CRISPR/Cas9 Knockout Confirmation and Characterization of a Novel Tumor Suppressor, OSTM1, in a B-cell Lymphoma Cell Line

Jack Centrelli, Muhammed Usama Tariq, and Wei-Xing Zong
Rutgers, The State University of New Jersey

B-cell malignancies are cancers that occur in an antibody-producing immune cell called B lymphocytes. A whole genome targeting CRISPR/Cas9 screen was performed in an interleukin 3 (IL-3) dependent murine pro-B cell line Ba/F3 to identify novel tumor suppressors. OSTM1, an E3 ubiquitin ligase frequently deleted in human B-cell lymphoma patients, was selected as a candidate gene for further analysis. Preliminary data show that OSTM1 targets phosphodiesterase 3B (PDE3B) for ubiquitination and subsequent proteasomal degradation, which leads to the inhibition of the tumor-suppressive cAMP/PKA pathway. To confirm this mechanism in human cancer cell lines, we attempted to silence OSTM1 by single-guide RNA (sgRNA) in ARH77, a multiple myeloma line with a high level of OSTM1. Single-cell clones were chosen to verify OSTM1 silencing and the corresponding expression level of PDE3B. Due to the lack of reliable antibodies, the single-cell clones were examined by PCR amplification of the CRISPR-targeting region followed by Sanger sequencing. OSTM1 gene editing was detected in two clones, which correlated with increased PDE3B expression and proliferation. Therefore, this preliminary study indicates that the OSTM1-PDE3B signaling axis is conserved between mice and humans. The OSTM1-silenced ARH77 clones can be used to further examine the molecular basis and biological relevance of this newly identified tumor-suppressive mechanism.