Lentiviral shRNA screen to test the validity of a gene signature of breast cancer stem cells using high throughput mammosphere assays

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Abstract
One in 8 women in the U.S. suffer from breast cancer and although current treatments have been successful in reducing the rate of cancer-related death over the past few years, one major problem that looms this field is the high likelihood of relapse within 5-15 years of initial therapy. One possible reason for this relapse might be the presence of cancer stem cells in the tumor which are not inhibited by traditional therapies and thus may require therapies that target the subpopulation of cells. The cancer stem cells hypothesis postulates that the cancer function in a hierarchical manner, similar to the breast tissue and contains a population of tumor initiating cells/cancer stem cells. We have identified a gene signature which is derived from an overlap between CD44+/CD24- cells and MS, a highly significant overlap (p<1.0E-9, one-sided Fisher’s exact). The test of the 282 genes demonstrated a sensitivity of non-small-cell lung cancer (NSCLC) cells. Using SUM159 cells that were transduced with individual lentiviruses shRNAs conferring sensitivity of non-small-cell lung cancer (NSCLC) cells. We found that 477 genes were significantly altered in MS, along with a positive control lentivirus targeting Bmi1, a polycomb group gene known to be required for self-renewal of many stem/progenitor cell types, a non-specific negative control lentivirus targeting breast cancer stem cells. This screen will be repeated in another cell line and a common set of therapies which target the cancer stem cells which would be used in conjunction with chemotherapy to target both the bulk of the tumor and the cancer stem cell population in order to reduce the possibility of relapse in breast cancer patients.

Background
A Model For “Cancer Stem Cells” In Treatment Resistance and Disease Recurrence

Method

1. A lentiviral shRNA screen to test the validity of a gene signature of breast cancer stem cells
2. Open Biosystems Lentiviral shRNA Library Screen to confirm the genes that regulate the cancer stem cell pathway

Results

1. Gene Expression Profiling of Chemoresistant, MS initiating population of cells by Affymetrix Gene Array
2. Expression of Control GFP shRNA genes in SUM 159 Cell Line and Dose Dependent effect of GSI Inhibitor on MS formation

Conclusions

- Genes identified in the gene signature were confirmed using the shRNA mediated Lentiviral Screen
- Growth Factor Signaling, Notch, Hedgehog and β-Catenin play an important role in Chemoresistant Cancer Stem Cells
- This data will be used to determine a list of candidate genes that will be inhibited along with Standard Chemotherapy for complete response

References
- Creighton et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor initiating features. Proc Natl Acad Sci U S A 2009 Aug 18;106(33):13820-5.

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