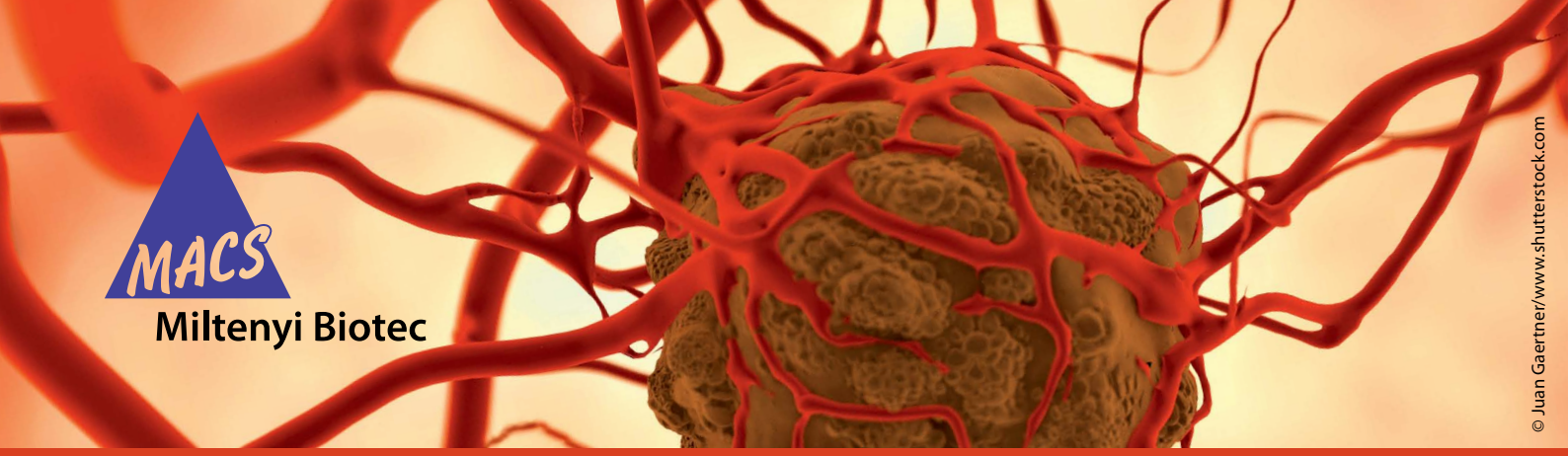

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Dissociation of mouse xenografts

Isolation of tumor cells from mouse xenografts using the gentleMACS™ Dissociator

Jen-Chieh Tseng and Kristen L. Jones
Lurie Family Imaging Center, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Boston, MA 02215, USA

Background

A robust and reliable method to isolate viable tumor cells from tumor tissue is of great importance for cancer research. There are two major applications for this technique. Isolated cancer cells can be directly used for biochemical or genetic characterization *in vitro*. Another application is to preserve and establish stable cell lines for downstream *in vivo* tumor model development. The sources of tumor tissues can be the human primary tumor samples or the tumor xenografts previously established in immune-compromised animals.

In this note, we describe two general protocols to isolate live cancer cells: from tumor fragments for solid cancer (glioblastoma) and from spleen tissue for disseminated hematological cancer.

Materials and methods

Materials

- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- MACSmix™ Tube Rotator in combination with wan incubator at 37 °C
- Centrifuge
- Cell strainer (mesh size 70 µm)
- RPMI 1640 medium (unsupplement)
- Suitable cell culture media (supplement with antifungal and other suitable selection antibiotics if necessary)

Additional requirements for isolation from solid tumor fragment (glioblastoma)

- Tumor Dissociation Kit, human

Additional requirements for isolation from spleen tissue for disseminated hematological cancer

- Spleen Dissociation Kit, mouse

Methods

Isolation from solid tumor fragment (glioblastoma)

1. Prepare enzyme mix of the Tumor Dissociation Kit, human according to the data sheet. Add 5 mL of enzyme mix in RPMI 1640 per gentleMACS C Tube.
2. Weight and cut tumors in small pieces of 2–4 mm in diameter. Avoid necrotic tumor tissue.
3. Transfer the tissue (1–2 g per C Tube) into the C Tube containing the enzyme mix.
4. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program **h_tumor_01**.
6. After the program finishes, detach C Tube from the gentleMACS Dissociator.
7. Incubate sample for 30 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **h_tumor_02**.
9. After the program finishes, detach C Tube from the gentleMACS Dissociator and incubate sample for 30 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
10. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **h_tumor_03**.
11. Resuspend sample and apply the cell suspension to a cell strainer, mesh size 70 µm, placed on a 50 mL tube.

12. Wash cell strainer with 2×10 mL of RPMI 1640 and centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
13. Suspend single-cell suspensions in cell culture media for expansion or in appropriate buffer for flow cytometry analysis. Healthy culture can be obtained usually within 72 hours after incubation.

Isolation from spleen tissue for disseminated hematological cancer

1. Prepare enzyme mix of the Spleen Dissociation Kit, mouse according to the data sheet. Add 2.5 mL of enzyme mix in Dissociation Buffer per gentleMACS C Tube.
2. Transfer the tissue (one spleen per C Tube) into the C Tube containing the enzyme mix.
3. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
4. Run the gentleMACS Program **m_spleen_02**.
5. After the program finishes, detach C Tube from the gentleMACS Dissociator.
6. Incubate sample for 15 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
7. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **m_spleen_03**.
8. Perform a short centrifugation and aspirate supernatant completely. Resuspend cell pellets and apply the suspension to a cell strainer, mesh size 70 µm, placed on a 50 mL tube.
9. Wash cell strainer with 2.5 mL of Dissociation Buffer and centrifuge cell suspension at 300×g for 10 minutes.
10. Suspend single-cell suspensions in cell culture media for expansion or in appropriate buffer for flow cytometry analysis.

Results

The method is capable of produce high-quality viable cancer cells from tumor xenografts, which can be further purified and directly analyzed. The isolated cells can be put into culture and we typically are able to establish a healthy cell line culture within 72 hours (fig. 1).

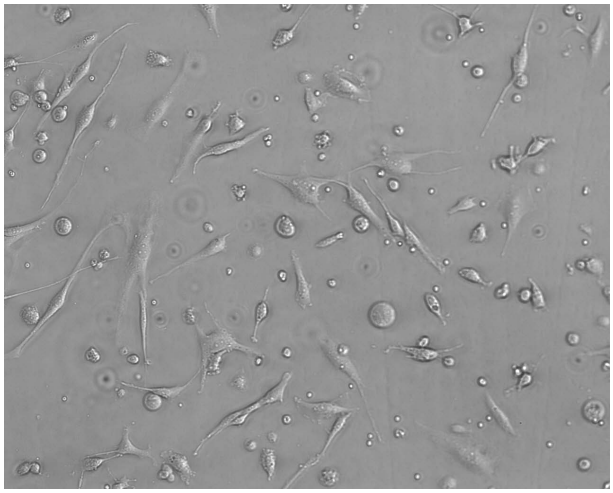

Figure 1: Typical result of cell line derived from tumor xenograft. Tumor fragments were harvested from a nude mice bearing U87 glioblastoma tumor xenograft. Single-cells suspension was prepared using the gentleMACS Dissociator and the Tumor Dissociation Kit. The viable single-cell suspension is capable to establish healthy culture within 24 hours after incubation in media.

Conclusion

Isolation of tumor cells from brain and spleen xenograft samples can be accomplished with ease using the gentleMACS Dissociator.

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