Molecular studies detected an original complex karyotype involving chromosomes 9, 11 and 14, with KMT2A-r in infant mixed-phenotype acute leukemia

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BACKGROUND

Acute Leukemia is the most common cancer in childhood. In 2-5% of AL cases present immunophenotypic profiles that cannot clearly fulfill any of the above mentioned specific lineage subtypes. Beyond this so-called biphenotypic, bilineal, ambiguous or mixed lineage leukemia (BAL/MPAL), the rearrangements of the KMT2A gene (KMT2A-r) may be implicated. The t(11;14)/KMT2A-r are a small set of very rare rearrangements with two partner genes reported in leukemia. Another rare abnormality in leukemia is the intra or extra-chromosomal amplification (11q gain/Amp11q). Infant leukemia with KMT2A-r usually shows features of poor prognosis. Besides, rare KMT2A-r cases are of interest to bust the pathological biology knowledge of this leukemia subtype, since KMT2A presents a great number of translocation partner genes (TPGs) diverging the outcome and the leukemic molecular mechanism depending on the TPG. In this work, we describe the clinical and molecular cytogenetic study of a novel three-way t(11;14;9) in a complex karyotype involving KMT2A-r in an infant with BAL/MPAL immunophenotype.

METHODS AND RESULTS

A 13-months-old boy with a clinical history of intermittent fever, sinusitis, diarrhea and a syncope episode. Patient's physical examination showed pallor, scattered lymphadenomegaly on cervical chain submandibular, inguinal and pelvic area, a mass on right mandible of 4cm, hepatosplenomegaly and mediastinal enlargement. Laboratory data showed anemia(hb: 8.8g/dl) and thrombocytopenia (platelet: 58 x 10^9/l), WBC of 5.2 x 10^9/l and LDH of 939U/l. The bone marrow (BM) presented 89% of lymphoid morphology. The immuphophenotypic profile showed 2 blast populations, both compatible with BAL/MPAL. The patient achieved complete remission at D33, but presented MRD$^+$ at D78(5x10^{-4}) and was negative at the subsequent follow-up points(MRD$^-$, < 10^{-5}). He was stratified to high-risk treatment arm according to ALL-BFM2013 criteria and is alive for eighteen months.

G banding cytogenetic analysis observed a karyotype presenting an additional material on 14 chromosome (Fig.1). The LSI-FISH screening, using MLL (KMT2A) break-apart observed split signal on chromosome derivative (der) 14 and on der 9, the chromosomes 11 presented normal MLL signals (Fig.2 A). This analysis also revealed a cryptical deletion on 11q13 and 9p12 confirmed by LSI-FISH analysis with IGH/CCND1 and CDKN2A probes (Fig.2 B-C). WCP for chromosomes 9, 11, and 14 were applied confirming the three-way translocation and the MCB refined the karyotype (Fig.3). The final karyotype was described as: 46,XY,der(9)t(9;11)(p21.3;q23.3),der(14)(14pter→ 14q32.3::11q13→ 11q23.3::p21.3→ 9pter),del(11)(q13).ish der(9)(3'KMT2A+,CDKN2A-),der(14)(IGH+;5'KMT2A+),del(11)(CCND1-).

