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Generating Stable Bioluminescent Cell Lines by Retroviral Transduction

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Introduction

It is difficult to accurately in vivo image orthotopic tumors using CT scans alone. Muriglo provides the capability to visualize in vivo bioluminescent signals emanating from intact tissues. We can coregister CT scans captured with the SARRP with luciferase signals captured with the Muriglo and locate tumor isocenters for image-guided radiation. We may then direct radiation to neoplastic tissue while also sparing the maximum amount of healthy tissue. To do this we must genetically engineer cells to express luciferase. We propose a protocol to use a retrovirus to create stable cell lines that express firefly luciferase for bioluminescence. We will also determine the optimal dosage of the G418 antibiotic for selection.

Materials and Methods

Retroviral Transfection

We first infected HEK293T cells using pcDNA3 gag pol, phCMV6 VSVG, and Luciferase mCherry plasmids and the transfecting reagent, Lipofectamine 3000. After 18 hours, we replaced the media (10% FBS). 48-72 hours later, we harvested the viral supernatant. We then filtered the supernatant using a 45-micron syringe filter and promptly froze the filtrate in -80º C. We assessed transfection efficiency with flow cytometry.

Determining G418 Dose for Selection

We grew Panc02 cells in a six well plate up to 80 percent confluence. We then replaced the media with media containing variant concentrations of our selection antibiotic, G418. After five days incubating at 37º C 5% CO_2, we took images and determined an appropriate concentration of G418 by finding the lowest concentration that killed all cells.

Retroviral Transduction

In a six well plate, we put different dilutions of the viral supernatant produced by the HEK293T cells in DMEM 10% FBS without penicillin streptomycin and Polybrene (10 µg/mL). Roughly 5 x 10^4 Panc02 cells were then placed into each well and the cells were incubated for 48-72 hours. We assessed transduction efficiency with flow cytometry.

AM12 gp Transfection

We seeded AM12 cells into four 10cm dishes at a density of 4 x 10^6 cells per 8mL DMEM 10% FBS wit penicillin and streptomycin. We then added 400 µL of LyoVec™ with 12 µg of GFP and mCherry Plasmid DNA to each dish according to the LyoVec™ protocol.

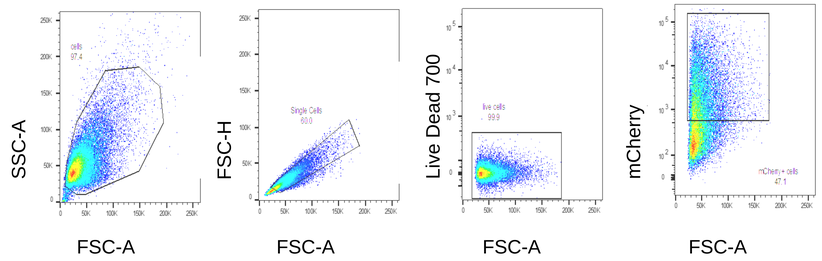


Figure 1 Flow Cytometry of Transfected HEK293T cells. The mCherry positive counts in the PE-A channel suggest that some HEK293T cells were successfully transfected. The viral supernatant collected from this sample however, did not successfully transduce Panc02 target cells.

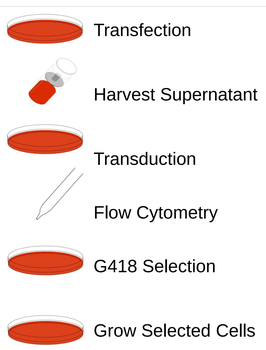
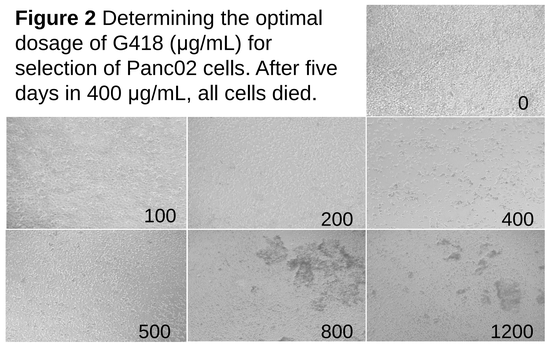


Figure 2 Determining the optimal dosage of G418 (µg/mL) for selection of Panc02 cells. After five days in 400 µg/mL, all cells died.



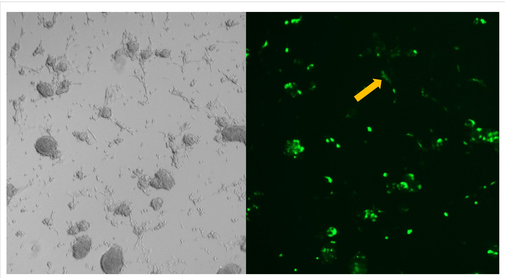


Figure 3 GFP Expression in transfected AM12 cells. Green signals indicate successfully transfected AM12 cells. Toxicity is suggested by the large population of dead cells. Photo was taking with 10x magnification.

Conclusions

Using flow cytometry, we were able to verify that we successfully transfected the mCherry plasmid into HEK293T cells. Based on our G418 toxicity test (Figure 2), over 200 µg/mL of G418 can be used for selection of Pano02 cells. We also found that AM12 cells can easily be transfected to produce virus.

