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CRISPR/Cas9 Technique for Identification of Genes Regulating Oxaliplatin Resistance of Pancreatic Cancer Cell Line

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Abstract

© 2016, Springer Science+Business Media New York.Genome editing approach based on prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats) system is a simple and useful way to investigate gene functions on a genome-wide scale. It is especially important for cancer research because of genetic contribution to tumor development. We applied this technique in a high-throughput screening format to find genes that could be involved in chemotherapy resistance of pancreatic cancer. We used AsPC1 cell line expressing doxycycline-inducible Cas9 to screen two sgRNA lentiviral libraries: (1) cell cycle genes (CC, 983 genes, ~12,000 sgRNA) and (2) genome-wide (GW, ~90,000 sgRNA). These sets of cells with different gene knockouts were treated with oxaliplatin to identify knockouts which increase sensitivity to the drug. We have performed screening both in vitro and in vivo settings. For the in vivo arm of our experiments, peritoneal carcinomatosis model in severe combined immunodeficiency (SCID) mice was created by intraperitoneal injection of AsPC1/Cas9 cells infected with sgRNA library. Genomic DNA from cells and animal tumor material was analyzed using next generation sequencing (NGS) to obtain data about representation of sgRNA. Preliminary data allowed us to identify genes potentially modulating oxaliplatin sensitivity.

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Keywords

CRISPR/Cas9, Drug resistance, Genome editing, Oxaliplatin, Pancreatic cancer

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