Drug Research in Inhibiting BCL6 in Ovarian Cancer

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Introduction

Ovarian cancer has a survival rate of 50 percent just five years after being diagnosed. Finding new ways to treat ovarian cancer is important to increase the survival of patients who have been diagnosed. BCL6 is a transcription factor that is involved with metastasis and promoting chemotherapy resistant cancer cells. Finding a way to inhibit BCL6 could help prevent these aspects of cancer and lead to a new treatment for patients with ovarian cancer. Looking into the gene that BCL6 modulates may offer new insights to treatments that can be researched to see if they are effective in inhibiting BCL6, which was the focus of my research. Using bioinformatics and cell culturing I researched drugs that had the possibility of inhibiting BCL6 and tested their effectiveness against ovarian cancer cell lines.

Background

- BCL6 has been found to be a pro-oncogene and is a negative prognosis factor of ovarian cancer.
- BCL6 is present in ovarian spheroids. Ovarian spheroids are also thought to hold a reserve of cancer cells that are not responsive to chemotherapy.
- BCL6 is shown to aid in the transformation of malignant cancer cells to metastatic cells, although how exactly is not clear yet.

Hypothesis

Using BCL6 target genes, we can identify inhibitors for ovarian cancer which can provide new treatments for inhibiting metastasis and the growth of ovarian cancer cells.

Methodology

BioBox
Use RNAseq data to identify genes associated with BCL6 to target

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CLUE
Use genes identified from RNAseq to find potential drug treatments

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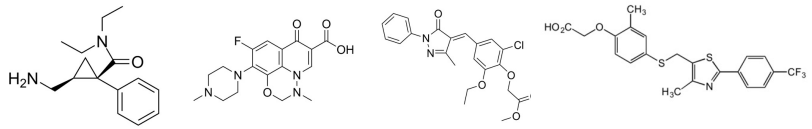
Cell Titer Glo Viability
Measure amount of ATP being produced by the cells.

Nucblue/PI
Staining for alive and dead cells.

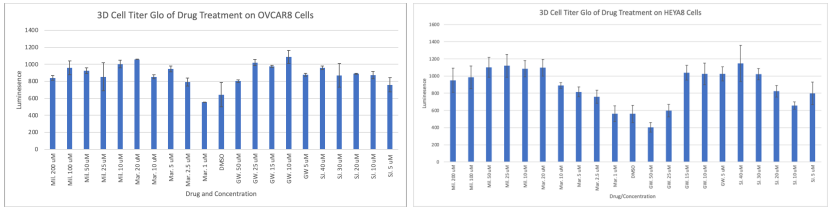
Clearance Assay
Measuring amount of mesothelial clearance by cancer cells.

Using CLUE to Identify BCL6 Inhibitors



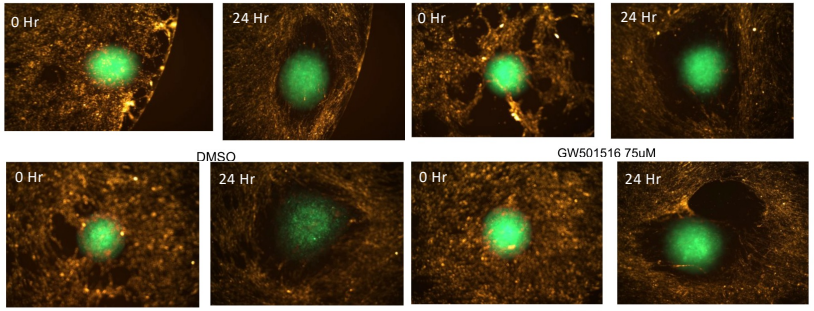
Drugs identified by CLUE as potential BCL6 inhibitors from left to right, Milnacipran (used to treat fibromyalgia in adults), Marbofloxacin (an antimicrobial), SJ-172550 (MDMX inhibitor), GW501516 (known to inhibit BCL6).

Cell Viability



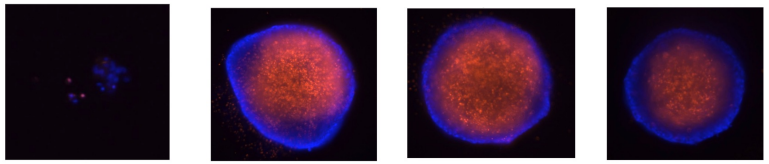
The drugs used were not effective at inhibiting cell growth compared to the control of DMSO. Ovarian spheroids were grown on an adherent 96 well plate for one day. The drugs above were added at their respective concentrations, these drugs were allowed to sit for six days and then 3D Cell Titer Glo was performed to see how effective the drugs were at inhibiting growth. DMSO was used as a negative control. The drugs expanded are Milnacipran, Marbofloxacin, GW501516, and SJ-172550.

Clearance Assay



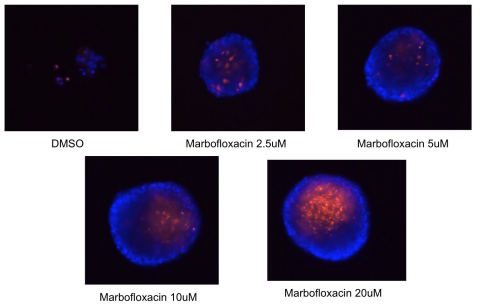
These images show the results of a clearance assay with the control of DMSO on OVCAR8 cells. The potential BCL6 inhibitors did not reduce mesothelial clearance. The 96 well plate with LP9 cells was incubated for three days before adding the drugged spheroids. The OVCAR8 cells had drug added to them one day before they were placed on the LP9 cells. The LP9 cells are stained orange and OVCAR8 cells were stained green. The images on the left was taken one hour after the spheroid had been added to the 96 well plate with LP9 cells and the next images was taken 24 hours later.

Nucblue/PI



There was some cell death in the middle of the spheroids, but this can not solely be accounted to the drugs as things like hypoxia can also cause this. OVCAR8 cells were grown as spheroids. Cells were treated for five days with the indicated drugs. Nucblue and PI were added to the spheres and imaged with the EVOS imager. PI stains dead cells red and the Nucblue stains alive cells blue.

Nucblue/PI



An increase in concentration of drug lead to a growth in ovarian cancer spheroid size. OVCAR8 cells were grown as spheroids. They were then treated for five days with the indicated drugs. Nucblue and PI were added to the spheres and imaged with the EVOS imager. PI stains dead cells red and the Nucblue stains alive cells blue.

Conclusions

- The results from the Cell Titer Glo viability assays suggest that that the drugs have little effect on the cells.
- The results of the Nucblue/PI images show that the drugs did not have any major effect on cell viability as well. In the case of Marbofloxacin the increasing concentration of drug appeared to make the spheroid larger which is the opposite of what would happen if the drug successfully inhibited the growth of the cancer cells.
- The clearance assay confirmed our previous results that the drugs are not effective at inhibiting the growth of the cells, and the drugs were not successful at preventing clearance.

Future Directions

- Analysis of a larger dose range of GW501516 on ovarian cancer cells since it is a known BCL6 inhibitor.
- Using BCL6 target genes we can identify and test other inhibitors for ovarian cancer.

Acknowledgements

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