Title: Functional Characterization of ACSL4 in ETV6::RUNX1-Positive B-Cell Acute Lymphoblastic Leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, with the *ETV6::RUNX1*-positive B-cell subtype (B-ALL) accounting for approximately 25% of the cases. Although this subtype generally has an excellent prognosis, a subset of patients responds slowly to the induction treatment, which is associated with a higher risk of relapse and poorer prognosis. To enhance patient outcomes and minimize chemotherapy-associated toxicity, it is essential to elucidate the molecular mechanisms underlying ALL pathogenesis.

A genome-wide perturbation screen (DepMap) identified *Acyl-CoA Synthetase Long-Chain Family Member 4 (ACSL4)* gene as a critical survival factor in *ETV6::RUNX1*-positive B-ALL. *ACSL4* encodes an enzyme that activates polyunsaturated fatty acids into acyl-CoAs that participate in several cellular processes, including ferroptosis and lipid metabolism. The aim was to examine the role of *ACSL4* in *ETV6::RUNX1*-positive B-ALL, as there are no previous studies regarding that.

Using the CRISPR-Cas9 genome editing tool, we generated *ACSL4* knockout *ETV6::RUNX1*-positive B-ALL cell line. Loss of *ACSL4* led to reduced colony-forming capacity but did not affect significantly to cell viability, proliferation, apoptosis or cell cycle. In RNA-seq analysis of *ETV6::RUNX1* patients, *ACSL4* expression did not differ between response groups; however, ferroptosis-regulating genes, a process in which *ACSL4* is involved, were more highly expressed in patients with a slow early response compared to those with a fast response. To validate and extend these findings, we developed an inducible *ACSL4* shRNA knockdown model, which is currently being used in multi-omics approaches, including transcriptomics and lipidomics, to define the role of *ACSL4* in leukemia.