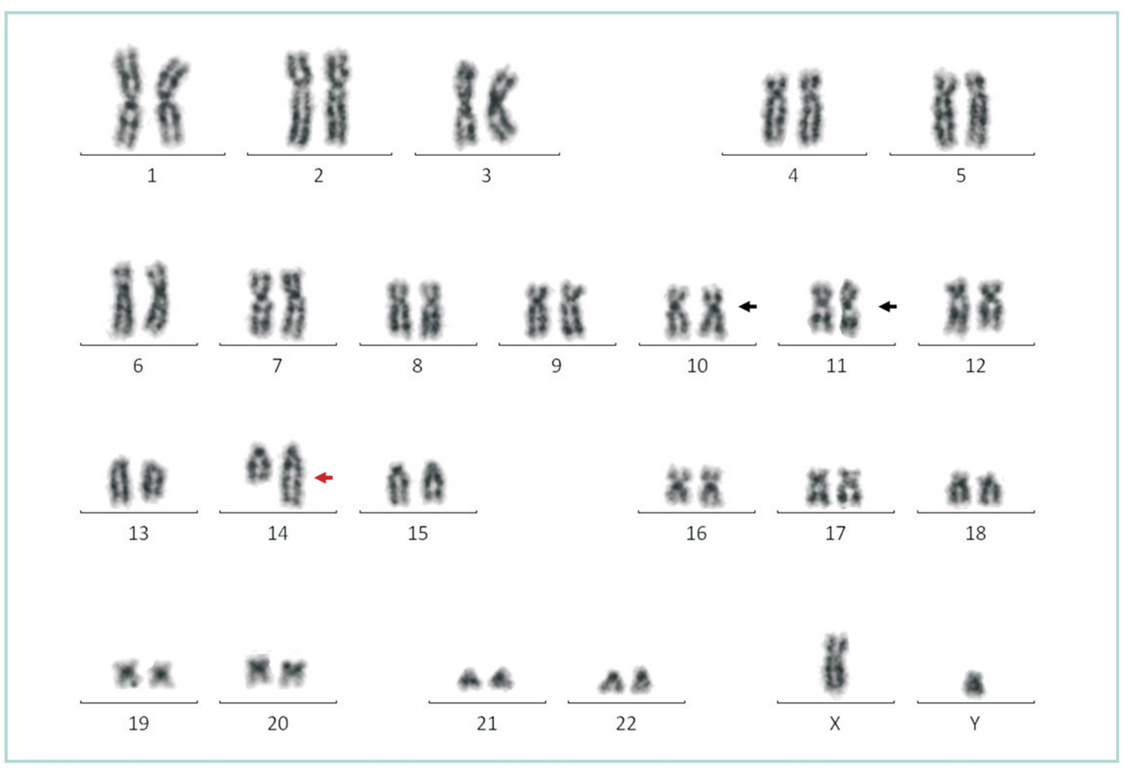

Figure 1: G-Banded Karyotype. Total karyotype showing additional chromosomal material on derivative chromosome 14 (red arrow) and no detectable abnormalities on chromosomes 9 and 11 (black arrow).

