello[3], Marcela Maidana[3], Carlos Martín[4], Javier Bordone[3], Irma Slavutsky[1]

From:

1. Laboratorio de Genética de Neoplasias Linfoides, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina, Buenos Aires, Argentina.
2. Centro Nacional de Genética Médica, Buenos Aires, Argentina.
3. Hospital de Alta Complejidad "Presidente Juan Domingo Perón", Formosa, Argentina.
4. Consultorio de Hematopatología, La Plata, Argentina.

Correspondence to: Irma Slavutsky, MD, PhD
Laboratorio de Genética de Neoplasias Linfoides
Instituto de Medicina Experimental
CONICET-Academia Nacional de Medicina
Pacheco de Melo 3081
1425 – Buenos Aires, Argentina

Phone: 5411 4805 8803
e-mail: islavutsky@hematologia.anm.edu.ar

Abstract

Translocation t(4;11)(q21;p15) is a rare recurrent change associated to T-cell acute leukemia. In most cases, this alteration appears as the only abnormality or as part of a simple karyotype. In this report, we present the first case of T acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) with the unbalanced translocation der(11)t(4;11)(q21;p15) as part of a very complex karyotype with multiple chromosome markers, most of them not previously described in the literature. FISH (fluorescence in situ hybridization) and spectral karyotype (HiSKY) analysis confirmed the presence of complex alterations. The patient, a 16 years-old male, showed poor response to treatment and short survival (11 months). A detailed review of previously reported cases with t(4;11)(q21;p15) is also provided. The description of this type of alterations may contribute to the identification of new molecular mechanism associated to neoplastic development.

Key words: T-cell acute leukemia/lymphoma; Cytogenetics; FISH; Spectral karyotype

Introduction

T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/LBL) is a neoplasm committed to the T-cell lineage that can be observed involving bone marrow (BM) and peripheral blood (T-acute lymphoblastic leukemia, T-ALL) or presenting with primary involvement of thymus, nodal or extranodal sites (T-lymphoblastic lymphoma, T-LBL) [1]. By convention, the term lymphoma is used when the process is confined to a mass lesion with no or minimal evidence of peripheral blood and BM involvement. If the patient presents with a mass lesion and lymphoblasts in the BM, the distinction between leukemia and lymphoma is arbitrary. T-ALL comprises about 15% of childhood ALL while T-LBL correspond to about 85-90% of all lymphoblastic lymphomas; both are most frequent in male adolescents. The annual incidence is 1.6/100,000 individuals in the general population [1]. In Argentina, data of the Onco-Hematology Argentine Registry [2] shows an incidence of 3.0/100,000 in children under 15 year-old.

Cytogenetic studies in this pathology have demonstrated an abnormal karyotype in about 50% of patients [3]. Thirty five percent of cases show translocations involving T cell receptors and different genes [4, 5]. These translocations usually result in oncogenes becoming juxtaposed to the promoter and enhancer elements of *TCR* genes, leading to their aberrant expression and the development of T-ALL/LBL. Alternatively, aberrant expression of one or more transcription factors is a critical component of the molecular pathogenesis of T-ALL/LBL [5]. On the contrary, the presence of recurrent reciprocal translocation without involvement of at least one *TCR* encoding gene is a rare event in this pathology. The translocation t(4;11)(q21;p15) is a very uncommon rearrangement described in 1985 [6] that, at the molecular level, induces the fusion **of the *RAP1GDS1* (RAP1, GTP-GDP dissociation stimulator 1) gene located at band 4q21, with the *NUP98* (nucleoporin 98kDa) gene mapped at 11p15** [7, 8], leading to a new chimeric transcript encoding a protein with dominant oncogenic properties: *NUP98-RAP1GDS1* [7]. In most cases, this alteration appears as the only abnormality or as part of a simple karyotype [9]. The literature shows only two cases with complex karyotype (three or more alterations) [10, 11] and only one case with a complex translocation [12]. In this study, we present the first case of T-ALL/LBL with an unbalanced translocation der(11)t(4;11)(q21;p15) as part of a very complex karyotype with multiple chromosome markers, most of them not previously described in the literature.

