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Cluster of Expressed Proteins Contribute to Wildtype PEAR1 Function in Acute Myeloid Leukemia Cell Lines

Luca Sax, BA; Cristina E. Tognon, PhD; Sunil K. Joshi, MD, PhD; Thomas J. Fernandez, BS; Jeffrey W. Tyner, PhD

Department of Cell, Developmental, and Cancer Biology, Department of Hematology and Oncology, Oregon Health & Science University

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Abstract

The mutational status of platelet-receptor PEAR1 was recently identified as a major factor for survival of young patients with Acute Myeloid Leukemia (Bottomly et al, 2022). While studies show that wildtype PEAR1 regulates hematopoiesis, little is known on how PEAR1's mutational status may impact downstream pathway proteins' activity (Krivtsof et al, 2007).

We generated triplicate Ba/F3 cell lines using retroviral vector transduction, stably expressing empty vector, wildtype, or mutated PEAR1. Lysates were probed with 491 antibodies bound to proteins of interest in Reverse- Phase Protein Arrays (RPPAs). We evaluated the data using hierarchical and k-means clustering and used the R-package SigClus to determine differences between and within clusters. Kruskal-Wallis testing determined expression differences for proteins of interest and STRING database searches highlighted signaling pathways identified in our analyses. A Principal Component Analysis (PCA) visually represented clustering of differences between triplicates.

PCAs showed comparable rotational degrees for ≥ 2/3 replicates for each condition and explained >70% of variance in the first two dimensions, supporting data stability. While no individual proteins showed significant expression differences in wildtype vs empty vector, a significant cluster of proteins was expressed in wildtype but not in empty vector. SigClus analyses did not identify subclusters within the wildtype cluster. This suggests that not an individual protein contributes to PEAR1 function, but rather that a combination of proteins expressed in wildtype lines does. This is further supported by STRING's significant protein-protein interaction p-value. KEGG and Reactome searches of these proteins showed enrichment in the EGFR/PI3K/ERBB2 pathway, suggesting that wildtype PEAR1 expression may increase pathway activity and that PI3K signaling downstream of PEAR1 may be picked up in RPPAs. Further experiments will help validate the changes in

identified pathway proteins across lines using Western blots. Subsequent drug treatments will evaluate the impact of PEAR1's mutational status on cell survival.