Abstracts of papers presented
at the 2021 *virtual meeting* on

GENOME ENGINEERING: CRISPR FRONTIERS

August 18–August 20, 2021

Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

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Arranged by

Jennifer Doudna, *University of California, Berkeley / HHMI*
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MILD PHENOTYPE OF KNOCKOUTS OF THE MAJOR APURINIC/APYRIMIDINIC ENDONUCLEASE APEX1 IN A NON-CANCER HUMAN CELL LINE

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A well-known approach to investigate protein functions is to generate knockout animals or cell lines. In the case of many base excision repair (BER) proteins (APEX1, POLB, LIG3, XRCC1) these knockout mice die at the early stages of embryonic development. The CRISPR/Cas9 genome editing technology offers a new way to generate knockouts without causing profound gene disruption. Using this technology, we have established human cell lines deficient in the major apurinic/apyrimidinic (AP) endonuclease, APEX1.

Three different single guide RNAs targeting the first and the second coding exons of the *APEX1* gene were designed. The most efficient sgRNA, as assessed by TIDE, was selected to obtain the knockout cell line. 293FT cell line was transfected with this sgRNA, sorted and diluted to single cells. Nineteen random clones were analyzed by restriction with DraIII endonuclease that has the recognition site within the protospacer. Six clones lost all copies of DraIII sites; for two of them (1C4, 2A9) we characterized the exact mutations introduced by genome editing into the hypotriploid 293FT genome. The 1C4 clone had two alleles with one-nucleotide deletion and one allele with a single-nucleotide insertion, while 2A9 carried three alleles with a single-nucleotide insertion. We proved the absence of APEX1 protein in whole cell extracts by Western blotting. Both clones showed no AP endonuclease activity on oligonucleotide substrates and were unable to support the repair of AP sites and uracil in DNA. After confirming the knockout state of the *APEX1* gene in the generated cell lines we had characterized them phenotypically. Both wild-type and knockout cells had approximately the same doubling time as well as the distribution of cells in different cell cycle stages. The APEX1 has a crucial role in BER, so the cells with APEX1 deficiency were expected to be more sensitive to genotoxic compounds. Both 1C4 and 2A9 cells demonstrated moderately enhanced sensitivity to methyl methanesulfonate compared to wild-type cells. However, no significant difference was found with respect to the oxidizing agents H_2O_2 and $KBrO_3$.

With the advent of CRISPR/Cas9 technology, it becomes possible to generate isogenic knockout cell lines to investigate complex systems such as DNA repair pathways. We believe that the *APEX1* knockout cell line will help to uncover the different ways to repair base lesions.

This research was supported by RFBR (grant 20-34-90092).