HEMO® CONGRESSO BRASILEIRO de HEMATOLOGIA, HEMOTERAPIA e TERAPIA CELULAR

MOLECULAR CYTOGENETIC CHARACTERIZATION OF PARTIAL TRISOMIES: SECONDARY CHROMOSOME ABNORMALITIES IN CHILDHOOD BURKITT LYMPHOMA/LEUKEMIA

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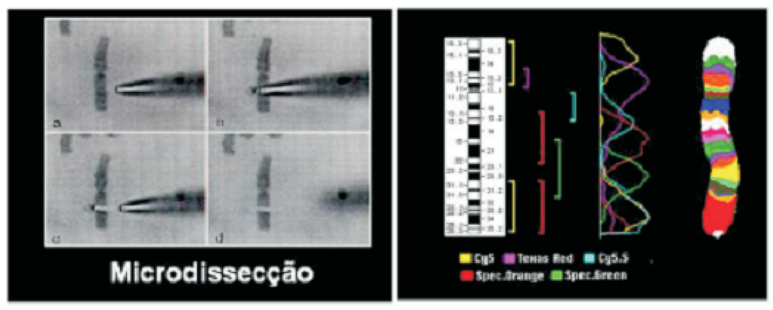
OBJECTIVES

The genetic hallmark of Burkitt lymphoma/Leukemia (BL/L) is MYC oncogene rearrangements, most commonly with immunoglobulin genes. The cure rate can reach 90%, although, for the majority of patients who relapse, there is no effective therapeutic alternative. Additional chromosomal abnormalities have been discussed as potential markers of unfavorable clinical prognosis, with controversial results in the literature. Previous studies from our group showed 71% of secondary chromosomal abnormalities in children and adolescents diagnosed with BL/L in bone marrow onset, mainly involving chromosomal regions 1q and 13q, which is in accordance with the world literature in relation to the chromosomes most commonly involved in secondary abnormalities in BL/L. Our aim here was to molecularly characterize secondary chromosome abnormalities in 4 cases from, our total sample, which presented secondary abnormalities primarly seen by G-banding cytogenetics.

Bone marrow aspirate from 4 BL/L patients were processed in a 24 hours cell culture, and studied by G-banding technique in the Cytogenetics Laboratory, INCA, RJ. Experiments for molecular characterization were performed in the Molecular Cytogenetics Laboratory at Jena University (Germany) using high-resolution molecular cytogenetics approaches, such as Fluorescence *in situ* hybridization (FISH), Multicolor Chromosome Banding (MCB), and Multicolor-FISH, with the application of locus specific, whole and partial chromosomic paintings, MCB and Bacterial Artificial Chromosome probes for accurate identification and description of the abnormalities. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN).



RESULTS

In all the cases we found partial trisomies, resulting from duplications or translocations, involving chromosomes 1q (in two samples), chromosome 8q and chromosome 13q. Besides, molecular cytogenetics allowed us to access the breakpoints involved in the abnormalities and revealed that these aberrations were part of complex karyotypes for some of the cases, that are characterized bellow.

Case 1

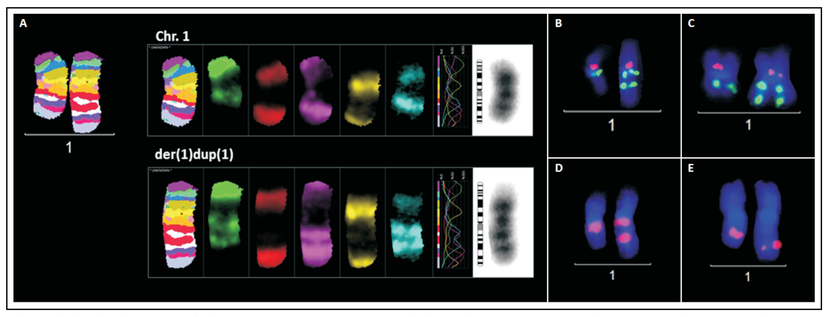


Figure 1: A) MCB1 confirmed the duplication of chromosome 1, suggesting the breakpoint q22;q33; B) Commercial LSI MCL1/1p12 probe characterizing one of the duplication breakpoints as q21.2; C) Commercial LSI MDM4/1p12 probe featuring the other breakpoint as q32.1; D) BAC RP11-301 M17 (1q21.2) in red confirming the breakpoint 1q21.2;) BAC RP11-57 I17 (q32.2) in red confirming the breakpoint 1q32.

