Controlling Noxa Protein Expression by a Novel Approach to Study Its Function in Leukemic Cells, Phase II

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Cancer develops when cells cannot activate apoptosis (“programmed cell death”) in response to damaging perturbations. Noxa, the focus of this project, plays a vital role in promoting apoptosis. This protein is not expressed in healthy cells, but is expressed constitutively in leukemic cells and has a pro-survival function due to phosphorylation of a serine residue. This study attempts to fill a critical gap in knowledge regarding regulatory functions of Noxa by controlling its expression in leukemic cells using a novel approach. In Phase I, each of three Noxa mutants created (SA, SE, and DA) revealed unique information about the functions of Noxa. Additionally, CRISPR/Cas9 technology was used to create a leukemic cell line with both alleles of the Noxa gene removed from its genome, so mutants could be expressed without interference. In Phase II, it was discovered that the cells did not survive double allele knock out of Noxa. This fascinating finding suggests that Noxa is vital to leukemic cell survival, which seems paradoxical. Given this result, shRNA was utilized to silence Noxa instead of completely eliminating it. Finally, an apoptosis assay was conducted to determine the mutants’ response to stress induced by glucose deprivation. Results demonstrate that the mutants are working as predicted, with SA accelerating apoptosis and SE and DA inhibiting it. This novel approach to controlling Noxa expression could lead to better insights into leukemia, its initiation, and progression. The knowledge gained can help to alter the proliferation and pathogenesis of cells to potentially find a cure.