Nucleic Acids

Major finding: I-motif DNA structures form in the nuclei of human cells in a cell-cycle and pH dependent manner.

Approach: Generation of a selective high-affinity antibody allowed the detection of i-motifs in human cells.

Impact: I-motifs may be potential therapeutic targets in cancer due to telomere and promoter regulatory functions.

I-MOTIF DNA STRUCTURES MAY REGULATE PROMOTERS AND TELOMERES

In vivo, DNA most commonly adopts the canonical B-form, a right-handed double helix secondary structure, with additional layers of DNA structure providing regulatory control. However, alternative non–B-form conformations have also been described including G-quadruplexes (G4), a regulatory structure formed within guanine (G)-rich regions. A similar structure, the intercalated motif (i-motif), has been identified within C-rich regions *in vitro*, but its existence has not been confirmed in human cells. I-motif structures form via a stack of intercalating hemiprotonated C-neutral C base pairs, which are stabilized in an acidic pH, and it is not clear if the conditions for i-motif formation occur in cells. Zeraati and colleagues generated and characterized an antibody fragment (iMab) that recognizes i-motif structures with high selectivity and affinity to allow detection of i-motifs in human cells. iMab bound i-motifs with diverse sequences, indicating that it is a structure-specific antibody, and it was able to differentiate i-motifs from G4s. Using iMab, i-motifs were detected in punctate foci in the nuclei of three human cancer cell lines, MCF7, U2OS, and HeLa, indicating that i-motifs can form under cellular conditions. The formation of i-motifs was cell-cycle dependent with increased formation at the G1/S boundary. Further, as had been demonstrated *in vitro*, the formation of i-motifs was pH-dependent with increased i-motif formation at lower intracellular pH levels. I-motifs were enriched at regulatory regions, colocalizing with TRF2 at telomeres and with E-box transcription factors at gene promoters, suggesting that i-motif structures may have regulatory functions at telomeres and in gene expression. The detection of i-motifs at regulatory regions in the nuclei of human cells suggests a role for i-motifs in genomic regulation and the potential for therapeutic targeting in cancer, though further experiments are needed to validate and characterize the proposed regulatory functions of i-motifs. ■

Zeraati M, Langley DB, Schofield P, Moye AL, Rouet R, Hughes WE, et al. I-motif DNA structures are formed in the nuclei of human cells. Nat Chem 2018 Apr 23 [Epub ahead of print].

Leukemia

Major finding: A p53-stapled peptide, ALRN-6924, blocks binding to MDMX and MDM2 to activate p53.

Concept: ALRN-6924 treatment suppressed the initiation and progression of leukemia *in vivo* and extended survival.

Impact: Dual MDMX/MDM2 inhibition with ALRN-6924 warrants further investigation for the treatment of AML.

DUAL TARGETING OF MDMX AND MDM2 HAS ANTILEUKEMIC ACTIVITY

The tumor suppressor p53 is frequently inactivated in patients with acute myeloid leukemia (AML) by overexpression of the endogenous inhibitors MDMX or MDM2. However, pharmacologic targeting of these proteins has proven challenging. Small-molecule MDM2 inhibitors are in clinical development, but these compounds lack affinity for MDMX. An α-helical p53-stapled peptide, ALRN-6924, has been developed that inhibits binding of both MDMX and MDM2 to p53. ALRN-6924 is being investigated in a phase I trial, but its mechanisms of action and activity in AML have not been determined, prompting Carvajal and colleagues to assess the activity of ALRN-6924 in AML. Analysis of data from The Cancer Genome Atlas and stem/progenitors from patients revealed that MDMX is highly expressed in AML. ALRN-6924 bound with high affinity to both MDMX and MDM2, blocking their interactions with p53, resulting in p53 pathway activation and potent cytotoxic activity in leukemia cells with wild-type p53. No functional effects were observed in p53-mutant cells. ALRN-6924 treatment rapidly increased p53-dependent transcription, upregulating target genes including *CDKN1A* (p21). Further, RNA sequencing found that ALRN-6924 enriched for p53-regulated gene expression signatures. ALRN-6924 suppressed proliferative, clonogenic, and serial replating capacity of AML cells and induced cell-cycle arrest and apoptosis. ALRN-6924 also suppressed the clonogenic capacity of primary human AML cells. *In vivo* in AML xenotransplantation models, ALRN-6924 reduced leukemia initiation and progression and extended survival. Based on these findings, a patient who had developed high-risk myelodysplastic syndrome with excess leukemic blasts was treated with ALRN-6924. It resulted in selective p53 activation in AML cells and a reduction of AML blasts, demonstrating on-target pharmacodynamic effects. Altogether, these findings indicate that dual inhibition of MDMX and MDM2 with ALRN-6924 is feasible and has antileukemic activity, supporting its further clinical investigation in patients with AML. ■

Carvajal LA, Neriah DB, Senecal A, Benard L, Thiruthuvanathan V, Yatsenko T, et al. Dual inhibition of MDMX and MDM2 as a therapeutic strategy in leukemia. Sci Transl Med 2018;10:eaao3003.