Case report

A 16-yr-old male was referred to the Hospital of High Complexity "Presidente Juan Domingo Perón", Formosa, Argentina, in November 2013 with cervical and inguinal lymphadenopathies and splenomegaly. Hematological data showed: white blood cells count $20x10^9$/L (28% neutrophils, 62% lymphocytes, 4% monocytes, 6% eosinophils), hemoglobin 15.4 g/dL, hematocrit 44% and platelet count $138x10^9$/L. Lactate dehidrogenase was raised to 448 IU/L (normal range 240-480 IU/L). The patient had a normal liver and kidney function and, negative serology for hepatitis B and C. The histopathological study of the lymph node showed complete replacement for a lymphoid population with diffuse pattern, composed of medium sized blasts with scant cytoplasm, condensed nuclear chromatin and indistinct nucleoli Immunohistochemical analysis showed co-expression of CD1a, Tdt, CD34 and CD10, and moderate reactivity of CD3 (**Figure 1a**). The Ki67 antigen disclosed 80% of proliferating cells. The bone marrow (BM) aspirate showed 90% lymphocytes with diffuse pattern, with typical T-ALL/LBL morphology. Flow cytometry showed proliferation of T-cell precursors, which accounted for 76.21% of cell population. The patient started chemotherapy according to the protocol of the Argentine Group of Acute Leukemia Treatment (GATLA) [13]. A complete hematological remission was achieved. Four months after consolidation, the patient showed hematological relapse and died three months later.

Methods

Cytogenetic analysis

Cytogenetic study was performed on BM cells cultured in RPMI 1640 medium supplemented with 20% fetal calf serum (Gibco) during 24 hs at 37ºC. G-banding technique was used. Chromosome abnormalities were described according to the International System for Human Cytogenetic Nomenclature (ISCN) [14].

FISH (fluorescence *in situ* hybridization) studies

FISH analysis was performed on the same material used for cytogenetic studies according to manufacturer's protocol. **Total chromosome paint (TCP) FISH probes (LiVE-Lexel, Buenos Aires, Argentina) for chromosomes 4, 11 and 12** as well as LSI MLL Dual color, Break Apart rearrangement probe (11q23) (Vysis-Abbott, Illinois, USA) were used. Image acquisition was performed using Cytovision 3.9 Software (Applied

Imaging Corporation, California, USA). In addition, High Resolution Spectral Karyotype (HiSKY) was performed using the ASI SKYPaint-KIT (Applied Spectral Imaging) and the BX61 HiSKY system (GenASIs Hyperspectral Platform) was employed for image acquisition.

Results

At diagnosis, cytogenetic study on the BM cells showed a 44~46,XY, der(11)t(4;11)(q21;p15),add(6)(q21),add(8)(q24),+del(12)(q11q13),-17,-19,add(20)(q11), +mar[cp9]/46,XY[10] karyotype. **TCP FISH probes for chromosomes 4 and 11 confirmed chromosome** marker der(11)t(4;11)(q21;p15) (**Figure 1b**) **as well as TCP12 confirmed** the interstitial deletion of chromosome 12: del(12)(q11q13). The analysis with the MLL probe showed a normal pattern of signals. In order to complete the characterization of this complex karyotype, we performed HiSKY technique. The combination of G-banding analysis, FISH and HiSKY allowed to define the following karyotype: 44~46,XY,der(4)t(4;10)(q12;**q?22**),+der(5)t(5;9)(?;?)x2,der(6)t(6;10)(q13;?), t(8;9)(q24;q34),-9,der(10)del**(10)**(p11)del**(10)**(q24),der(11)t(4;11)(q21;p15),+del(12) (q11q13),+inv(13)(p11q14),+del(15)(q15),-17,-19,der(20)t(13;20)(?;q11),der(20)t(9;20) (?;q13),-21,**-21**[cp17]/46,XY[11] (**Figures 1c-d**). In addition, the following chromosome markers, most of them complex rearrangements, were observed in only one cell each: der(2)t(2;10)(q11;?), **der(10)t(1;10)(?;q11),** del(14)(q24). Among these alterations, only t(8;9)(q24;q34) [15] and der(20)t(13;20)(?;q11) [16] have been described in only one patient each, del(15)(q15) were observed in five cases as part of complex karyotypes [9], meanwhile monosomies 9, 17 and 19 are frequent numerical alterations in T-ALL/LBL patients [9].

