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Title: A Rapid Screening Workflow to Identify Potential Combination Therapy for GBM Using Patient-Derived Glioma Stem Cells

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees self-record interview statements. JoVE can provide support for this option.

4. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

Current Protocol Length

Number of Steps: 13
Number of Shots: 31

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ziyi Hu:** Glioma Stem Cells are a subpopulation cells in the glioma which are usually resistant to chemo-/radiotherapy. Here we described a method for rapid identification of combination therapies targeting GSCs instead of differentiated glioma cells **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Tingting Zhou:** Compared with other methods which may be laborious and complicated, this protocol is relatively simple and rapid to identify potential useful drug combinations **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

1.3. GBM specimen was acquired from a patient during a routine operation after obtaining fully informed consent and approval by the human research ethics committee of The First Affiliated Hospital of Nanjing Medical University.

Protocol

2. Preparing Luciferase-tagged GSCs

2.1. Begin by collecting the glioma stem cells, or GSCs, from the culture medium **[1]** and centrifuging them at 70 x *g* for 3 minutes at room temperature **[2].**

2.1.1. WIDE: Establishing shot of talent collecting GSCs.

2.1.2. Talent placing the GSCs in the centrifuge.

2.2. After removing the supernatant **[1]**, digest the cells with accutase for 4 minutes at 37 degrees Celsius **[2]**. Using a 200-microliter tip, pipette the cells repeatedly to dissociate and resuspend the cell pellet **[3].**

2.2.1. Talent removing the supernatant.

2.2.2. Talent adding accutase to the cells.

2.2.3. Talent repeatedly pipetting the cell pellet.

2.3. Add the GSCs at a density of 200,000 cells in 1 milliliter culture medium into each well of a 12-well culture plate **[1]** and incubate them overnight **[2].**

2.3.1. Talent adding cells to the culture plate.

2.3.2. Talent placing the cells in the incubator. *Videographer: Obtain multiple usable takes because this will be reused in 2.4.3*

2.4. On the next day, add 30 microliters of luciferase-EGFP virus supernatant into each well of the plate **[1-TXT].** Centrifuge the cells at 1,000 x *g* for 2 hours at 25 degrees Celsius **[2]** and incubate them overnight **[3].**

2.4.1. Talent adding viral supernatant to the plate wells. **TEXT: Viral titer >10^8 TU/mL**

2.4.2. Talent centrifuging the cells.

2.4.3. *Use 2.3.2*

2.5. On the following day, replace the medium in the wells by collecting the GSCs **[1]**, centrifuging them **[2]**, removing the supernatant **[3]**, resuspending them in fresh medium, and re-plating them. Culture the cells for another 48 hours **[4]**.

2.5.1. Talent collecting GSCs.

2.5.2. Talent placing the GSCs in the centrifuge.

2.5.3. Talent removing the supernatant.

2.5.4. Talent resuspending and re-plating the cells.

2.6. Observe the plate under a fluorescent microscope **[1]** and confirm the appearance of GFP positive cells **[2]**. *Videographer: This step is difficult and important!*

2.6.1. Talent observing the plate under a fluorescent microscope.

2.6.2. LAB MEDIA: image001

2.7. Sort and collect the GSCs with a high fluorescence using a flow cell sorter and culture them further **[1].**

2.7.1. Talent using a flow cell sorter to collect the cells.

3. Combination Screening

3.1. Coat 96-well optical bottom plates with an extracellular matrix mixture such as Matrigel **[1-TXT]** and incubate them for 1 hour at 37 degrees Celsius **[2].** Remove the excess mixture **[3]** and gently rinse the plates once with PBS **[4].** *Videographer: This step is important!*

3.1.1. Talent adding the extracellular matrix mixture. **TEXT: 40 µL of 0.15 mg/mL ECM mixture per well**

3.1.2. Talent placing the 96-well plate in the incubator.

3.1.3. Talent removing excess mixture.

3.1.4. Talent rinsing the plate with PBS.

3.2. Then, seed XG387-Luc cells at a density of 1,000 cells in 100 microliters of culture medium into each well of the coated plates **[1]** and culture them overnight **[2].** On the following day, observe the cells under a microscope **[3]** to confirm their attachment to the plate **[4]**.

3.2.1. Talent seeding cells.

3.2.2. Talent placing the plates in an incubator.

3.2.3. Talent observing the cells under a microscope.

3.2.4. LAB MEDIA: image002 and image003.

3.3. Next, prepare a 200 micromolar temozolomide solution and 2 micromolar solutions of the targeted agents in the culture medium **[1].** *Videographer: This step is important!*

3.3.1. Talent preparing 200µM of temozolomide and 2µM of targeted agents.

3.4. For combination drug screening, remove the blank medium **[1]** and add the medium containing either 200 micromolar temozolomide or 2 micromolar targeted agent, or a combination of both, into each well for three technical replicates per treatment **[2]**.

3.4.1. Talent removing blank medium.

3.4.2. Talent adding temozolomide and targeted agents into the wells.

3.5. Incubate the plate at 37 degrees Celsius and 5% carbon dioxide for 3 days **[1].**

3.5.1. Talent placing the plate in the incubator.

3.6. Take images of the cellular bio-luminescence in the plate using the IVIS *(eye-vis)* spectrum imaging system **[1]**. Using the built-in software, create multiple circular areas of the region of interest and quantify the cellular bio-luminescence **[2]**. *Videographer: This step is difficult and important!*

3.6.1. Talent using the IVIS spectrum imaging system.

3.6.2. SCREEN: 62312_ screen shot_1.mp4. 00:04-00:06 + 00:40-00:55 + 1:27-1:34. *Video Editor: Add the three time-code clips and speedup the video to play.*

Results

4. Results: Identification of Potential Combination Therapy Candidates for Glioblastoma

4.1. To identify potential candidates that could enhance the anti-glioblastoma effect of temozolomide, 20 target-selective small molecule inhibitors were analyzed through drug combination screening **[1]**.

4.1.1. LAB MEDIA: Figure 2B.

4.2. The sensitive index values of 13 targeted agents were above 0, and 5 of them were above 0.1 **[1].** The sensitive index of the top two candidate drugs, UMI-77 *(pronounce 'oo-me-seventy-seven')* and A 83-01 *(pronounce 'A-eighty-three-zero-one')*, were higher than 0.25, suggesting their potential to synergize with temozolomide **[2].**

4.2.1. LAB MEDIA: Figure 2B. *Video Editor: Emphasize the red points*

4.2.2. LAB MEDIA: Figure 2C. *Video Editor: Emphasize the SI values of UMI-77 and A 83-01*

4.3. The combined treatment of temozolomide and UMI-77 was evaluated using an anti-proliferative assay in a six-by-six dose titration matrix **[1]**.

4.3.1. LAB MEDIA: Figure 3A

4.4. The combination index values were less than 1 **[1]** and the high single agent values were greater than 0 for most of the combinations of temozolomide and UMI-77 at different concentrations, suggesting an overall synergistic interaction of temozolomide and UMI-77 in both XG387 and XG328 GSCs **[2]**.

4.4.1. LAB MEDIA: Figure 3B. *Video Editor: Emphasize the CI values being less than 1*

4.4.2. LAB MEDIA: Figure 3C.

Conclusion

5. Conclusion Interview Statements

5.1. **Tingting Zhou:** This protocol can also be applied in finding drug combinations using adherent cancer cells by simply removing the plate coating step **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.