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

XIX Congress of the Italian Society of Experimental Hematology ABSTRACT N. P072

Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

FOCAL ADHESION KINASE FAK INTERPLAYS WITH ROR1 IN CHRONIC LYMPHOCYTIC LEUKEMIA

V. Gramegna, M. Castronuovo, A. Beltrame, G. Capasso, P. Fantato, E. Pagnin, F. Angotzi, A. Cellini, A. Serafin, M. Facco, A. Visentin, L. Trentin, F. Frezzato.

Hematology Unit, Department of Medicine, University of Padua.

Introduction: Chronic lymphocytic leukemia (CLL) strongly depends on the tumor microenvironment, which supports cell survival, proliferation, and migration. Two key molecules emerge as crucial regulators in these processes: Focal Adhesion Kinase (FAK) and Receptor tyrosine kinase-like Orphan Receptor 1 (ROR1). FAK has been implicated in CLL progression, affecting the microenvironment dynamics. It interacts with molecules such as HS1 and cortactin, also involved in CLL pathogenesis. ROR1 shares connections with both these molecules and FAK pathways, suggesting a molecular network. This study investigates the potential interplay between FAK and ROR1 in CLL progression and aggressiveness.

Methods: CD19+/CD5+ cells from therapy-free or ibrutinib-treated CLL patients were isolated by density gradient centrifugation. Flow cytometry and cell sorting were used to analyze FAK and ROR1 expression in different CLL subpopulations, the proliferating ($CXCR4^{dim}/CD5^{bright}$) and quiescent ($CXCR4^{bright}/CD5^{dim}$) ones. According to the experiments, leukemic cells were treated with 200nM Wnt5a, 200nM CXCL12, and 5μM defactinib, for the appropriate time. Cell migration was assessed using transwell assays, with migrated cells counted by flow cytometry. Protein expression and activation of FAK, ROR1, and HS1 were quantified by western blotting.

Results: Our analysis found a significant correlation between FAK activation and ROR1 expression in CLL cells ($r=0.41$, $p<0.05$). Stimulation with Wnt5a, a ligand of ROR1, enhanced FAK phosphorylation at Y397, suggesting a link between ROR1 signaling and FAK activation. We also examined different CLL subpopulations. Both FAK and ROR1 were overexpressed in the proliferative ($CXCR4^{dim}/CD5^{bright}$) compared to the resting fraction ($CXCR4^{bright}/CD5^{dim}$, $p<0.001$). Analysis of *ex vivo* CLL cells from ibrutinib-treated patients revealed elevated FAK and ROR1 levels in circulating lymphocytes during early treatment-induced lymphocytosis, suggesting their role in CLL cell mobilization. In this context, migration assays demonstrated that the FAK inhibitor defactinib significantly reduced CLL cells motility induced by Wnt5a and CXCL12. Importantly, we observed a positive correlation among the expression levels of FAK, ROR1, and HS1, particularly pronounced in poor-prognosis CLL patients, suggesting a complex interplay between these molecules in disease progression.

Conclusions: Our findings highlight the interplay between FAK and ROR1 in CLL cell migration and response to therapy. Their overexpression in proliferative CLL cells and elevated levels during ibrutinib-induced lymphocytosis suggest a crucial role in proliferating CLL cell in lymphoid organs. The effects of FAK inhibition underscore its potential as a therapeutic target, thus combining FAK (and ROR1) inhibitors with BTK inhibitors could offer a promising strategy to enhance treatment efficacy by addressing both cell migration and survival of the leukemic clone.