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Title: Bone Marrow Transplantation Procedures in Mice to Study Clonal Hematopoiesis

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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 similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

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 outside of the filming date. JoVE can provide support for this option.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length

Number of Shots: **0** All shots should be provided in single megafile from authors. Each shot should be slated

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Kenneth Walsh**: Our protocol will help researchers understand the different physiological effects of three different bone marrow transplantation methods and how they affect experimental outcomes in a clonal hematopoiesis setting **[1]**.

1.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Kenneth Walsh**: Total-body irradiation bone marrow transplantation can negatively impact the cardiovascular organs and alter disease pathogenesis. Thus, our lab has developed two alternative methods to minimize or avoid possible side effects **[1]**.

1.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Kenneth Walsh**: Demonstrating the procedures will be Eunbee Park, a graduate student, and Megan Evans, a post-doctoral fellow, both from my laboratory **[1][2][3]**.

1.3.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

1.3.3. ADDED SHOT: The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Virginia.

Protocol

2. Partially Shielded Irradiation

2.1. For thorax and abdomen shielding, place the adjustable tray in the X-ray irradiator at the correct distance to achieve uniform irradiation **[1]** and place the anesthetized mice on a flat lead plate inverted to each other in the supine position **[2-TXT]**.

2.1.1. LAB MEDIA: To be provided by Authors: Talent placing adjustable tray in the irradiator

2.1.2. LAB MEDIA: To be provided by Authors: Mouse being oriented inverted to each other on tray *Videographer: More Talent than mouse in shot* **TEXT: Anesthesia: ketamine 80-100 mg/kg + xylazine 5-10 mg/kg i.p.**

2.2. Secure the paws to the plate with tape **[1]** and place the lead shielding so that the lower end aligns with the xiphisternum bone and the upper end of the lead shield sits near the thymus **[2]**.

2.2.1. LAB MEDIA: To be provided by Authors: Paw being taped

2.2.2. LAB MEDIA: To be provided by Authors: Shielding being placed

2.3. After shielding, place the mice into the irradiator **[1]** and expose the animals to two, 5.5-grey fractions of irradiation separated by a 4-24-hour interval **[2-TXT]**.

2.3.1. LAB MEDIA: To be provided by Authors: Talent placing mice into irradiator.

2.3.2. LAB MEDIA: To be provided by Authors: Talent initiating radiation dose. **TEXT: See text for abdominal shielding details**

2.4. For head shielding, carefully tape the forepaws of the mouse to the abdomen **[1]**.

2.4.1. LAB MEDIA: To be provided by Authors: Forepaw(s) being taped **T**

2.5. Place the mouse in a conical restrainer **[1]** and slide the restrainer into the slot within the lead shield so that mouse's head and ears are completely covered **[2]**, leaving the rest of the mouse's body exposed for irradiation **[3]**.

2.5.1. LAB MEDIA: To be provided by Authors: Talent placing mouse into restrainer *Videographer: More Talent than mouse in shot*

2.5.2. LAB MEDIA: To be provided by Authors: Restrainer being slid into slot/head being covered

2.5.3. LAB MEDIA: To be provided by Authors: Shot of exposed body

2.6. After shielding, place the mice into the irradiator **[1]** and expose the animals to two, 5.5-grey fractions of irradiation separated by a 4-24-hour interval **[2]**.

2.6.1. LAB MEDIA: To be provided by Authors: Talent placing mice into irradiator *Videographer: More Talent than mouse in shot*

2.6.2. LAB MEDIA: To be provided by Authors: Talent initiating radiation dose

2.7. After each irradiation treatment, place the anesthetized mice in a cage on a heated mat with monitoring until fully recovered **[1]**.

2.7.1. LAB MEDIA: To be provided by Authors: Talent placing mice into cage on heating mat *Videographer: More Talent than mouse in shot*

3. Bone Isolation

3.1. To isolate the bones, disinfect the skin of the donor mouse with 70% ethanol **[1-TXT]** and make a small, transverse skin incision below the rib cage. **[2]**.