Discussion

The translocation t(4;11)(q21;p15) is a very infrequent rearrangement. This anomaly characterizes a subset of T-ALL/LBL **originating** from an early precursor with variable co-expression of CD10 and myeloid markers. It is found mainly in patients older than 15 years, and associated with poor clinical outcome [17]. The translocation t(4;11)(q21;p15) may be a unique, recurring abnormality, as it appears to be associated with *de novo* acute leukemia (myeloid and lymphoid), secondary leukemias, as well as lymphoma. As mentioned, this translocation **results in** the fusion of *NUP98 and*

RAP1GDS1 genes. *NUP98* codes for a component of the nuclear pore complex [18] and is involved in numerous translocations described in hematological disorders including acute and chronic myeloid leukemias, T-cell ALL, myelodysplastic syndrome and secondary acute myeloid leukemia, suggesting an important role in leukemogenesis [19-22]. *NUP98* has been found rearranged with 19 different partner genes subclassified in three different groups, with different distribution among pathologies [20]. Thus, *HOX* and *NSD* genes are mainly associated with *de novo* myeloid malignancies; TOP family genes and *DDX10* are related to therapy-associated myeloid disorders, and *RAP1GDS* gene is involved in T-ALL. NUP98/RAP1GDS1 fusion represents 9% of the *NUP98* fusion genes, and half of these patients are less 20 years old. As regards to *RAP1GDS* gene, it encodes a cytoplasmic protein that regulates actin assembly associated with the folding of the membrane. When the rearrangement with *NUP98* gene occurs, the entire coding region of *RAP1GDS* merged to the GLFG repeat domain of *NUP98* to form the fusion transcript NUP98-RAP1GDS1 [7].

At present, there are 20 cases reported in the literature (Table 1): 12 patients with T-ALL, 2 with T-ALL/LBL, 2 with T-LBL, 1 with B-ALL, and 3 with acute myeloid leukemia. Six out of 16 (37.5%) cases with T-cell disease were children. Among them, eight patients showed the **t(4;11)** as the only karyotypic abnormality, in one of them as a result of a complex **translocation,** four cases had deletions of chromosomes 5, 12 or 13, one case had a different balanced translocation in the karyotype, **three cases showed hyperdiploid karyotypes.** From the three cases with diagnosis of AML, two had the **t(4;11)** as a single alteration and one case showed a hyperdiploid karyotype with multiple numerical alterations and the t(4;11)(q21;q23) involving the MLL gene. Thus, our case is, to our knowledge, the first description of this anomaly as an unbalanced translocation in the context of a very complex karyotype. In addition, complex rearrangements were observed in single cells. As known, changes in gene dose can potentially generate copy-number alterations as well as critical combinations of deleted and gained regions that are important driver of cancer, some times associated to complex karyotypes (23). This remodeling of the genome may confer significant selective advantages to clonal cells that can promote cancer development and progression, associated to adverse clinical behavior of the disease. It is consistent with those observed in our patient that showed a poor clinical outcome with a very short survival.

Certainly the description of this type of alterations may contribute to the knowledge of the biological and clinical characteristics of different subgroups of patients. It is also interesting to note the importance of using molecular cytogenetic techniques in cases with complex chromosome rearrangements in order to a better characterization of the karyotype. It constitute a contribution to a higher accuracy in the diagnosis and/or prognosis of patients and to guide future studies that enable the identification of new molecular mechanisms associated with neoplastic development that may be the basis for new therapeutic approaches.

