Primary Tumor Preparation

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[Abstract] This protocol has been developed for culturing primary glioblastoma cells. We have most experience in using it on rodent preparations, but it can also be used in culturing cells from other species.

Materials and Reagents

1. Phosphate buffered saline (PBS)
2. Fetal bovine serum (FBS)
3. Trypsin (Corning, Cellgro®, catalog number: 25-054-CI)
4. Minimum essential medium (MEM)
5. HEPES
6. Sucrose
7. B104 conditioned media
8. N2 supplement (Life Technologies, Invitrogen™, catalog number: 17502-048)
9. T3 (Sigma-Aldrich, catalog number: T2877)
10. Penicillin/streptomycin/amphotericin (Life Technologies, Invitrogen™, catalog number: 15240-062)
11. DMEM (Life Technologies, Invitrogen™, catalog number: 11965-167)
12. Poly-L-lysine
13. PDGF-AA (Sigma-Aldrich)
14. FGFb (Life Technologies, Gibco®)
15. Basal media (in DMEM) (see Recipes)

Equipment

1. Mesh (BD Biosciences, Falcon®)
2. Shaking bath
3. Centrifuges
4. 6-well tissue culture plates

Procedure

1. Perform *ex vivo* gross total resection of the tumor, shred and mince the tissue in a small amount of PBS.
   Note: Enzymatic and mechanical dissociation followed a modified protocol (Gensert and Goldman., 2001).
2. Treat the shredded tissue with 11 ml per sample digestive enzyme (1:1,000 dilution of Trypsin 2.5% in MEM containing 20 mM HEPES) for 30 min in a 37 °C shaking bath. After the digestion, filter the dissociated cells through a 70 µm mesh.
3. Add 5 ml of 10% heat-inactivated FBS to inactivate the trypsin. Centrifuge cells for 10 min at 450 *x g* and re-suspend in MEM containing 20 mM HEPES and 30% sucrose.
4. Centrifuge cells for 20 min at 770 g and re-suspend in media.
5. Plate tumor cells onto 6-well tissue culture plates coated with poly-L-lysine (concentration) at a concentration of 2 million cells per well.
6. Grow the cells in culture media containing a 2:1 mixture of basal media and B104 conditioned media (Canoll *et al.*, 1996), further supplemented with 10 ng/ml PDGF-AA and 10 ng/ml FGFb.
7. Basal media contained N2 supplement, 20 ng/ml T3, 0.5% FBS, and penicillin/streptomycin/amphotericin in DMEM.
8. Collect B104 conditioned media from confluent cultures of the B104 neuroblastoma cell line maintained in basal media for 48 h.

Recipes

1. Basal media (in DMEM)
   N2 supplement
   20 ng/ml T3
   0.5% FBS
   Penicillin/streptomycin/amphotericin

Acknowledgments

This protocol was developed in Dr. Peter Canoll's lab at Columbia University, NY, USA. Please cite (Lei *et al.,* 2011) if you use this protocol in your publications.

References

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