3.1.1. LAB MEDIA: To be provided by Authors: Talent disinfecting skin **TEXT: Euthanasia: cervical dislocation without anesthesia**

3.1.2. LAB MEDIA: To be provided by Authors: Incision being made

3.2. Holding the skin tightly at either side of the incision, tear in opposite directions toward the head and feet **[1]** and peel the skin from all of the limbs **[2][3]**.

3.2.1. LAB MEDIA: To be provided by Authors: Skin being torn

3.2.2. LAB MEDIA: To be provided by Authors: Skin being peeled away from limb

3.2.3. ADDED SHOT: LAB MEDIA: To be provided by Authors: Skin being peeled away from fore limb NOTE: Authors added this shot but skin being peeled from only one set of limbs is necessary. Feel free to include this second shot or not, depending on timing/video editor's preference

3.3. Cut over the shoulders and elbow joints **[1]** and use a lab wipe to remove the attached muscles and connective tissues from the humeri **[2]**.

3.3.1. LAB MEDIA: To be provided by Authors: Muscle being cut

3.3.2. LAB MEDIA: To be provided by Authors: Bone being cleaned from muscles and connective tissue

3.4. Carefully dislocate the hip joints between the femur and hip bones **[1]** and use blunt scissors to cut along the femur head to detach the legs **[2]**.

3.4.1. LAB MEDIA: To be provided by Authors: Hip joint being dislocated

3.4.2. LAB MEDIA: To be provided by Authors: Femur head being cut/detached

3.5. Cut over the knee joint to separate the femur and tibia **[1]** and use lab wipes to carefully remove the attached muscles and connective tissues from the bones **[2]**.

3.5.1. LAB MEDIA: To be provided by Authors: Knee joint being cut

3.5.2. LAB MEDIA: To be provided by Authors: Bone(s) being cleaned from muscles and connective tissue

3.6. Then pool the bones from mice of the same genotype into individual 50-milliliter conical tubes containing 20 milliliters of ice-cold sterile PBS on ice **[1]**.

3.6.1. LAB MEDIA: To be provided by Authors: Talent adding bones to tube

4. Bone Marrow Isolation

4.1. To isolate the bone marrow cells, in a biosafety class two cabinet, use an 18-gauge needle to make a small hole in the bottom of one sterile 500-microliter microtube per genotype **[1]** and place the tubes into individual sterile 1.5-milliliter microcentrifuge tubes containing 100 microliters of ice-cold sterile PBS per tube **[2]**.

4.1.1. LAB MEDIA: To be provided by Authors: Talent at hood, making hole in tube

4.1.2. LAB MEDIA: To be provided by Authors: Talent placing 0.5-mL tube into 1.5-mL tube, with PBS container visible in frame

4.2. When all of the tubes have been prepared, remove the PBS from the tube containing the isolated bones **[1]** and transfer the bones onto a sterile 100-millimeter cell culture dish **[2]**.

4.2.1. LAB MEDIA: To be provided by Authors: Talent aspirating the PBS from tube of bones

4.2.2. LAB MEDIA: To be provided by Authors: Talent placing bones into dish

4.3. Use fine forceps and small scissors to carefully remove the epiphyses from the ends of each bone **[1]** and place up to six bones into each 500-microliter tube **[2]**.

4.3.1. LAB MEDIA: To be provided by Authors: Bone being cut

4.3.2. LAB MEDIA: To be provided by Authors: Bone being placed into prepared tubes

4.4. When all of the bones have been cut, extract the bone marrow by centrifugation **[1-TXT]**. If all of the marrow has been removed, the bones should appear white and translucent **[2]** with a relatively large red pellet at the bottom of the 1.5-milliliter microcentrifuge tube **[3]**.