Future Research

Create stable cell lines that can express a bioluminescent marker Trials involving in vivo imaging of bioluminescent tumors using SARP and Muriglo.

Expression in Aromatase Inhibitor Responder vs. Non-Responder HR+ Breast Cancer Cohorts

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Introduction

Hormone receptor positive (HR+) breast cancer patients have an excellent prognosis with over 85% cure rates. However, 20,000 women with HR+ breast cancer die annually from tumor recurrence. Those at highest risk for recurrence are those women whose tumors continue to proliferate through estrogen blockade (Figure 4). Addition of chemotherapy for these patients failed to improve outcomes. We retrospectively identified women with HR+ breast cancer treated neoadjuvantly with aromatase inhibitors (AI), and selected matched patient cohorts for responders and non-responders determined by post-AI Ki67 of <10% or ≥10%. The pathology and molecular genomics core extracted RNA from FFPE slides from the surgical resection tissue and performed Nanostring sequencing using the Pan Cancer IO 360 panel, Breast Cancer 360 panel, and custom probes totaling 1,249 sequenced transcripts. We proposed to utilize this Nanostring analysis to identify drug able immuno-oncology targets to improve outcomes for these women at high risk of recurrence.

Materials and Methods

We used the Nanostring technology, nSolver to normalize our RNA count data. We then used R to generate heat maps and differential expression plots. Using the fold change determined on nSolver, we used Cytoscape and Reactome pathway visualization software to create pathway maps.

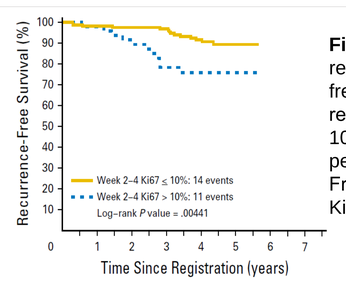

Figure 4 Time since registration vs. recurrence-free survival. Five years after registration, patients with ≤ 10% Ki67 have a higher percentage of Recurrence-Free Survival than ≥ 10% Ki67 patients.

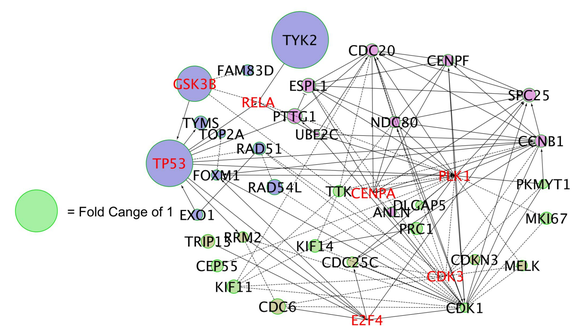

Figure 5 Cytoscape Reactome pathway visualization. Sizes of circles were determined by fold change. Interactor genes are labeled in red. All genes here are threshold by an adjusted p-value of 0.1.

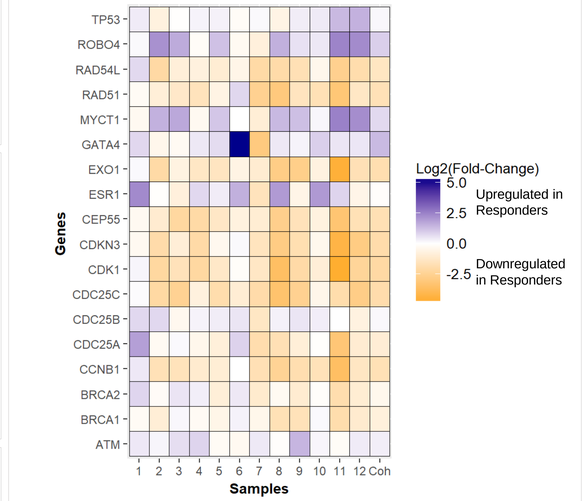

Figure 6 Heatmap of ER pathway genes vs. patient pairings shows upregulation of DNA Damage genes and downregulation of angiogenesis genes in non-responders.

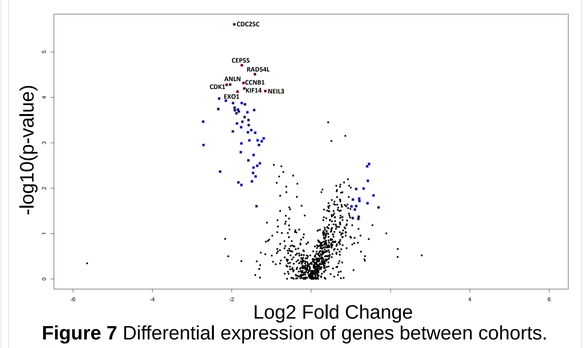

Figure 7 Differential expression of genes between cohorts. Statistically significant genes were mainly downregulated in the responder cohort.

Conclusions

In non-responders, genes regulating to cell cycle an DNA repair were upregulated. In responders, genes regulating endothelial mesenchymal transition, cytokine signaling, chemokine signaling, and angiogenesis were slightly upregulated.

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