Development and Characterization of a Novel Zinc-Finger Construct for Targeted Epigenetic Modification

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Altered epigenetic landscapes play an integral role in the deregulation of genes. In diffuse large B-cell lymphoma (DLBCL), tumor cells overexpress BCL6, a transcription factor that normally regulates B-cell proliferation and activation. Current DLBCL treatment produces adverse side effects and is unable to permanently prevent recurrence. ZF-KRAB, a construct that fuses a zinc-finger (ZF) targeted towards the first noncoding exon of BCL6 with a Krüppel associated box (KRAB) that represses transcription, was developed to address this issue. Previous research showed that when endogenously produced by lymphoma cells, ZF-KRAB successfully represses BCL6 expression. However, since this approach cannot be used as a therapeutic, the ZF-KRAB must be made exogenously. Using E. coli to produce protein, ZF-KRAB was used to treat lymphoma cells but was unable to knock down BCL6, a problem with three possible causes: failed nuclear localization, low dosage, and non-specific binding. Fluorescence microscopy and a cellular fractionation assay revealed that the ZF-KRAB was predominantly located in the nucleus after direct delivery. The exogenous protein dosage was compared to the minimum viable concentration of endogenous ZF-KRAB for treatment via western blot and confirmed as sufficient. Ultimately, it was deemed that ZF-KRAB was unable to specifically bind to the first exon of BCL6 through a binding assay. To explore the generalized applicability of ZF-KRAB as a therapeutic, exogenous protein treatments were performed on breast cancer cells and found to reduce BCL6 expression. ZF-KRAB therefore represents a novel epigenetic approach to therapy that responds to the pressing need for improved cancer treatment.