Transfection of MCF-7 Cells for Targeted Knockout of PFK-2 Using CRISPR/Cas9 System

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CRISPR/Cas9 system which enables site-directed genome modifications at the basal level of gene expression is a popular genome engineering technology due to its simple design and easy operation. This research focuses on modifying a vital gene, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*pfkfb3*), which codes for the phosphofructokinase 2 (PFK-2) enzyme involved in the regulation of glycolysis, cell cycle, and apoptosis, through CRISPR/Cas9 system. The intent is to control the growth and survival of cancer cells, by decreasing their oxygen consumption rate. To obtain a successful knockout of PFK-2, 20 nucleotide-long crRNA sequence, and donor template with homology arms integrable into the target locus upon double-strand break following homology-directed repair pathway, were designed after determining a suitable knockout target site through bioinformatic analyses. The chemically synthesized crRNA sequence was cloned into pSpCas9(BB)-2A-Puro plasmid, and the accuracy of crRNA cloning was verified by a colony polymerase chain reaction performed on transformed *Escherichia coli* followed by plasmid sequencing. A high yield of extracted recombinant plasmids was used to transfect MCF-7 cells using polyethylenimine (PEI) as the transfecting agent. Transfected cells were lysed using NP-40 buffer, and the cell lysate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blotting to detect the 6×His tag of truncated PFK-2. Several protein bands around 37 kDa (size of truncated PFK-2) were observed on gel after staining, as expected. The successfulness of the knockout should be further analyzed statistically after performing detection steps for the 6×His tag of the truncated PFK-2 on the blot. This study indicates that the transfection of MCF-7 cells with the afore-designed construct has the potential for targeted knockout of PFK-2 using CRISPR/Cas9 system.

Keywords: *Cancer, CRISPR/Cas9, Genome Editing, Knockout, PFK-2*