Case 2

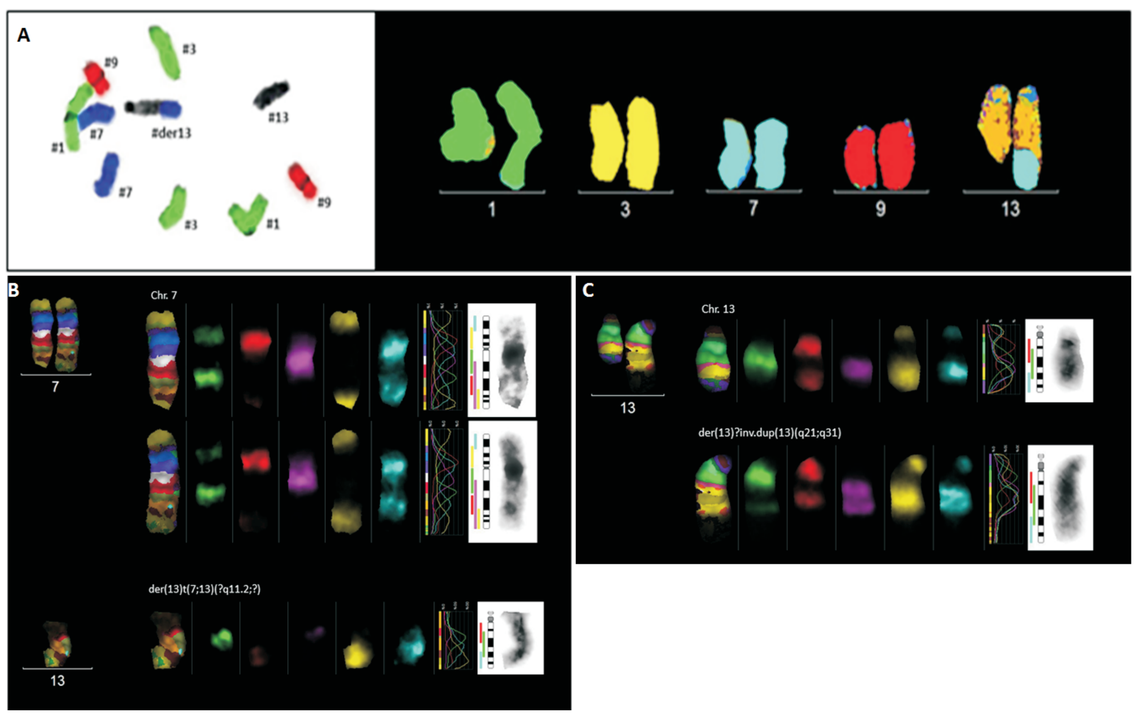


Figure 2: A) Chromosome paintings for chromosomes 1 (green), 3 (yellow), 7 (blue) and 9 (red) characterized that der(13) had a portion of chromosome 7; B) MCB7 showing the presence of normal chromosomes 7 not involved in the alteration and characterizing a partial trisomy of chromosome 7 in the derivative chromosome 13; C) MCB13 characterizing a duplication on chromosome 13 and suggesting the breakpoint dup(13)(?q21;?q31). In addition, MCB13 characterized that in addition to duplication of chromosome 13, there was an extra portion on that chromosome, that was latter observed to be from

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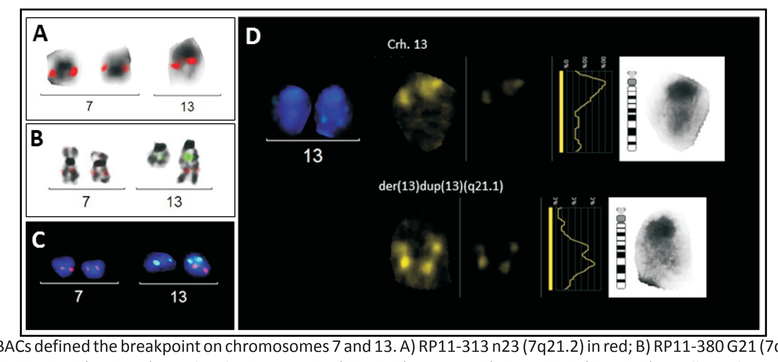

Figure 3: Application of BACs defined the breakpoint on chromosomes 7 and 13. A) RP11-313 n23 (7q21.2) in red; B) RP11-380 G21 (7q21.3) in red and RP11-520 F9 (13q21.31) in green; C) RP11-90 N9 (7q21.11) in red and RP11-538 C21 (13q21.1) in green; D) RP11-98 F3 (13q21.1) in yellow. The application of BACs enabled the characterization of the breakpoints of der(13) as der(13)t(7;13)(13pter->13q34::13q21->13q34::7q21->7qter).

