XIX CONGRESSO NAZIONALE SIES2026
Firenze | 4-6 marzo 2026
Palazzo degli Affari

Acute Leukemias

FUNCTIONAL STUDY OF CIRCRNA WITH ALTERED EXPRESSION IN T-ALL AND CIRCRNA-DRIVEN T-ALL SUBTYPE CLASSIFICATION

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Omics studies in T cell acute lymphoblastic leukemia (T-ALL) are uncovering disease mechanisms and progressively explaining the disease heterogeneity. Recently, circular RNAs (circRNAs) have gained traction in cancer research as versatile regulators of cellular activities, with distinct mechanisms and roles in driving oncogenic axes. The dysregulation and pathogenetic relevance of circRNAs in T-ALL have been demonstrated in recent studies (Buratin et al. 2020, 2023). We aim to deepen our understanding of circRNAs' functional roles in T-ALL and to explore the utility of circRNA expression for classifying T-ALL molecular subtypes. We considered three cohorts of pediatric patients with T-ALL profiled by RNA-seq, comprising 25 (TALL25), 264 (TALL264) and 1300 (TALL1300) cases at diagnosis and 19 samples of immature thymocytes from healthy donors. We quantified circRNA expression using CirComPara2 and ccp2tools and applied best-practice methods for normalization and batch-effects removal. CircRNA differential expression was assessed using DESeq2 and Limma with a significant threshold of adj $p < 0.01$. The uwot R package was used for UMAP analysis, XGBoost tree methods for classification and the Seurat package with the Leiden algorithm for unsupervised analysis. We quantified circRNA expression in 10 T-ALL cell lines by RT-qPCR using divergent primers designed with CircPrimer2. The back-splicing junctions were confirmed by Sanger sequencing. RNA pull-down experiments were performed using ElementZero probes, followed by RNA-seq. Comparison of TALL25 and TALL264 with normal thymocytes identified 1,009 and 551 circRNAs with significantly altered expression, respectively, which were predominantly (85%) overexpressed in malignant cells. By overlapping results across the two cohorts, we defined a robust set of 256 circRNAs concordantly dysregulated in T-ALL. The quantification in a panel of T-ALL cell lines of 16 circRNAs among the most upregulated and abundant in T-ALL defined and prioritized candidates for functional investigation. Loss-of-function studies are ongoing. For one selected circRNA, RNA pull-down experiments yielded specific and reproducible results, with high efficiency across three replicates, enabling isolation of interacting RNAs. CircRNA expression in TALL1300 has been considered in relation to the annotated molecular subtypes, characterized by typical lesions and gene expression profiles, and associated with T cell maturation stages. The classification based on circRNA expression mostly aligns with the known subgroups.

We identified and validated several circRNAs that were markedly upregulated in T-ALL, conducted functional investigations using RNA pull-down experiments and circRNA silencing *in vitro*. The development of a machine learning model for circRNA-based T-ALL subtype classification has the potential to identify the most informative dysregulated circRNA for each molecular subgroup, thereby increasing the understanding of the disease heterogeneity and suggesting new players in T-ALL pathogenesis.