INCA

LDI-PCR STUDIES DETECTED A NOVEL GENE FUSION *SEC16A-KMT2A*, AND *KMT2A-MLLT1* CRYPTICALLY PRESENT IN THE TRANSLOCATION t(9;11)(q34;q23) IN AN INFANT WITH ACUTE LYMPHOBLASTIC LEUKEMIA: AN ORIGINAL CASE REPORT

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Objectives

Rearrangements involving the lysine (K)-specific methyltransferase 2A gene (*KMT2A*) also known as MLL gene, are reported in 18% of pediatric patients with acute myeloid leukemia (AML) and 6-8% of pediatric patients with acute lymphoblastic leukemia (ALL). However, in infants (<1 year of age), 70% of patients with ALL and 35-50% of patients with AML harbor *KMT2A* gene rearrangements.

KMT2A fusion partner genes play a significant role in the transcriptional activation of *KMT2A* target genes. *KMT2A* partners comprise (a) genes that encode nuclear proteins implicated in transcriptional activation, and (b) genes that encode cytoplasmic proteins with multiple functions.

Most *KMT2A* rearrangements are detectable by G-banding. However, about 20% of them occur in karyotypes in which the *KMT2A* partner gene cannot be identified. In such cases, the *KMT2A* rearrangement must be characterized by more sensible techniques. In this sense, cytogenomic approaches, aiming a more reliable and precise *KMT2A* fusion characterization, are important to better understand the role of recurrent and novel *KMT2A* fusion partner genes in the genesis of pediatric acute leukemias.

Herein, we present an original case report of an infant with ALL in which a combination of cyto-molecular and cytogenomic techniques detected a novel fusion gene *SEC16A-KMT2A*, and a *KMT2A-MLLT1*, cryptically present in the translocation t(9;11)(q34;q23).

**This project was approved by the research ethics committee of INCA (#077/08).*

Material and Methods

Patient and Methods

At the diagnosis, sample from a 2-month-old boy was referred to the Laboratory of Cytogenetics – INCA. Immunophenotyping showed 100% of blast Cells, and a profile compatible with Pro-B-ALL diagnosis, being treated under the ALL-BFM-2009 protocol.

The patient could not experience any treatment response, and died due to an early relapse.

Cytogenetic analysis was performed at diagnosis, in bone marrow samples cultured for 24 hours, according to the standard protocol. FISH experiments were conducted using homemade and commercial probes, according to the manufacturers' instructions.

LDI-PCR assays were used to identify the *KMT2A* partner genes and their corresponding breakpoints.

Results

Conventional cytogenetics by G-Banding technique revealed the karyotype 47,XY,+X,t(9;11)(q34;q23) **(Figure 1A).**

FISH assay with the MLL break apart, dual color probe revealed a *KMT2A* gene rearrangement **(Figure 1B)**.

The LDI-PCR sequencing revealed a novel fusion gene *SEC16A-KMT2A* and the fusion *KMT2A-MLLT1* **(Figures 2 and 3)**.

