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Title: Discovery of Metastatic Regulators Using a Rapid and Quantitative Intravital Chick Chorioallantoic Membrane Model

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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? **YES**

   If **Yes**, can you record movies/images using your own microscope camera?

   NO

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Zeiss Lumar.V12

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

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 wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **NO**

   If **Yes**, how far apart are the locations?

Current Protocol Length

Number of Steps: 16
Number of Shots: 46

Introduction

1. Introductory Interview Statements

REQUIRED: NOTE: 1.1-1.3, take 1 is not good, take 2- WS good, take 3- MS good

1.1. **Konstantin Stoletov:** Our protocol permits an efficient identification of gene or gene combinations that block cancer cell invasion in vivo.

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

1.2. **Konstantin Stoletov:** Chicken ex ovo culture does not require advanced animal facilities for maintenance and Whole shRNA or gene overexpression libraries can be screened in a matter of several weeks *in vivo*.

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

OPTIONAL:

1.3. **Konstantin Stoletov:** This ex ovo screening system will help researchers discover novel therapeutic gene targets, or gene target combinations, that can be used to block cancer metastasis.

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

Ethics Title Card

1.4. All experiments were performed in accordance with the regulations and guidelines of the Institutional Animal Care and Use Committee at the University of Alberta.

Protocol

2. Intravenous injection of cancer cells for metastatic colony formation

2.1. For IV injection, concentrate cells to 0.5 to 1 million cells per milliliter, using ice cold 1x PBS to dilute or resuspend the cells. Expect that approximately 1 milliliter of cell suspension will be needed for every ten embryos **[1]**.

2.1.1. Talent diluting or resuspending the cells using PBS.

2.2. To assemble the injection apparatus, mount a needle onto the syringe **[1]** and then extend the syringe needle with a 3- to 5-centimeter-long piece of tubing **[2]**. Break the tip of the borosilicate needle using fine forceps **[3]**.

2.2.1. Talent mounting the needle onto syringe.

2.2.2. Talent extending the syringe needle with tubing.

2.2.3. Talent breaking the borosilicate needle.

2.3. Load the syringe with 50 to 200 microliters of the cancer cell suspension **[1]** and insert the borosilicate needle into the tubing **[2]**. Inspect the needle for cell clogging and air bubbles **[3]**.

2.3.1. Talent loading the syringe with cancer cell suspension.

2.3.2. Talent inserting the needle into tubing.

2.3.3. Talent inspecting the needle.

2.4. If cell clogging is observed within the capillary, remove the capillary **[1]**, re-suspend the cells **[2]**, and replace it with a new capillary **[3]**. Use the plunger to push out any bubbles **[4]**.

2.4.1. Talent removing the clogged capillary.

2.4.2. Talent resuspending cells in capillary.

2.4.3. Talent replacing new capillary.

2.4.4. Talent using plunger to push out air bubbles.

2.5. Remove the cover lid **[1]** and transfer the embryo under the stereoscope **[2]**. Identify the appropriate vein to be injected on the CAM *(pronounce as one word, 'cam')* surface, which is normally only slightly wider than the diameter of the borosilicate

needle tip and located midway between the embryo and the weighing dish wall **[3-TXT]**.

2.5.1. Talent removing the cover lid.

2.5.2. Talent transferring the embryo under stereoscope.

2.5.3. Talent identifying the vein to be injected. **TEXT: CAM- chorioallantoic membrane**

NOTE: The scope camera's gear to control zoom have become worn out over time and the focal length does not stay set. There was much difficulty getting these scope shots hopefully they work out.

2.6. Find the ideal vein to inject cells which is only slightly wider than the diameter of borosilicate needle tip and located midway between the embryo and the weighing dish wall. It is generally easier to inject at the point immediately adjacent to the vein bifurcation **[1]**. *Videographer: This step is important!*

2.6.1. SCOPE: Good vein for injection. NOTE: Optional Side shot in case Scope shot isn't enough; Use Take 1

2.7. Press the tip of the needle against the blood vessel wall and apply gentle pressure in the same direction as the blood flow. If necessary, use a cotton swab to help anchor or stabilize the vessel that is being injected **[1]**. Gently depress the syringe plunger for 2 to 10 seconds until the desired volume is injected **[2]**. *Videographer: This step is difficult and important!*

2.7.1. SCOPE: Talent pressing the tip of needle against the blood vessel. NOTE: Take 1 and Take 2: Optional Side shot in case Scope shot isn't enough; Use Take 1

2.7.2. Talent depressing the syringe.

2.8. Discard the embryo if excessive bleeding or clear liquid accumulation appears at the injection site **[1]**. Remove the needle from the CAM and gently dab the injection site with a cotton swab to remove any blood or excess cancer cells **[2]**.

2.8.1. Excessive bleeding or clear liquid accumulation at the injection site.

2.8.2. Talent removing the needle from CAM and dabbing the injection site with cotton swab.

2.9. Cover the injected embryo in the weighing dish with a lid **[1]** and return it into the incubator. Repeat the procedure until all embryos are injected **[2]**.

2.9.1. Talent covering the embryo with lid.

2.9.2. Talent keeping the injected chick embryo in incubator.

3. Embryo maintenance during the metastatic colony growth and their isolation

3.1. Visually inspect the embryos for bacteria or mold contamination or death **[1]** and discard contaminated embryos according to laboratory disposal procedures **[2]**.