Acknowledgements

This work was supported by grants from the National Research Council (CONICET), the National Agency of Scientific and Technical Promotion (ANPCyT), and the National Cancer Institute from Argentina. The authors want to thank the technical assistance of the National Center of Medical Genetics for spectral analysis of chromosome alterations and Dr Alejandro Laudicina that kindly provided some of the FISH probes used in this study.

References

[1] Borowitz MJ, Chan JKC. T lymphoblastic leukaemia/lymphoma. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. (Eds.): WHO classification of Tumours of Haematopoietic and Lymphoid Tissue. IARC: Lyon, 2008, p. 176-178.

[2] Registro Onco-Hematológico Argentino http://www.msal.gov.ar/inc/index.php/investigacion-y-epidemiologia/registro-/roha, 2015.

[3] Van Vlierberghe P, Pieters R, Beverloo HB, Meijerink JP. Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. Br J Haematol 2008;143: 153–68.

[4] Cauwelier B, Dastugue N, Cools J, Poppe B, Herens C, De Paepe A, et al. Molecular cytogenetic study of 126 unselected T-ALL cases reveals high incidence of TCR beta locus rearrangements and putative new T-cell oncogenes. Leukemia 2006; 20: 1238-44.

[5] Harrison CJ. Acute Lymphoblastic Leukemia. Clin Lab Med 2011; 31: 631-47.

[6] Inoue S, Tyrkus M, Ravindranath Y, Gohle, N. A variant translocation between chromosomes 4 and 11, t(4q;11p) in a child with acute leukemia. Am J Pediatr Hematol Oncol 1985; 7: 211-4.

[7] Hussey DJ, Nicola M, Moore S, Peters GB, Dobrovic A. The (4;11)(q21;p15) translocation fuses the NUP98 and RAP1GDS1 genes and is recurrent in T-cell acute lymphocytic leukemia. Blood 1999; 94: 2072-9.

[8] Mecucci C, La Starza R, Negrini M, Sabbioni S, Crescenzi B, Leoni P, et al. t(4;11)(q21;p15) translocation involving NUP98 and RAP1GDS1 genes: characterization of a new subset of T acute lymphoblastic leukaemia, Br J Haematol 2000; 109: 788-93.

[9] Mittelman F, Johansson B, Mertens F, eds. Mitelman Database of Chromosome Aberration and Gene Fusions in Cancer. http://cgap.nci.nih.gov/Chromosomes/Mitelman, 2015.

[10] Secker-Walker LM, Stewart EL, Chan L, O'Callaghan U, Chessells JM. The (4;11) translocation in acute leukaemia of childhood: the importance of additional chromosomal aberrations. Br J Haematol 1985; 1: 101-11.

[11] Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012; 481:157-63.

[12] Douet-Guilbert N, Morel F, Le Bris MJ, Herry A, Le Calvez G, Marion V, et al. t(4;11)(q21;p15), including one complex translocation t(1;4;11)(p32;q21;p15), in adult T-cell acute lymphoblastic leukemia. Leuk Res 2003; 27: 965-7.

[13] Grupo Argentino de Tratamiento de la Leucemia Aguda (GATLA). http://www.gatla.org.ar/index.php, 2015.

[14] ISCN. An international system for human cytogenetic nomenclature. LG Shaffer, JJ McGowan and M Schmid, eds., 2013.

[15] Kim HJ, Cho HI, Kim EC, Ko EK, See CJ, Park SY, et al. A study on 289 consecutive Korean patients with acute leukaemias revealed fluorescence in situ hybridization detects the MLL translocation without cytogenetic evidence both initially and during follow-up. Br J Haematol 2002; 119; 930-9.

[16] Chapiro E, Radford-Weiss I, Cung HA, Dastugue N, Nadal N, Taviaux S, et al. Chromosomal translocations involving the IGH@ locus in B-cell precursor acute lymphoblastic leukemia: 29 new cases and a review of the literature. Cancer Genet 2013; 206; 162-73.

[17] Braoudaki M, Tzortzatou-Stathopoulou F. Clinical cytogenetics in pediatric acute leukemia: An Update. Clin Lymph, Myeloma Leuk 2012; 12: 230-7.

