PRELIMINARY RESULTS OF *MLL* MOLECULAR LEVEL CHARACTERIZATION THROUGH AN EPIDEMIOLOGICAL POINT OF VIEW

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BACKGROUND

An epidemiological study has been conducted in Brazil, registering infant leukemia (IL) patients, in order to explore the effect of maternal environmental exposures during pregnancy.[1] The precise identification of *MLL* aberrations is necessary for clinical decisions and minimal residual disease.

AIM

We aim now to address its importance for epidemiological purposes. In this context, we describe herein the preliminary results of *MLL* specific fusion sites obtained from Brazilian IL cases included in the epidemiological survey.

MATERIALS AND METHODS

✓ We isolated genomic DNA from prescreened (cytogenetics, FISH, NG2 positivity or RT-PCR) bone-marrow aspirates from 13 IL *MLL*+ cases (Figure 1);

✓ The *MLL* specific breakpoint region was identified at DCAL using long-distance

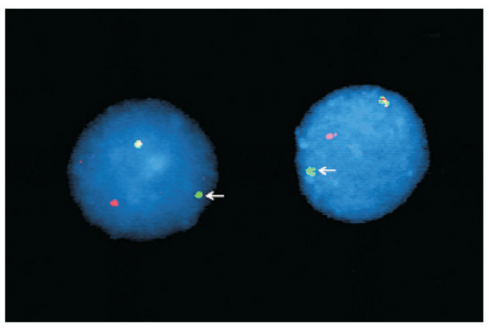

Figure 1. IL cases were previously screened for *MLL* rearrangements. In this example, we show a positive case using FISH assay. The probe was hybridized to interphase nuclei and displayed one split hybridization (arrow) signal that indicates translocation with an unknown partner gene. *V.M. Cóser et al. / Cancer Genetics and Cytogenetics 198 (2010) 151-154.*

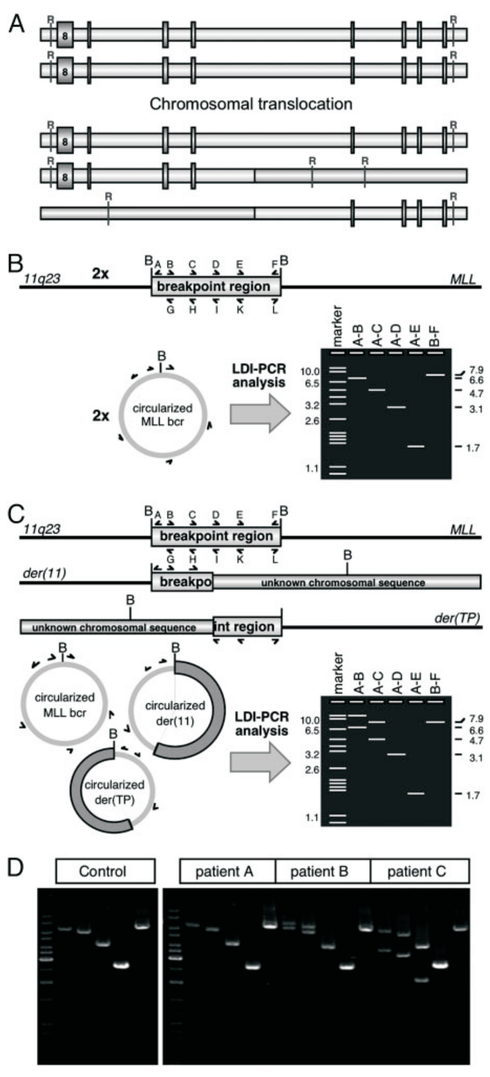

Figure 2. Principles of the LDI-PCR method and exemplary analyses. *(A)* Chromosomal translocations are creating restriction polymorphic DNA fragments that are targeted by the LDI-PCR approach. R, restriction site. *(B)* Nonrearranged *MLL* alleles:*Bam*HI digestion and religation of the*MLLbcr* will lead to twoDNAcircles that can be amplified by the primer combinations A–B, A–C, A–D, and A–E. The primer combination B–F serves as internal control. B, *Bam*HI restriction recognition site; bcr, breakpoint cluster region. *(C)* Presence of a rearranged *MLL* allele. *Bam*HI digestion and religation of the two *MLL* alleles will lead to three different DNA circles [der(11) and der(TP); TP, translocation partner] that can be amplified by the designated primer combinations A–L. Non-germ-line PCR amplimers can be analyzed by sequence analysis using oligonucleotideAor L. *(D)* GenomicDNA of patients was tested with four different oligonucleotide combinations (A–B, A–C, A–D, and A–E). *(Left)* Size marker and control DNA (placenta). *(Right)* Analyses of different patients (A–C; not listed in Table 2). Non-germ-line amplimers can be isolated and analyzed by sequence analysis. *Meyer et al., PNAS 2005, 102, 449-454.*

RESULTS

✓ There were 9 boys and 4 girls, median age 10.6 (0-23 months); 9 (69%) were classified as pro-B acute lymphoblastic leukemia (ALL) and 4 (31%) as acute myeloid leukemia (AML-M4/M5);

Table 1. Main demographical and laboratorial features of patients, *MLL* partner gene and breakpoint region

