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Therapeutic Effects of Knocking out Validated Oncogene Targets kRAS and MYC in Ovarian Cancer Cell Lines

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THERAPEUTIC EFFECTS OF KNOCKING OUT VALIDATED ONCOGENE TARGETS KRAS AND MYC IN OVARIAN CANCER CELL LINES

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Abstract

Ovarian cancer ranks fifth in cancer deaths among women and accounts for the most deaths of any other cancer involving the female reproductive system. In 2019, there were an estimated 22,000 new cases of ovarian cancer, with an estimated death toll of 14,000. Two oncogenes, MYC and kRAS, which when amplified are associated with more aggressive disease, have been validated as potential targets in ovarian cancer cell lines to possibly mediate cell death or enhance chemotherapeutic efficacy. The CRISPR CAS9 system allows for gene editing and thus the knocking out of specifically selected genes. Here, we investigate the effects of knocking out oncogenes, MYC, kRAS, and both in a double knock, in ovarian cell lines with amplified kRAS (OV56, SKOV-3) in terms of their morphology and chemotherapeutic efficacy. Successful transfection of OV56 and SKOV-3 cells with MYC and kRAS combination knockouts will allow for several further investigations on the effect on growth of the cancer cells, ability to form spheroids, and susceptibility to standards of care.

Methods

Cell Culture: OV56 and SKOV3 ovarian cancer cells were cultured and grown to confluence in 5% CO_2 at 37°C

Transfection: 150,000 cells/well were plated in a 6 well plate and transfection of CRISPR-CAS9 gene-editing system was performed the next day with jetPRIME (Polyplus transfection) using 1µg of DNA. Cells were transfected with different CRISPR-CAS9 plasmids targeting specific oncogenes to provide 6 conditions per cell line: CAS9 (control), MYC KO, kRAS near KO, kRAS mid KO, MYC + kRAS near KO, MYC + kRAS mid KO.

Colony Selection: Transfected cells were grown to confluency over 2 days, before being harvested and serially diluted in 96 well plates from 6,000 cells/well down in 1:3 dilutions. Wells were monitored for single cell originated islands, which were then subsequently expanded to 6 well plates and then t25 flasks when confluent.

DNA Extraction/PCR: DNA extraction of selected cells were carried out using Wizard Genomic DNA Purification. Amplification of isolated DNA was achieved using polymerase chain reaction utilizing kRAS and MYC primers. For samples with kRAS primers, initial denaturation occurred at 95°C for 30s, followed by 30 cycles with the following conditions: 95°C for 15s, 56°C for 30s, and 72°C for 30s. For samples with MYC primers, initial denaturation occurred at 95°C for 30s, followed by 30 cycles with the following conditions: 95°C for 15s, 52°C for 30s, and 72°C for 30s.

Gel electrophoresis: PCR products were separated by size by agarose gel electrophoresis with 1x Gelgreen dye to begin the verification of target knockouts.

