

ACUTE LEUKEMIAS

## A NOVEL HIGHLY RECURRENT FUSION CIRC RNA FROM THE *KMT2A::AFF1* FUSION GENE IMPACTING MITOCHONDRIAL METABOLISM IN B-ALL WITH THE T(4;11)(Q213-Q221;Q233) TRANSLOCATION

D. Tolomeo<sup>1</sup>, M. Bardini<sup>2</sup>, S. Venuto<sup>1</sup>, G. Visci<sup>1</sup>, A. Lonoce<sup>1</sup>, L. Calamo<sup>1</sup>, I. Cifola<sup>3</sup>, M. Severgnini<sup>3</sup>, M. Mastrodonato<sup>1</sup>, D. Semeraro<sup>1</sup>, L. Valsecchi<sup>2</sup>, M. Mauri<sup>4</sup>, R. La Starza<sup>5</sup>, M. Lucarelli<sup>5</sup>, A. Azzariti<sup>6</sup>, S. Serrati<sup>6</sup>, A. Ferrari<sup>7</sup>, G. Martinelli<sup>8</sup>, N. Santoro<sup>9</sup>, M. Fumagalli<sup>2</sup>, G. Cazzaniga<sup>2,10</sup>, G. Fazio<sup>2,10</sup>, C. T. Storlazzi<sup>1</sup>

<sup>1</sup>Department of Biosciences, Biotechnologies, and Environment, University of Bari Aldo Moro; <sup>2</sup>Tettamanti Center, Fondazione IRCCS San Gerardo dei Tintori; <sup>3</sup>Institute for Biomedical Technologies (ITB), National Research Council <sup>CNR</sup>; <sup>4</sup>School of Medicine and Surgery, University of Milano-Bicocca; <sup>5</sup>Hematology and Bone Marrow Transplantation Section, Department of Medicine and Surgery, University of Perugia, Centro di Ricerche EmatoOncologiche, Azienda di Perugia; <sup>6</sup>IRCCS Istituto Tumori "Giovanni Paolo II"; <sup>7</sup>Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"; <sup>8</sup>Department of Hematology and Oncology Sciences, Institute of Haematology "L. and A. Seràgnoli", S. Orsola University Hospital; <sup>9</sup>Pediatric Oncology AOUC of Policlinico of Bari; <sup>10</sup>School of Medicine and Surgery, University of Milano-Bicocca.

**Introduction:** Fusion circRNAs (f-circRNAs) may be generated by the back-splicing of linear fusion transcripts derived from genomic rearrangements. Recently, f-circRNAs from the *KMT2A::AFF1* fusion gene, originated by a balanced t(4;11)(q21.3-q22.1;q23.3), were detected by bioinformatics approaches in the B-cell Acute Lymphoblastic Leukemia (B-ALL) RS4;11 cell line, as well as in patients, though their contribution to leukemia has not yet been studied. In this framework, we identified a novel *KMT2A::AFF1* f-circRNA back-splicing junction (BSJ) in B-ALL cell lines, investigated its oncogenic role *in vitro*, and determined its possible recurrence in B-ALL patients harboring the t(4;11) rearrangement.

**Methods:** *KMT2A::AFF1* BSJs were investigated by PCR approaches and Sanger sequencing in RNase R-treated and untreated samples of SEM, ALL-PO, and RS4;11 B-ALL cell lines with the t(4;11) translocation, as well as in pediatric and adult B-ALL patients, both with and without the t(4;11) translocation. The f-circRNA functional role was explored using total RNA-seq profiling of Dicer substrate small-interfering RNA (DsiRNA) BSJ knockdown cells, combined with cell death assays, confocal laser scanning microscopy (CLSM), Seahorse analysis, and transmission electron microscopy (TEM).

**Results:** RT-PCR/Sanger sequencing revealed a BSJ fusing *AFF1* exon 8 to *KMT2A* exon 2 (AK\_8\_2), enriched in RNase R-treated versus untreated samples, in three B-ALL cell lines, all carrying the t(4;11)(q24;q23)/*KMT2A::AFF1* translocation. This sequence was also found in 8/10 pediatric and 7/10 adult t(4;11)-positive B-ALL patients, including three paired diagnosis-relapse samples, whereas it was absent in 23 t(4;11)-negative B-ALL samples. Total RNA-seq profiling of AK\_8\_2 knocked-down SEM cells identified upregulated genes involved in oxidative stress response and apoptosis regulation. Cell death analysis confirmed the pro-apoptotic impact of AK\_8\_2 silencing. Confocal analyses and Seahorse bioenergetic profiling demonstrated overproduction of reactive oxygen species, increased mitochondrial membrane potential, and enhanced mitochondrial function, accompanied by dysmorphic mitochondria, as observed by TEM, after AK\_8\_2 knockdown.

**Conclusions:** We identified AK\_8\_2 as a novel f-circRNA BSJ recurrent in t(4;11)-positive B-ALL patients at both diagnosis and relapse. Our data provide evidence of the functional role of this RNA molecule in apoptosis and mitochondrial metabolism, suggesting its potential involvement in B-ALL leukemogenesis.