Novel Method of Identifying and Confirming Candidate Genes Causing Resistance to Acute Myeloid Leukemia using CRISPR Cas9

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This year, an estimated 19,950 people in the United States will be diagnosed with AML, the second most common leukemia. A protein called BCL2 inhibits apoptosis (programmed cell death) among cells and is often upregulated in many types of leukemia. This results in cancer cells bypassing apoptosis and multiplying rapidly. To counteract BCL2 activity, an oral therapeutic drug called Venetoclax (ABT199) was created. It inhibits BCL2 in cancer cells and is extremely effective in Chronic Lymphocytic Leukemia with a response rate of 88%. It is currently showing promise in treatment of AML as well. However, as with any drug, patients develop insensitivity (also called resistance) to these drugs. To test the genes for their relevance to ABT199 resistance, I hypothesized that they should be expressed in leukemia patients cells and should confer resistance when inactivated individually. I began with an analysis of over a thousand gene candidates, choosing the top 26 genes expressed in leukemia patients. I then cloned these sgRNA's (single guide RNA) into lentiviral vectors carrying Cas9 proteins to make lentiviruses. These viruses were used to infect Molm13 cells by means of spinoculation. Through this process, targeted genes were knocked out individually with use of CRISPR-Cas9. After 10 days, the ABT199 drug was printed on the cells at different concentrations and compared against a control cell line for survival, after 4 and 6 days. Successful clones showed more living cells at higher drug concentrations than the control. I concluded that 11 of the candidate genes have a potential to be relevant in ABT199 resistance. These genes can now be further tested for expression in patients who show resistance to ABT199.

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