Establishment and characterization of gene knockout tumor cell lines by CRISPR/Cas9

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BACKGROUND: Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated nuclease 9(CRISPR/Cas9) gene editing system is a new type of gene editing technology developed based on the immune mechanism of archaea resisting the invasion of exogenous nucleic acid. Now, this gene engineering technologies is enabling a broad range of applications from basic biology to biotechnology and medicine. Exciting is that this technology in cancer research and treatment also showed great potential.

HYPOTHESIS: In this study, we tried to demonstrate that CRISPR/Cas9 system can efficiently deletion genes in tumor cell lines and used for the studying of cancer-associated genes.

METHODS: The plasmid of pMJ920 and sgRNA were transfected into different tumor cell lines, used to knockout VDR and FOXM1. Screening of the target protein deficient mutant cell lines from the sorting out of the monoclonal system by Western Blotting. Wounding scratch assay and transwell assay were performed to determine the the healing ability and migration and invasie capacity of gene knockout cells.

RESULTS: Generation of VDR and Foxm1 knockout tumor cell lines using the CRISPR/Cas9 system. Screening mutant cell lines from these monoclonal cell lines and verifying by sequencing. Knockout of VDR promotes colon cancer cells migration and migration and invasie capacity in vitro. Foxm1 knockout alters wound healing, migration and invasion abilities of colon cancer cells.