[18] Powers MA, Forbes DI, Dahlberg JE, Lund E. The vertebrate GLFG nucleoporin, Nup98, is an essential component of multiple RNA export pathways. J Cell Biol 1997; 136: 241-50.

[19] Yasar D, Karadoganb I, Alanogluc G, Akkaya B, Luleci G, Salim O, et al. Array comparative genomic hybridization analysis of adult acute leukemia patients. Cancer Genet Cytogenet 2010; 197: 122-9.

[20] Romana SP, Radford-Weiss I, Ben Abdelali R, Schluth C, Petit A, Dastugue N, et al. NUP98 rearrangements in hematopoietic malignancies: A study of the Groupe Francophone de Cytogénétique Hématologique. Leukemia 2006; 20: 696-706.

[21] Hollink I, Heuvel-Eibrink M, Arentsen-Peters S, Pratcorona M, Abbas S, Kuipers JE, et al. *NUP98/NSD1* characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct *HOX* gene expression pattern. Blood 2011; 118: 3645-56.

[22] Akiki S, Dyer SA, Grimwade D, Ivey A, Abou-Zeid N, Borrow J, et al. NUP98-NSD1 fusion in association with FLT3-ITD mutation identifies a prognostically relevant subgroup of pediatric acute myeloid leukemia patients suitable for monitoring by real time quantitative PCR, Genes Chrom Cancer 2013, 11: 1053-64.

[23] Stratton, MR, Campbell, PJ, Futreal, PA. The cancer genome. Nature 2009; 458, 719-24.

[24] Kawakami K, Miyanishi S, Amakawa R, Hayashi T, Kurata M, Nakamura F, et al. A case of T-lineage lymphoblastic lymphoma/leukemia with t(4;11)(q21;p15) that switched to myelomonocytic leukemia at relapse. Int J Hematol 1999; 69:196-9

[25] Hardingham JE, Peters GB, Dobrovic A, Dale BM, Kotasek D, Ford HE, et al. A rare translocation (4;11)(q21;p14-15) in an acute lymphoblastic leukemia expressing T-cell and myeloid markers. Cancer Genet Cytogenet 1991; 56: 255-62.

[26] Pui CH, Raimondi SC, Head DR, Schell MJ, Rivera GK, Mirro J Jr, et al. Characterization of childhood acute leukemia with multiple myeloid and lymphoid markers at diagnosis and at relapse. Blood 1991; 78:1327-37.

[27] Bloomfield CD, Goldman AI, Alimena G, Berger R, Borgström GH, Brandt L, et al. Chromosomal abnormalities identify high-risk and low-risk patients with acute lymphoblastic leukemia. Blood 1986; 67:415-20.

[28] Thangavelu M, Huang B, Lemieux M, Tom W, Richkind KE. A t(4;11)(q21;p15) in a case of T-cell lymphoma and a case of acute myelogenous leukemia. Cancer Genet Cytogenet 2002; 132: 109–115.

[29] Kobzev YN, Martínez-Climent J, Lee S, Chen J, Rowley JD. Analysis of translocations that involve the NUP98 gene in patients with 11p15 chromosomal rearrangements. Genes Chrom Cancer 2004; 41: 339-52.

[30] Radtke I, Mullighan CG, Ishii M, Su X, Cheng J, Ma J, et al. Genomic analysis reveals few genetic alterations in pediatric acute myeloid leukemia. Proc Natl Acad Sci USA 2009; 31: 12944-9.

Legend

Figure 1: a) Lymph node biopsy stained with Hematoxilin & Eosin showing complete replacement by lymphoblastic lymphoma, and immunohistochemical technique showing: moderate reactivity of CD3; Tdt expression; CD1a expression (400x); b) G-banding partial karyotype showing normal chromosomes 4 and 11 and der(11)t(4,11)(q21;p15); c) Spectral karyotype of the patient showing chromosome markers: der(4)t(4;10)(q12;**q?22**), der(5)t(5;9)(?;?)x2, der(6)t(6;10)(q13;?), t(8;9)(q24;q34), der(10)del**(10)**(p11)del**(10)**(q24), der(11)t(4;11)(q21;p15), del(15)(q15), der(20)t(13;20) (?;q11) (arrows), as well as losses of chromosomes 17, 18, 21 and 22; d) G-banding and Spectral partial karyotypes showing: normal chromosomes 12 and del(12)(q11q13), normal chromosomes 13 and inv(13)(p11q14) and normal chromosome 20 and der(20)t(9;20)(?;q13).

