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Acute Leukemias

TARGETING ONCOGENE-INDUCED SENESCENCE IN ETV6::RUNX1 PRE-LEUKEMIC CELLS

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Introduction: The t(12;21)(p13;q22) is the most common chromosomal translocation in pediatric B cell precursor acute lymphoblastic leukemia (BCP-ALL), occurring in 5% of healthy newborns. This alteration generates the ETV6::RUNX1 (E::R) fusion gene, encoding an aberrant transcription factor that is insufficient to cause leukemia directly, but establishes a clinically silent pre-leukemic progenitor not yet fully characterized. We previously showed that E::R expression in the murine pro-B BaF3 cells caused the slowdown of cell cycle progression and increased phospho-histone H2AX levels, both features of Oncogene Induced Senescence (OIS).

Methods: We explored whether E::R induces OIS in immature hematopoietic cells to uncover therapeutic targets for pre-leukemia. We used two E::R+ pre-leukemic models: an inducible BaF3 Pro-B cell system and Sca1-E::R transgenic mice, where E::R is expressed in immature Lin−Sca1+ cells.

Results: We observed that E::R caused a senescence-like phenotype in BaF3 cells, characterized by altered morphology, increased β-galactosidase activity (% SA β-Gal positive cells: ctr = 8.05 ± 10,61; E::R = 50.02 ± 9.95, p<0.0001), elevated reactive oxygen species (ROS) (fold change of CM-H2DCFDA MFI, E::R versus ctr = 1.42 ± 0.41, p<0.001), and Senescence-Associated Secretory Phenotype (SASP) factor secretion. It dysregulated genes within the p53 pathway, including senescence-related genes, causing the accumulation of p53 protein and alteration in its post-translational modifications. In E::R positive cells, while p53-mediated cell cycle arrest occurred, apoptosis was impaired, providing a survival advantage under genotoxic stress mediated by etoposide (% annexin V+ ctr versus E::R cells: 0.5 µg/ml etoposide = 70 ± 6.6 versus 51 ± 6.7, p<0.01; 0.75 µg/ml etoposide = 76 ± 4.9 versus 64 ± 9.4, p=0.014). Multiple therapeutic approaches targeting these vulnerabilities were tested. Senolytics SSK1 and piperlongumine selectively eliminated E::R+ cells by exploiting elevated β-gal activity and ROS levels, respectively. TM5441 leveraged caspase-3 inhibitor PAI-1 upregulation to induce apoptosis. Furthermore, using Sca1-E::R transgenic mice, we validated E::R-induced OIS in the pre-leukemic Lin-Sca1+ immature population by observing an increased proportion of cells in G0 phase (% G0 cells: ctr = 59.34 ± 12.74; E::R = 73.19 ± 6.841, p = 0.0263), and enhanced SA β-Gal activity compared to WT mice (SA β-Gal MFI: ctr = 1941 ± 939.8; E::R = 2764 ± 1108, p = 0.03). We also observed reduced pre-B colony formation after SSK1 treatment (Ratio E::R pre-B colonies treated versus untreated: 1.6 pM SSK1 = 0.63 ± 0.11, p<0.01; 8 pM SSK1 = 0.61 ± 0.07, p < 0.01).

Conclusions: These findings demonstrate E::R's dual role in inducing OIS and conferring apoptosis resistance, highlighting the potential of senescence-targeted therapies to prevent leukemia progression and relapse in E::R carriers.