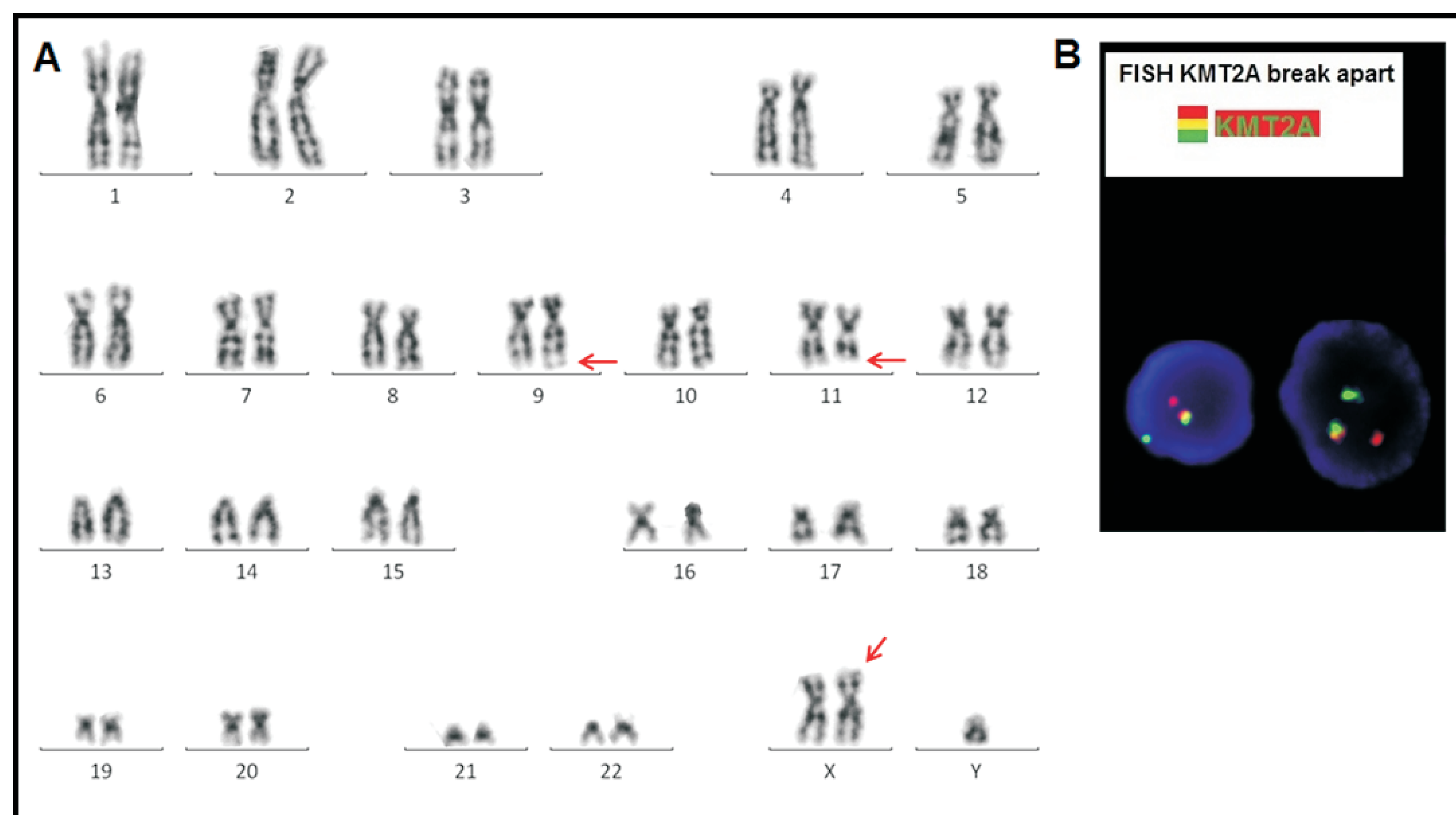

Figure 1: Cytogenetic analysis: **(A)** G-banding karyotype. The red arrows show the derivative chromosomes 9 and 11, and the trisomy X. **(B)** FISH analysis in interphase nuclei showing the *KMT2A* gene rearrangement. The fused signals show the normal gene. The green and red signals broke apart confirm the rearrangement.

SEC16A-MLL (+/-)

SEC16A: Intron 15
MLL: Intron 11

CATCCGAGCATCGCCCTGTGAGCTGTGAACTCTTCTGGACACTTCACTCATCATCGTCTCCC
ACCCACCACGCCCGCTGACCACAGTCCTAGTCCCTGTTCCCCAAGGGATTTCAAGAGCAAAG
TAAACGACTAAGCTGGTAGCAAACTCACTCTGCTTTCCCCCCAACCCCTTTCATGGGGCCAC
TCGGCTCTCACAGTCACAACGCCCCAAGAGCACGGCACTGAGACCACTGCCTTCCGTG•TA•
TCCTAGTTAGTATTTTTGAAAATCCTATATCAATATGAAAATAACTTATTTCTAAAA*TTAGA*
AATGGAGGCTGGGCGTGGTGGCTCACGCCTATAATCCCAGCACTTTGGGAGGCCGAGGCAGG
CAGATCACAAGGTCAGGAGATTGAGACCATCCTCGCTAACACAGTGAAACCCCATCTCTACT
AAAAATACAAAAAATTAGCCAGGTGTGGTGGCACGCGCCTGTGATCCCAGCTACTCAGGAGA
CTGAGGCTGGAGAATCGCTTGAACCCAGGAGGCGGAGGTTGCAGTGAGTCGAGATCGCACCA
*CTGCACCCCAGCCTGGGCGACAGCGAGACTCCGTCTCAAAAAAATAAATAAATAAAAAT*TAA
AACAATTAAAAAAATAAAATTACAAATGGAAAGGACAAACCAGACCTTACAACTGTTTCGTA
TATTACAGAAAACGTTTAAACCCTCCCTATTTCCCCCACCCCACTCCTTTATATTCCCATAG