Table 1: Cases with t(4;11)(q21;p15) reported in the literature

Reference	Age/ Sex (years)	Karyotype	Diagnosis	Methodology
Hussey et al [7]	49/M	46,XY,t(4;11)(q21;p15),del(5)(q13q31)	T-ALL	Cytog, PCR
	25/F	46,XX,t(4;11)(q21p14-15),del(12)(p13), del(13)(q12q14)	T-ALL	Cytog, PCR
Inoue et al [6]	14/M	46,XY, t(4;11)(q21p14-15),12p-/46,XY, t(4;11)(q21p14-15)	T-ALL	Cytog
Kawakami et al (24)	51/F	46,XX,t(4;11)(q21;p15)	T-ALL /LBL	Cytog
Hardingham et al [25]	21/M	48,XY,t(4;11)(q21;p15),+2mar	T-ALL	Cytog
Pui et al. [26]	6/F	46,XY, t(4;11)(q21;p14-15)	T-ALL	Cytog
Bloomfield et al. [27]	40/M	46,XY, t(4;11)(q21;p15)	T-ALL	Cytog
Mecucci et al. [8]	16/F	47,XX,t(4;11)(q21;p15),+8	T-ALL	Cytog, FISH
	38/F	46-47,XX,t(4;11)(q21;p15),+mar	T-ALL	Cytog, PCR?
Thangavelu et al. [28]	11/M	46,XY,t(4;11)(q21;p15)	T-LBL	Cytog, FISH
	36/M	46,XY,t(4;11)(q21;p15)	AML	Cytog, FISH
Douet-Guilbert et al. [12]	40/F	46,XX,t(4;11)(q21;p15)/46,XX	T-ALL	Cytog, FISH
	25/M	46,XY,t(1;4;11)(p32;q21;p15)/46,XY	T-ALL	Cytog, FISH
Kobzev et al. [29]	18/M	46XX,t(2;21)(q11;q11),t(4;11)(q21;p15)/ 46,XX	T-LBL	FISH
Romana et al. [20]	19/M	46,XY,t(4;11)(q21;p15)	T-ALL	Cytog, FISH, PCR
	10/F	46,XY,t(4;11)(q21;p15)	T-ALL	
Yasar el al. [19]	32/M	46,XY,t(4;11)(q21;q23)/46,XY,t(4;11) (q21;p15)	B-ALL	CGH Array
Zhang et al. [11]	12/F	46,XX,t(4;11)(q21;p15),del(5)(q22q35),del(12) (p12)	T-ALL /LBL	Cytog, PCR
Radtke et al. [30]	?/F	46,XX,t(4;11)(q21;p15)	AML	Cytog
Secker-Walker et al. [10]	1/F	38~53,XX,+4,t(4;11)(q21;p15),t(4;11)(q21;q23), +6,-8,-9,-10,+11,-18,+19	AML	Cytog
		44~46,XY,der(4)t(4;10)(q12;**q?22**),+der(5)t(5;9)		

This report	16/M	(?;?)x2,der(6)t(6;10)(q13;?),t(8;9)(q24;q34), -9,der(10)del**(10)**(p11)del**(10)**(q24),der(11) t(4;11)(q21;p15),+del(12)(q11q13),+inv(13)(p11 q14), +del(15)(q15),-17, -19, der(20)t(13;20) (?;q11),der(20)t(9;20)(?;q13),-21**,-21**[cp17]/ 46,XY[11]	T-ALL /LBL	Cytog, FISH, HiSky

M:male; F: female; Cytog: cytogenetics; PCR: polymerase chain reaction