Transfection of OV56 and SKOV3 cell lines

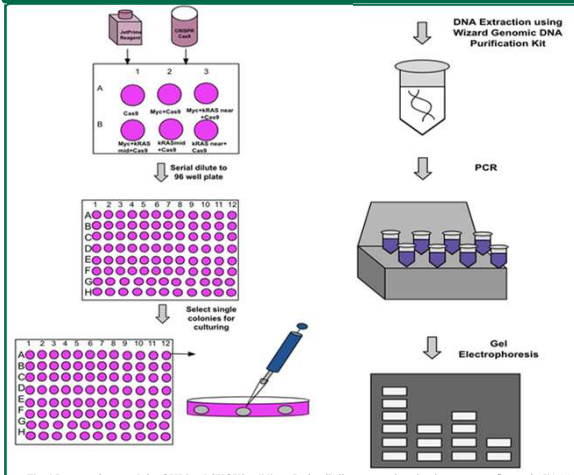

Fig. 1 Process of research for OV56 and SKOV3 cell lines. Both cells lines were cultured and grown to confluence in 5% CO_2 at 37°C. In a 6 well plate, transfection of 6 different conditions (CAS9 (control), MYC KO, kRAS near KO, kRAS mid KO, MYC + kRAS near KO, MYC + kRAS mid KO) was performed using the CRISPR/CAS9 gene editing system and 2 µl of jetPRIME reagent + 1 µg of plasmid DNA per well. After transfection, the cells were serially diluted to a 96 well plate where single cell colonies were selected to be grown in a t25 flask. DNA was extracted from these cells using the Wizard Genomic DNA Purification Kit. Following extraction, transfection was verified by amplifying samples using polymerase chain reaction utilizing kRAS and MYC primers and running a gel electrophoresis.

Beginning of Transfection Verification via Gel Electrophoresis

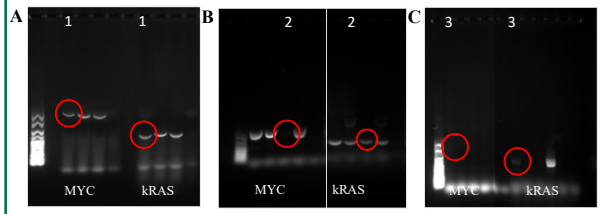

Fig. 2 Gel Electrophoresis of CRISPR Samples. (A) kRASnear KO sample (1). MYC primer band present, with kRAS primer band lower relative to other samples. (B) MYC +kRAS near sample (2) with MYC band not appearing. (C) MYC +kRAS near sample (3) with MYC band not appearing. kRAS band knocked down.

Morphology and Cytotoxicity of Parental Cells

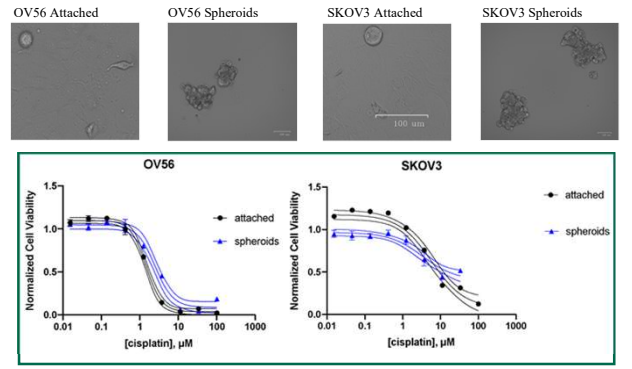

Fig. 3 Morphology and Cytotoxicity curves of OV56 and SKOV3 Parent cell lines. Parental (Non-transfected) OV56 and SKOV3 cells. (A) Morphologies of parental OV56 and SKOV3 cells both attached and as spheroids. (B) Cytotoxicity 72 hours post cisplatin treatment, the standard of care for ovarian cancer. Calculated IC50 values [µM]: OV56 att 1.4; Sph-att 2.5; Sph 2.4; SKOV3 (estimated at) att 5.8; Sph-att 4.5; Sph 3.4.

Future Work with Transfected Cell Lines

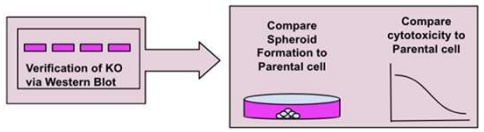

Fig. 4 Verification of Transfection via Western Blot. There must be further verification that the oncogenes were successfully abrogated in the selected cell colonies. This will be done using a Western Blot analysis. Further experimentation will determine whether or not the knockout cells will form spheroids, how their morphology is affected and any changes in sensitivity to chemotherapy (e.g. ΔIC_{50} values compared to the parental cell line.)

Conclusion

Various presumably transfected OV56 cell lines were seen to have a morphology change. Possible morphology changes while grown into spheroids, a 3D model which may more closely represent *in vivo* ovarian tumors and stem cells, will continue to be monitored after transfection confirmation. Changes in cell cytotoxicity of pre and post knockouts of both cells line when treated with chemotherapeutics will be compared. Successful transfection of OV56 and SKOV-3 cells with MYC and kRAS combination knockouts will allow for several further investigations to proceed.