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Poster Session

Molecular Pharmacology, Drug Resistance Poster II

Development of a Novel Polyamide-Based Agent to Inhibit EVI1 Function

Géraldine Sicot[1,*], Marion Vogel[1,*], Yi Zhang[1,*], Daniel A Harki, Ph.D.[2,*], Kimberly Lezon-Geyda, Ph.D.[3,*], Peter Dervan[2,*] and Archibald S. Perkins, MD, PhD[1]

[1] Pathology and Lab Medicine, Univ. of Rochester, Rochester, NY, USA, [2] California Institute of Technology, [3] Comprehensive Cancer Center, Yale Univ. School of Med., New Haven, CT, USA

Abstract

The EVI1 gene at chromosome 3q26 is associated with acute myeloid leukemogenesis, due to both chromosomal rearrangement and to overexpression in the absence of rearrangement. Some rearrangements such as t(3;3) and inv(3) result in overexpression of EVI1 protein, while translocation t(3;21) yields an AML1-MDS1-EVI1 (AME) fusion protein. EVI1 possesses two zinc finger domains, an N-terminal domain with fingers 1–7, which binds to GACAAGATA, and a C-terminal domain (fingers 8–10) which binds GAAGATGAG. Inhibition of EVI1 function with a small molecule compound may provide a targeted therapy for EVI1-expressing leukemias. As a first step towards inhibiting the leukemogenic function of EVI1, we performed structure-function studies on both EVI1 and AME protein to determine what domains are critical for malignant transformation activity. Assays were

1. Rat1 fibroblasts in a soft agar colony forming assay for EVI1;
2. primary bone marrow cells in a serial replating assay for AME.

Both assays revealed that mutation of arginine 205 in zinc finger 6 of EVI1, which completely abrogates sequencespecific DNA binding via the N-terminal zinc finger domain, resulted in complete loss of transforming activity; mutations in other domains, such as the C-terminal zinc finger domain, CtBP binding domain, and the domains of AML1 had less of an effect or no effect on transforming activity. In an effort to inhibit EVI1 leukemogenic function, we developed a **polyamide**, DH-IV-298, designed to block zinc fingers 1–7 binding to the GACAAGATA motif. DNAseI footprinting revealed a specific interaction between DH-IV-298 and the GACAAGATA motif; no significant interaction was observed elsewhere; a mismatch **polyamide** failed to footprint at equivalent concentrations; and DH-IV-298 failed to bind to a control DNA lacking the GACAAGATA motif. Electromobility shift assay showed that, at a 1:1 **polyamide**:DNA ratio, DH-IV-298 lowered EVI1:DNA affinity by over 98%, while mismatch was significantly less effective (74% reduction). To assess the effect of DH-IV-298 on EVI1 binding to DNA in vivo, we performed CAT reporter assays in a NIH-3T3-derived cell line with a chromosome-embedded tet-inducible EVI1-VP16 as well as a EVI1-responsive CAT reporter. Removal of tetracycline resulted in a four-fold increase in CAT activity that was completely blocked by DH-IV-298. The mismatch **polyamide** was significantly less effective than DH-IV-298. Further studies are being performed to assess the effect on endogenous gene expression, and on growth of leukemic cells that express EVI1. These studies provide evidence that a cell permeable small molecule compound may effectively block the activity of a leukemogenic transcription factor.

Footnotes

Corresponding author

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