3.1.1. Talent inspecting the embryos. NOTE: 3.1.1a Take 1 and Take 2: Additional shot if needed

3.1.2. Talent discarding the contaminated and dead embryos. NOTE: 3.1.2a: Extra shot CU on biohazard symbol

3.2. Ensure that metastatic colonies appear uniform in shape during the first 1 to 3 days post-injection and identify invasive or non-invasive metastatic colonies at days 4 to 5 post injection **[1]**. *Videographer: This step is important!*

3.2.1. SCOPE: Metastatic colonies. NOTE: Take 1 is ok and Take 2 is better

3.3. At day 5 post injection, remove the embryos from the incubator **[1]** and inspect and locate the embryo CAMs for metastatic colony distribution **[2]**.

3.3.1. Talent removing embryos from the incubator.

3.3.2. Talent placing the embryos under the microscope. NOTE: Take 1 good and Take 2 better.

3.4. Under the dissection microscope, gently pull the CAM tissue that contains the metastatic colony of interest upwards using fine forceps and cut it off with surgical scissors **[1]**. Transfer the CAM tissue into an empty, sterile 1.5-milliliter tube on ice **[2]** and close the tube lid **[3]**. *Videographer: This step is important!*

3.4.1. SCOPE: Talent pulling the CAM tissue containing metastatic colony and cutting it off. *Videographer take a side shot as well.* NOTE: Take 1: Optional Side shot in case Scope shot isn't enough. Scope shot- Attempt 2 in clip is best

3.4.2. Talent transferring the cut CAM tissue into a sterile tube. NOTE: 3.4.2 and 3.4.3 are combined

3.4.3. Talent closing the lid of the tube.

3.5. Repeat the excision procedure until all the colonies of interest are collected into separate tubes **[1]**. Gently mince the CAM tissue in a microcentrifuge tube using a

separate sterile 18 Gauge needle for each colony **[2]**. Add 100 microliters of 1x collagenase solution **[3]** and incubate for 30 minutes at 37 degrees Celsius **[4]**.

3.5.1. Multiple tubes with colonies. NOTE: Mislabeled slate 3.4.3

3.5.2. Talent mincing the tissue with needle.

3.5.3. Talent adding 1x collagenase solution.

3.5.4. Talent incubating the tube at 37 degrees.

3.6. Spin down the cells and CAM tissue at 300 times *g* for 5 minutes at ambient temperature **[1]**. Aspirate the collagenase solution **[2]** and resuspend the cells in complete media for the cell line of interest **[3]**, then spin the cells and tissue again **[4]**.

3.6.1. Talent spinning the CAM tissue.

3.6.2. Talent aspirating the collagenase solution. NOTE: 3.6.2a: Additional shot if needed

3.6.3. Talent adding complete media to the tube.

3.6.4. Talent spinning the tube. NOTE: 3.6.4a: Additional shot if needed

3.7. Resuspend cells and tissue pieces in 1 milliliter of complete media and selection factor, if any **[1]**, then transfer into a single 12-well tissue culture dish well **[2]**. For the next 1 to 3 weeks monitor the cancer cells daily for growth and contamination **[3]**.

3.7.1. Talent resuspending the cells in complete media.

3.7.2. Talent transferring the cells in culture dish.

3.7.3. Talent checking the cells.

3.8. When the cells reach 70 to 80% of confluency, transfer them into a larger volume culture dish **[1]**. Proceed to sequencing or the next round of selection as soon as adequate cancer cell numbers are reached **[2]**.

3.8.1. Talent transferring the confluent cells into larger culture dish. NOTE: 3.8.1a Take 1: Author may have lab media to add to this section of protocol and 3.8.1 Take 2 CU: A couple shots were not at an ideal angle in final location as there was maintenance workers in the room next this vent station fixing some incubators.

3.8.2. Talent proceeding to sequencing.

Results

4. Metastatic colony isolation and representative results for different steps of the screening protocol

4.1. Embryos displaying a buildup of cancer cells due to unsuccessful injection can be seen in most of the capillaries **[1]**.

4.1.1. LAB MEDIA: Figure 2E. *Video editor focus on the region that is pointed to by the red arrow.*

4.2. When the optimal cell concentration and duration of injection is achieved, day 5 post injection transduced cells should produce a wide variety of colony phenotypes, with the majority of the colonies invasive as determined by the cancer cells appearing scattered in the CAM tissue **[1]**.

4.2.1. LAB MEDIA: Figure 3A.

4.3. Attention should be paid to the metastatic colonies that appear “compact” and are located far enough from neighboring colonies that they can be excised with forceps and scissors in one piece of CAM tissue **[1]**.

4.3.1. LAB MEDIA: Figure 3C.

4.4. An isolated positive screen hit should display the compact colony phenotype upon reinjection **[1]**. For measurement of the cancer cell-blood vessel contacts, attention should be paid to the vascular wall stain brightness and the appropriate amount of lectin should be injected for the vascular wall signal **[2]**.

4.4.1. LAB MEDIA: Figure 3B.

4.4.2. LAB MEDIA: Figure 3D and 3E.

Conclusion

5. Conclusion Interview Statements NOTE: Take 1-MS good, Take 2-WS good

5.1. **Konstantin Stoletov:** When attempting this protocol, avoid under or overinjecton and remove excess bleeding or liquid buildup.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7.1., 2.7.2., and 2.8.1, 2.8.2.*

5.2. **Konstantin Stoletov:** Several novel genes that are driving cancer cell invasion in vivo have been identified using this technique.

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

5.3. Special thanks to the University of Alberta. *Video Editor: Please include this in the credits*