LRRK King for a Cure
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Background

- Leucine-rich repeat kinase 2 (LRRK2) is large (280 kD) protein involved in vesicle trafficking.
- LRRK2 is overexpressed in kidney cancer cells and is required for their growth in cell culture.
- Previous experiments in our lab have shown that when LRRK2 is knocked down, the golgi apparatus is fragmented, which arrests cell growth and causes cell death.

Goals

- We hypothesized that a therapy could be designed that combines a LRRK2 inhibitor drug with a chemotherapy drug. The combination drug would be just as effective but less toxic.
- The LRRK2 inhibitor drugs we used were GNE, L2N1, PFE and the chemotherapy drug Vincristine. In order to test this hypothesis we used a cell viability assay.
- We also hypothesized that LRRK2 inhibitor drugs would affect cell growth and cell migration. We tested this hypothesis by using a soft agar assay and a scratch assay, respectively.

Experiments

Soft Agar Assay

- Purpose: Test if LRRK2 inhibitors affect 3D cell growth/anchorage independence.
- Method: Grow renal cancer lines in soft agar gels and treat with LRRK2 inhibitor drugs.
- Data: After three weeks, colonies are fixed and stained with crystal violet, then imaged using a simple scanner.

Cell Viability Assay (Cell Titer-glo)

- Purpose: Determine the cell viability after treatment with Vincristine. The Assay measures ATP levels which can be used to determine number of live cells present. We compared the effects of varying concentrations of Vincristine on the cancer cell lines.
- Method: Plated cancer cell lines on 96 well plates and treated with varying concentrations of vincristine. After 48 hours of drug treatments wells were stained and preserved with crystal violet.
- Data: Data was collected by a plate reader and quantified.

Scratch/Wound Healing Assay

- Purpose: Test if LRRK2 inhibitors slow cell migration.
- Method: Cancer cell lines were plated on 24 well plates and grown until 100% confluent. A scratch was made on the bottom of the plate and LRRK2 inhibitor drugs were added to the media. After a treatment period of 12 hours, wells were stained and preserved with crystal violet.
- Data: Images were taken of the stained plates stained with crystal violet, then imaged using a simple scanner.

Results

Soft Agar

A workflow for analysis of soft agar growth was established using ImageJ software. This allowed us to count the number of colonies and determine their average size.

Cell Viability

Results from a 3-fold serial dilution treatment of Vincristine on SKRC39 renal cells in a 48 hour period.

Scratch Assay

LRRK2 inhibitors GNE-7915, LRRK2-IN-1, and PFE-475 were tested for their effect on cell migration in a scratch assay.

Conclusions

Soft Agar:

- We were able to determine the appropriate plating density for three cancer cell lines in soft agar, and are using ImageJ software to quantify the number/size of colonies per well.

Cell Viability:

- LD50 and concentration was determined for SKRC39 cell line; we are still testing to determine whether addition of LRRK2 inhibitors will shift the LD50 value.

Scratch Assay:

- Different growth factors increase wound healing rate, though the LRRK2 inhibitors did not have a significant effect in any of the conditions we tested.

Future Direction

- Add LRRK2 inhibitors with Vincristine to cancer cell lines to determine if they increase the potency of Vincristine.
- Include LRRK2 inhibitors in soft agar assays to assess effect of blocking LRRK2 kinase function on tumorigenecity of renal cancer cells.
- Repeat scratch assay with LRRK2 inhibitors to validate our preliminary findings.
- Perform transwell migration assays to confirm the lack of LRRK2 inhibitor effects on cell migration.

All three experiments were designed to test the effects of LRRK2 inhibitors on the following aspects of cancer cells: viability, tumorigenicity, and migration. Further experiments will be used to confirm our data from this summer and provide new direction for this project.

References