Case 3

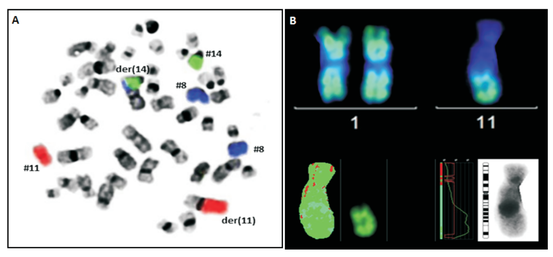

Figure 4: A) FISH using chromosome paintings WCP8 in blue, WCP11 in red and WCP14 in green showing t(8;14) and a derivative chromosome 11 - der(11); B) Due to the DAPI counterstain suggestive of the extra chromosome portion in der(11) coming from chromosome 1, the chromosome painting WCP1 was applied, confirming this assumption, characterizing a partial trisomy of chromosome 1, through a t(1;11).

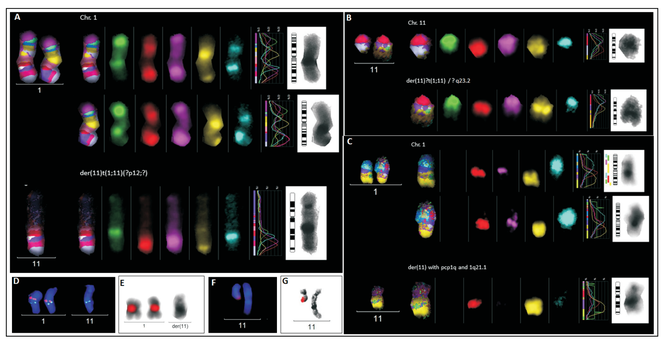

Figure 5: A) MCB1 illustrates the portion of chromosome 1 that is in der (11). The MCB suggested the breakpoint of the partial trisomy in derivative 11 as 1?p12; B) MCB11 illustrating a normal chromosome 11 and der(11). MCB suggested that der(11) lost its terminal portion from the band 11q23.2->qter; C) mFISH submix1 with probes PCP1p (blue), PCP1q (yellow), RP11-130 B18-1q12 (pink), RP11-35 B4-1q21.1 (red) showing that der(11) has several portions of the chromosome 1; D) LSI MCL1/1p12 specific locus probe showing the presence of this gene in der(11); E) Centromeric probe for chromosome 1 (red) showing that the der(11) does not carry the centromere 1; F-G) BACs RP11-567 M27 from region 11q24.3 (left) and RP11-356 E17 from region 11q23.3 (right), respectively, both in red, proving the breakpoint suggested by the MCB.

Case 4

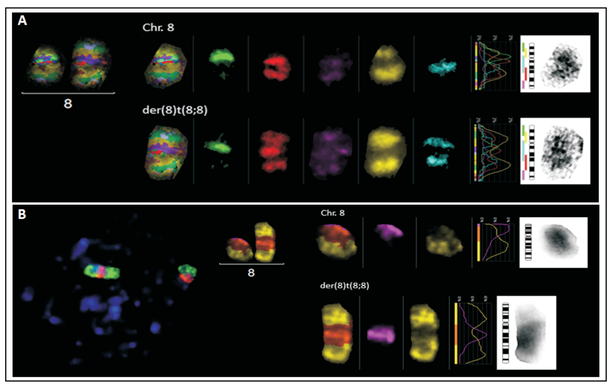

Figure 6: A) G-banding diagnosis initially suggested an isochromosome 8. A) After application of MCB8 probe, a t(8;8) was suggested since it had the short arm held; B) PCP8p (pink/red) and PCP8q (green/yellow) probes confirming the presence of the short arm in the der (8).

For case 4, we also performed expression approaches (data not shown) in order to endorse the diagnosis of BL/L, once it may fit to the new classification of World Health Organization for cases of BL/L without MYC translocation. Although literature also describes chromosome losses as seconday abnormalities associated with t(8;14)(q24;q32) or its variants in BL, so far, the great majority of our childhood Burkitt Lymphoma cohort, have been presenting partial trisomies generated by duplications and/or, less commonly, duplications followed by translocations.

DISCUSSION

Once secondary abnormalities in BL/L seem to play an adverse role in the prognosis of this disease, a detailed characterization of the breakpoints is of great importance, once they may be involving putative genes that can influence the phenotype of the disease. Interestingly, although we can find in the literature recurrent losses of chromosomic regions among BL/L secondary abnormalities, in our cohort, duplications generating partial trisomies have been most commonly seen. The reason and mechanisms for the occurrence of these partial trisomies in BL/L remain to be elucidated.

CONCLUSION

This observation can lead us to think that some set of genes may be deregulated, which could influence the unfavorable outcome observed in the majority of patients harboring such kind of abnormalities. This hypothesis remain to be elucidated by other methodological approaches in a larger amount of cases.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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