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Authors

Zong, Yang
Goldstein, Andrew S
Witte, Owen N

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Protocol

Preparation of Urogenital Sinus Mesenchymal Cells for Prostate Tissue Recombination Models

Yang Zong,[1] Andrew S. Goldstein,[2,3,5,6,7] and Owen N. Witte[1,2,4,5,6]

[1]*Howard Hughes Medical Institute, University of California, Los Angeles, California 90095;* [2]*Department of Molecular and Medical Pharmacology, University of California, Los Angeles, California 90095;* [3]*Department of Urology, University of California, Los Angeles, California 90095;* [4]*Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, California 90095;* [5]*Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, California 90095;* [6]*Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, California 90095*

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An appropriate microenvironment provided by the mesenchyme is important for establishing tissue recombination models for epithelial cancer. Urogenital sinus mesenchymal (UGSM) cells derived from embryonic rodent show potent inductive effects for prostate regeneration. Genetic manipulation of these mesenchymal cells allows us to define the contribution of the tumor microenvironment to prostate cancer development. This protocol describes preparation and propagation of murine UGSM cells in culture.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPE: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

BFS growth medium <R>
Collagenase type I (1900 units/mL in RPMI medium)
DMSO (for freezing cells)
DNase I
Dulbecco's Modified Eagle Medium (DMEM)
Fetal bovine serum (FBS)
Mice (C57/BL6), at breeding age
Phosphate-buffered saline (PBS)
Trypsin (1%)

Equipment

Cell strainer (100 µm)

[7]Correspondence: andrew.goldstein@ucla.edu

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Centrifuge (benchtop)
Dissecting macroscope with lower LED illumination
Dissecting tools (scissors and forceps)
Glass slides (concave)
Incubator
Insulin syringes with 27-gauge needles
Shaker
Tissue culture dishes
Tubes (15-mL conical)

METHOD

1. Set up timed pregnancies and sacrifice female mice at Day 16 of pregnancy.
2. Remove the embryos from the uterus, dissect away the amniotic sac and the placenta, and place the embryos in sterile dishes containing DMEM + 10% FBS.
3. Cut the embryos in half below the liver. Place the lower half of the torso into 1× PBS.
4. Under a dissecting macroscope, locate the fetal bladder, also called the rostral urogenital sinus (UGS), on the midline of the torso and gently pull out the whole UGS with forceps.

   The whole UGS consists of the rostral, pelvic, and caudal parts, which give rise to bladder, prostate (in males) or upper vagina (in females), and urethra, respectively.
5. Dissect the pelvic UGS by removing the attached tubular structures and cutting off the fetal bladder and urethra.
6. Pool all of the pelvic UGS tissues onto a concave glass slide containing DMEM + 10% FBS, and wash the tissues with 1× PBS twice by aspiration with a pipette.
7. Add 250 µL of 1% trypsin and digest the UGS tissues at 4°C for 90 min.
8. Remove the trypsin and inactivate any residual enzyme by adding DMEM + 10% FBS to the tissues.
9. Replace the medium with fresh DMEM + 10% FBS containing 500 units of DNase I, and let the tissues sit at room temperature for 2 min.
10. Wash the UGS tissues twice with DMEM + 10% FBS, and separate the mesenchymal tissue fragments from the epithelial fragments under the macroscope using two insulin syringes with 27-gauge needles.

    With lower LED illumination, the centrally localized epithelia show a translucent, cylinder-shaped appearance, whereas the surrounding mesenchyme is more opaque and fluffy.
11. Collect all of the mesenchymal fragments into a 15-mL conical tube, and add 9 mL of DMEM + 10% FBS and 1 mL of RPMI containing collagenase type I (1900 units/mL). Incubate on a shaker at 37°C for 90 min.
12. Filter the UGSM cells through a 100-µm cell strainer, then wash the cell strainer with 30 mL of DMEM + 10% FBS.
13. Centrifuge the cells at 550*g* for 5 min. Resuspend the pellet with 10 mL of BFS growth medium.
14. Culture the UGSM cells in BFS growth medium in tissue culture dishes at 37°C, and passage cells when they are 80%–90% confluent.

    *After two passages of propagation, UGSM cells can be used for in vivo prostate regeneration (see Protocol: **Dissociated Prostate Regeneration under the Renal Capsule** [Zong et al. 2014]) or frozen in BFS growth medium supplemented with 10% DMSO for long-term storage in liquid nitrogen. The UGSM cells will lose their inductive effects if they are passaged beyond five generations.*

RECIPE

BFS Growth Medium

Reagent	Final concentration
Dulbecco's Modified Eagle Medium (DMEM)	1×
Fetal bovine serum (FBS)	5% (v/v)
Nu-Serum IV	5% (v/v)
Glutamine	2 mM
Penicillin	100 units/mL
Streptomycin	100 µg/mL
Insulin	5 µg/mL

Store medium at 4°C for up to 1 mo.

REFERENCE

Zong Y, Goldstein AS, Witte ON. 2014. Dissociated prostate regeneration under the renal capsule. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot078063.

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