**CRISPR/CAS9 GENOME EDITING OF THE HUMAN TOPOISOMERASE IIα INTRON 19 5' SPLICE SITE CIRCUMVENTS ETOPOSIDE RESISTANCE IN HUMAN LEUKEMIA K562 CELLS**

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An essential function of DNA topoisomerase II*α* (TOP2*α*; 170 kDa, TOP2*α*/170) is to resolve DNA topologic entanglements during chromosome disjunction by introducing transient DNA double-stranded breaks. TOP2*α*/170 is an important target for DNA damage-stabilizing anticancer drugs, whose clinical efficacy is compromised by drug resistance often associated with decreased TOP2*α*/170 expression. We recently demonstrated that an etoposide-resistant K562 clonal subline, K/VP.5, with reduced levels of TOP2*α*/170, expresses high levels of a novel C-terminal truncated TOP2*α* isoform (90 kDa, TOP2*α*/90). TOP2*α*/90, the translation product of a TOP2*α* mRNA that retains a processed intron 19 (I19), heterodimerizes with TOP2*α*/170 and is a resistance determinant through a dominant-negative effect on drug activity. We hypothesized that genome editing to enhance I19 removal would provide a tractable strategy to circumvent acquired TOP2*α*-mediated drug resistance. To enhance I19 removal in K/VP.5 cells, CRISPR/Cas9 was used to make changes (GAG//GTAA**AC**→GAG//GTAA**GT**) in the TOP2*α* gene’s suboptimal exon 19/intron 19 5′ splice site (E19/I19 5′ SS). Gene-edited clones were identified by quantitative polymerase chain reaction and verified by sequencing. Characterization of a clone with all TOP2*α* alleles edited revealed improved I19 removal, decreased TOP2*α*/90 mRNA/protein, and increased TOP2*α*/170 mRNA/protein. Sensitivity to etoposide-induced DNA damage (*γ*H2AX, Comet assays) and growth inhibition was restored to levels comparable to those in parental K562 cells. Together, the results indicate that our gene-editing strategy for optimizing the TOP2*α* E19/I19 5′ SS in K/VP.5 cells circumvents resistance to etoposide and other TOP2*α*-targeted drugs.