Patient		Age (months)	GENDER	WBC x10^9/L	DIAGNOSIS	PARTNER GENE	*MLL* BREAKPOINT
1	188/01	21	Male	107.5	AML-M2	*EPS15*	Intron 10
2	372/01	8	Male	182.0	Pro-B ALL	*AFF1*	Intron 9
3	319/02	11	Female	101.6	Pro-B ALL	*MLLT10*	Intron 10
4[a]	624/02	3	Male	25.6	Pro-B ALL	*AFF1*	Intron 11
5	010/04	5	Female	140.0	Pro-B ALL	*MLLT1*	Intron 11
6	060/05	8	Male	30.5	Pro-B ALL	*MLLT3*	Exon 10
7	042/06	21	Female	85.6	AML-M4	*MLLT3*	Intron 10
8[b]	107/07	4	Male	1600.0	Pro-B ALL	*MLLT1*	Intron 11
9	446/07	23	Male	61.0	Pro-B ALL	*MLLT3*	Intron 10
10	582/08	11	Male	106.0	AML-M5	*NEBL*	Intron 9
11	1042/08	0	Male	143.0	Pro-B ALL	*AFF1*	Intron 11
12	270/07	20	Male	65.0	AML-M4	1q34	Intron 9
13	291/08	4	Female	130.0	Pro-B ALL	11p11	Intron 11

[a]46XY,-4,add(4)(q21), add(11)(q23); [b]46,XY,16qh+;

✓ TPGs were successfully identified in 11 cases, being *AFF1/AF4*(27%), *MLLT1/ENL*(18%) and *MLLT3/AF9*(27%) responsible for 8 of them;

✓ Other TPGs included *MLLT10/AF10*, *EPS15/AF1P* and *NEBL* (first described) (Figure 3);

One case displayed an *MLL* translocation involving *MLL* intron 9 fused to a region at 1q34 and the other showed *MLL* intron 11 fused to 11p11, but further characterization was not retrieved due to insufficient DNA;

✓ Chromosomal breakpoints were found within *MLL* introns 9(n=3), 10(n=4) and 11(n=5). One case *(MLL-AF9)* showed a recombination event affecting *MLL* exon 10;

✓ This patient is an 11-month-old boy, native Amerindian, who was diagnosed with AML-M5. He died one month after initiation of treatment. This is an interesting case because leukemia development among Amerindians is not commonly described. People living in remote places under the influence of ancient cultural customs use vegetable roots, fruits, nuts, infusion herbs, and oils with phytotherapeutic properties that remain unknown. Additionally, indiscriminate use of pesticides is common because agriculture is the major activity in this region. We speculate that this child was continuously exposed to chemicals that could facilitate DNA damage and subsequently acquired the identified *MLL* rearrangement[2].

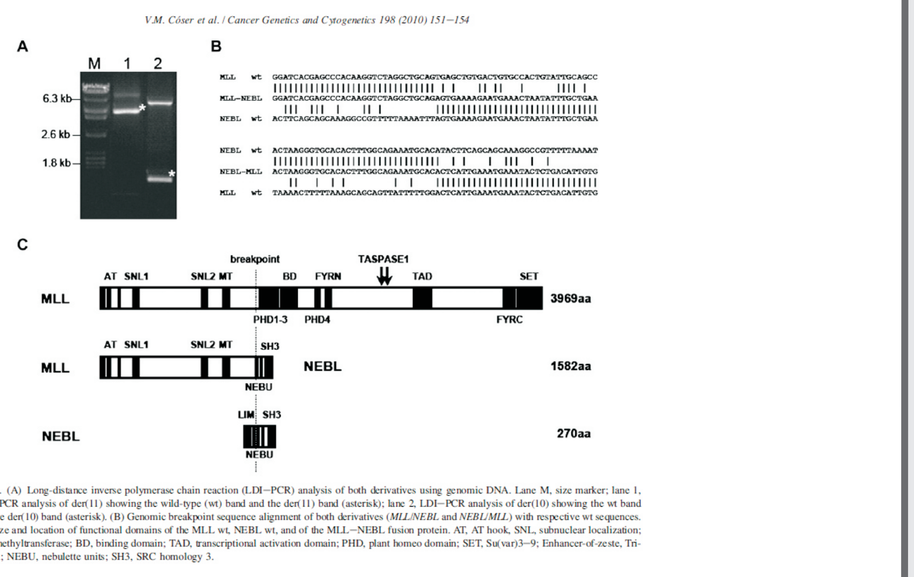

Fig. 3. (A) Long-distance inverse polymerase chain reaction (LDI–PCR) analysis of both derivatives using genomic DNA. Lane M, size marker; lane 1, LDI–PCR analysis of der(11) showing the wild-type (wt) band and the der(11) band (asterisk); lane 2, LDI–PCR analysis of der(10) showing the wt band and the der(10) band (asterisk). (B) Genomic breakpoint sequence alignment of both derivatives (*MLL/NEBL* and *NEBL/MLL*) with respective wt sequences. (C) Size and location of functional domains of the MLL wt, NEBL wt, and of the MLL–NEBL fusion protein. AT, AT hook; SNL, subnuclear localization; MT, methyltransferase; BD, binding domain; TAD, transcriptional activation domain; PHD, plant homeo domain; SET, Su(var)3–9; Enhancer-of-zeste, Trithorax; NEBU, nebulette units; SH3, SRC homology 3.

CONCLUSIONS

Concerning *MLL* TPGs and breakpoints distribution, our results are in line with recently recombinome published data. Moreover, the high number of cases included in the epidemiological study constitutes a rich source for future discoveries and, the continuous *MLL* recombinome analysis will allow us to determine whether certain environmental risk-factor maternal exposures are associated to specific *MLL* rearrangements.

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