Figure 2: Results of LDI-PCR assay. The LDI-PCR sequencing identified the *SEC16A-KMT2A* fusion gene, and the corresponding breakpoints.

MLL-MLLT1

MLL: Intron 11
MLLT1: Intron 1

AATTTTTTAAACTTTTTATGTTGACATGATTTCAGACTTACAAAAAAACTATGAGT*TGTA*
*CAGA*C*GAATTC*TAAGTACCCCTCACCCAAATTCCCTAAGTGTTAATATGTTTCTCTGTGTG
TATATATTTTACAAAATAACAAATAAAATACATATACACATTTTACCTGTAGATACACAT
GTATCTAAAAATTTGAGAACAAGTTGCAGACATAAACCATTTTACCTCTAAATATTTTAG
TGTATATTTTTAAAAATCAAGGACGTTCTCGTATTTAACCATGGTATAATTACCAAATCA
GGAAATTAACACACTGGTACATTACTATTATCTGATCTATAGGCCTTATTTAGGTTTGAC
CAATTGTCCCAATAATTCCTTTATGGCAAAAGAAAATTCTGGATTATCCTAG•TT•GTTG
GAATGCGGTGGTGTTCAGGCAGACACTCGTGCTTGGCCTGAGTATTGCCATGCCGGTGCT
CAGCCCTCACCTCGTGTGTTTGGTGGGGGTACTTTTACCTGAGATCCTCATGAATCCGCA
GCGCCGGGGGTTTATGAGTGCGCCTGATCTTCCGTCCATCCCCACTTTCTTCCCACACTG
GGGTCGTGAATGTCCCTGCTGCCCGGATCTTGGGGTTACCTGAATGTCCTCCACCCTCGG
GTCCTGTCAGTTCTGTGGCTTTGGGCTGTGTTCACCCCTTTTTCCAGCCACTCCTTCTGG
TTGCCTCCTCATGGGCTGGGGTTGCTGTTAGTTTGCCATTCGCCCTTTTCTGCCTGCACC
CCAGGCCTTACACAGAGGGCCGGGGGCTTTGACTCCGAGACCGTTTCCCATATCTGTCA
GCCACTTATGACGACGGGTTATTTCTTTTGTCTCCGGCCTGATCCCAGGCACCCCCTGCA

Figure 3: Results of LDI-PCR assay. The LDI-PCR sequencing identified the *KMT2A-MLLT1* fusion gene, and the corresponding breakpoints.

Discussion and Conclusion

Using the G-banding technique, it was possible to observe an alteration in the band pattern of both chromosomes 9 and 11, informing a t(9;11).

The FISH analysis confirmed the *KMT2A* gene rearrangement.

Thus, we performed LDI-PCR sequencing, which interestingly revealed two gene fusions, the novel *SEC16A-KMT2A*, and the recurrent *KMT2A-MLLT1* cryptically present in the context of the t(9;11)(q34;q23).

The *SEC16A* (SEC16 Homolog A) gene encodes a protein that forms part of the Sec16 complex. This protein has a role in protein transport from the endoplasmic reticulum (ER) to the Golgi and mediates COPII vesicle formation at the transitional ER. Alternative splicing results in multiple transcript variants that encode different protein isoforms. Although, more similar cases need to be studied to determine its role and prognostic impact as a *KMT2A* partner gene.

Here we describe, for the first time in the literature, a novel gene fusion *SEC16A-KMT2A* cryptically present in the translocation t(9;11)(q34;q23) in an infant with ALL. Future studies will be necessary to properly characterize the role of this novel fusion proteins and its importance in the genesis of acute lymphoblastic leukemia.

Acknowledgements

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