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CRISPR/Cas9-mediated K/O of the allosteric activator binding domain of PFK-1 as a novel therapeutic approach for cancer

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Cancer is one of the most deleterious diseases and is the second leading cause of death around the world. Current treatment methods available for cancer affect both cancerous and surrounding non-cancerous cells, leading to several adverse side effects. Therefore, there is an urgent need to develop effective new treatments that selectively target tumor cells to reduce the risk of side effects. The metabolic profile of cancer cells typically includes increased consumption of glucose and preferential production of lactate, even in the presence of oxygen (Warburg effect). Therefore, therapeutics that can decrease the rate of glycolysis would be particularly effective against cancer cells and may have minimal adverse effects on the noncancerous cells. The main goal of this research project is to downregulate the activity of PFK-1 in human MCF-7 cancer cells by knocking out the allosteric activator binding domain (C-terminal domain) of the enzyme using the CRISPR/Cas9 genome-editing tool. A specific target site that is needed for the K/O of the regulatory domain was elucidated via bioinformatic tools, followed by the design of target-specific crRNA sequence via the CRISPR design tool by 'Horizon discovery group company' and the repair donor template via the ApE tool. Then the crRNA, which is specific for the PFK-1 was cloned into pSpcas9 (BB)-2A-Puro plasmid and the recombinant plasmid was verified using colony PCR and sequencing. Next, the recombinant plasmids were transformed Into the *Escherichia coli* Top 10 cells for the amplification and upon extraction, those recombinant plasmids were transfected into MCF-7 cells. In there, an optimization was also performed to find the most suitable ratio of PEI:DNA for the transfection and then the transfected cells were identified using the puromycin selection. From the results obtained so far, it was found out that the effectively designed recombinant plasmid with the CRISPR construct is successfully transfected into the MCF-7 breast cancer cells and the most effective PEI:DNA ratio for the transfection is 6:1. Future work includes clonal expansion, SDS- PAGE, and a western blot to verify the truncated protein. Finally, the effect of the gene modification is expected to be assessed in the context of tumor proliferation, growth, and survival.

Keywords: Cancer, CRISPR/Cas9, PFK-1, regulatory domain, crRNA

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