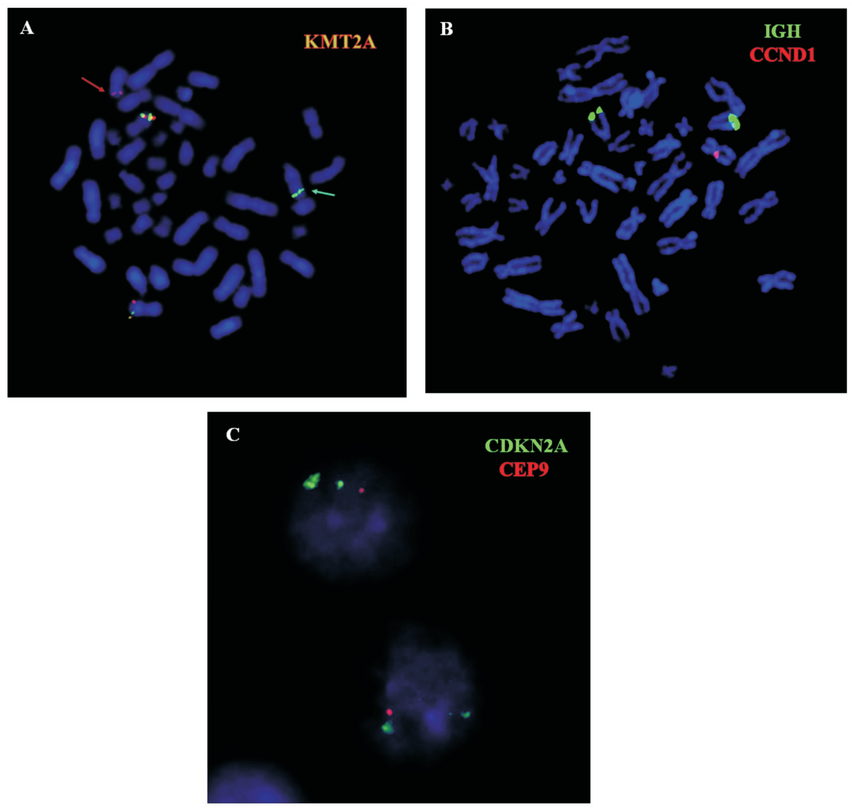

Figure 2: LSI FISH experiments: A: analysis with LSI MLL (KMT2A) break-apart probe showing two normal *KMT2A* signals, and one extra copy of *KMT2A* gene with split signals; the proximal *KMT2A* signal on der(14) (green signal – green arrow) and distal *KMT2A* signal on der(9) (red signal – red arrow); **B**: *IGH/CCND1* probe showing a heterozygous loss of *CCND1* gene (loss of red signal); **C:** analysis with *CDKN2A*/CEP9 probe showing heterozygous deletion of *CDKN2A* (loss of red signal)

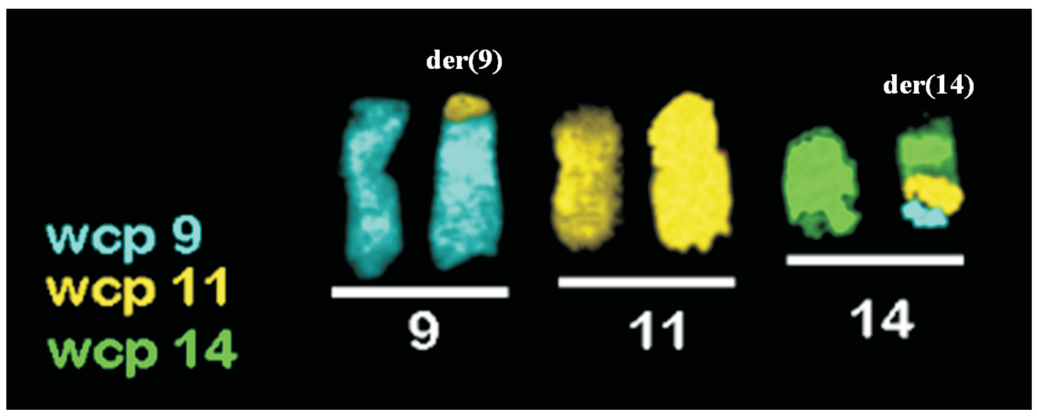

Figure 3: FISH with WCP probes for chromosomes 9 (blue), 11 (yellow), 14 (green), confirming the presence of 11 chromosomal material o der(9), two normal 11 chromosomes, and, presence of 9 chromosomal material and additional 11 chromosomal material on der(14), confirming unbalanced three-way translocation

CONCLUSION

The cytogenetic and genetic findings are considered decisive to differential diagnostic, categorization of some BAL/MPAL subtypes and therapeutic decision. In cases of KMA2A-r, WHO criteria consider this entity a special category in the classification. Hypothetically, a low expression of CCND-1 and CDKN2A could lead to abnormal DNA repair by miss function mechanism due to heterozygous deletion of both genes in this leukemogenesis.

In the case presented here, KMT2A gene was inserted in the der(14) and rearranged with a third chromosome in a three-way translocation. This karyotype complexity could only be solved after an additional molecular cytogenetic investigation that revealed the *KMT2A-MLLT3* fusion gene. A *KMT2A* TPGs multicentric worldwide study observed that translocation t(9;11)/*KMT2A-MLLT3* comprises 18% of all infant leukemia (lymphoid and myeloid lineage), but this study did not include the BAL/MPAL immunophenotype classification. Thus, we contribute new data for the infant leukemia with a complex karyotype harboring a new KMT2A-r and BAL/MPAL immunophenotype.

We highlight that banding cytogenetics combined with FISH-based molecular cytogenetic techniques is a crucial approach to better characterize the rearrangements, disclose the abnormalities, and properly adjust the risk stratification for the patient.

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