4.4.1. LAB MEDIA: To be provided by Authors: Talent placing tube(s) into centrifuge **TEXT: 35 s, 10,000 x g, 4 °C**

4.4.2. LAB MEDIA: To be provided by Authors: Shot of white, clear bone(s)

4.4.3. LAB MEDIA: To be provided by Authors: Shot of red pellet

5. Bone Marrow Cell Transplantation

5.1. For bone marrow cell transplant, dilute the isolated bone marrow cells in serum-free RPMI media **[1-TXT]** and load 200 microliters of the cell suspension into one 0.5-milliliter insulin syringe per mouse to be injected **[2]**.

5.1.1. LAB MEDIA: To be provided by Authors: Talent adding media to cells, with media container visible in frame **TEXT: See text for bone marrow cell isolation and preparation details**

5.1.2. LAB MEDIA: To be provided by Authors: Talent loading cells into syringe

5.2. After confirming a lack of response to pedal reflex **[1]**, slowly inject the entire volume of cells into the retro-orbital vein of each anesthetized recipient animal **[2-TXT]**.

5.2.1. LAB MEDIA: To be provided by Authors: Toe being pinched

5.2.2. LAB MEDIA: To be provided by Authors: Cells being delivered by retro-orbital injection **TEXT: Anesthesia: 5% isoflurane**

5.3. Then place a drop of proparacaine onto the eye for pain relief **[1]** and allow the mouse to regain consciousness while being monitored **[2]**.

5.3.1. LAB MEDIA: To be provided by Authors: Eye drop being placed on eye surface

5.3.2. LAB MEDIA: To be provided by Authors: Talent placing mouse into recovery cage

Results

6. Results: Representative Donor and Recipient Cell Expansion After BMT

6.1. In this representative analysis, in the peripheral blood of recipient mice that received total body irradiation **[1]**, monocytes, neutrophils, and B cells were largely ablated **[2]** and replaced by the progeny of donor bone marrow-derived cells **[3]**.

6.1.1. LAB MEDIA: Figure 2A
6.1.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize grey mono, Neut, and B cell Blood data bars*
6.1.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize blue Blood data bars*

6.2. In addition, the resident recipient cardiac monocyte and neutrophil populations **[1]** were almost completely replaced by donor-derived cells **[2]**.

6.2.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize grey mono and Neut Heart data bars*
6.2.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize blue Heart data bars*

6.3. In the partially shielded irradiation group **[1]**, the donor-derived cardiac immune cell replacement was modest **[2]**.

6.3.1. LAB MEDIA: Figure 2B
6.3.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize red Heart data bars*

6.4. The recipient mouse bone marrow cells within the shielded regions likely contributed to the lower level of peripheral blood reconstitution **[1]** compared to mice that received total body irradiation **[2]**.

6.4.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize grey Blood data bars*
6.4.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize red Blood data bars*

6.5. In the group without BMT pre-conditioning **[1]**, donor-derived cells were detectable in the peripheral blood and hearts of recipient mice at 4 weeks post-BMT **[2]**.

6.5.1. LAB MEDIA: Figure 2C
6.5.2. LAB MEDIA: Figure 2C *Video Editor: please emphasize red data bars*

6.6. In addition, Tet2-deficienct donor cells gradually expanded over time **[1]**.

6.6.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize red data bars and/or sequentially emphasize red data bars from 4-16 weeks or otherwise indicate gradual expansion*

6.7. In comparison, recipient mice engrafted with wild type donor cells showed minimal clonal expansion of donor cells **[1]**.

6.7.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize black data bars*

Conclusion

7. Conclusion Interview Statements

7.1. **Eunbee Park**: The optimization of these experimental models will enable more rigorous studies of the clonal hematopoiesis driver genes that contribute to all-cause mortality, such as cardio-metabolic disease and cancer **[1]**.

7.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

7.2. **Megan A Evans**: We hope that our protocols will allow researchers to perform studies on clonal hematopoiesis to investigate how it contributes to cardiovascular disease development and other disease states **[1]**.

7.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera