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Customer protocol

Isolation of CD133+ cells from human ovarian tumors using the gentleMACS™ Dissociator

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Background

It has been suggested that tumor regrowth, as well as chemotherapy resistance and metastasis, are dependent on a small sub-population of cancer cells within the tumor that are thought to represent cancer stem cells (CSCs). The presence of CSCs in solid tumors has been proposed for diverse human cancers including breast, colon, pancreatic, prostate, and ovarian cancer. Solid tumor stem cells have been defined as a small subset of cancer cells that constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor. Several cell surface markers, including CD133, have been used to sort populations of putative cancer stem cells from primary tumor cultures or cell suspensions obtained from tumor biopsies.
Cancers of epithelial origin, such as ovarian cancer, feature two distinct cell stages: epithelial and mesenchymal phenotypes. Transitions between epithelial and mesenchymal phenotypes, namely epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET), are reversible and have been accredited pivotal roles during a number of processes including embryonic development, tissue repair, cancer progression, as well as the acquisition of stem cell properties.
We demonstrated that the combined assessment of key antigens associated with cancer stem cells and the epithelial-mesenchymal transition can distinguish the phenotype of ovarian cancer stem cells from more differentiated cells.

This protocol describes our procedure to isolate CD133+ cells from ovarian tumors using the gentleMACS™ Dissociator and MACS® MicroBeads.

Materials and methods

Materials

- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- Centrifuge
- Incubator (37 °C)
- Cell strainer (mesh size 70 µm)
- CD133/2 (293C3)-Biotin, human
- Anti-Biotin MicroBeads
- Digestion buffer (RPMI with 1 mg/mL Collagenase D)
- DNase solution (10 mg/mL)
- $CaCl_2$ solution (100 mM)
- RPMI medium with 10% fetal bovine serum (FBS)
- Phosphate-buffered saline (PBS)
- Versene solution (contains calcium chelating agent EDTA)

Methods

1. Carefully cut tumor biopsies in small pieces of 5 mm.
2. Transfer up to 2 g of tissue into the gentleMACS C Tube containing 5 mL of digestion buffer.
3. Add 5 µL of $CaCl_2$ solution.
4. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program **h_tumor_01** twice.
6. After termination of the program, detach the C Tube from the gentleMACS Dissociator and incubate sample for 20 minutes at 37 °C
7. Attach the C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **h_tumor_02** twice.
8. Repeat steps 6 and 7.
9. After termination of the program, detach C Tube from the gentleMACS Dissociator. Add 5 mL of Versene solution and incubate sample for 20 minutes at 37 °C.
10. Attach the C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **h_tumor_03** twice.

11. Repeat steps 9 and 10.
12. Resuspend sample and apply the cell suspension to a cell strainer (70 µm) placed on a 50 mL tube.
13. Wash cell strainer with 25 mL of RPMI and centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
14. Add 4.5 mL of RPMI and 500 µL of DNase solution (1 mg/mL final solution). Transfer sample into a 15 mL bluecap tube and incubate for 15 minutes at 37 °C.
15. Add 10 mL of RPMI and centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
16. Resuspend cell pellet with an appropriate amount of buffer to isolate CD133$^+$ cells usingCD133/2 (293C3)-Biotin and Anti-Biotin MicroBeads according to the protocol in the data sheet.

Results

In this study, we provide supporting data for one of the key features of CSCs, by demonstrating multipotency, i.e., the ability to give rise to phenotypically heterogeneous daughter cells (with the ability to differentiate into a limited number of different lineages). Our data suggest that in ovarian cancer cells these features are intrinsically linked with the phenomena of EMT and MET.

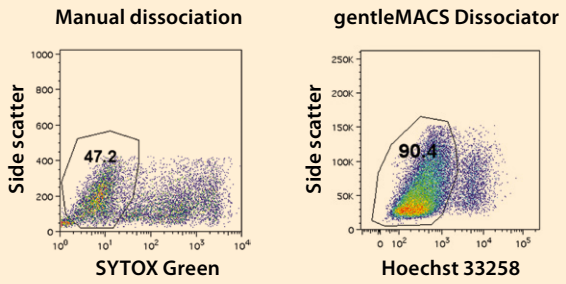

Figure 1: Comparison of ovarian tumor dissociation with a manual method and with the gentleMACS Dissociator. Cells used for transplantation were sorted by flow cytometry and non-viable cells were excluded using SYTOX® Green and Hoechst 33258. Cell viability is increased from around 50% to approminately 90% using the gentleMACS Dissociator.

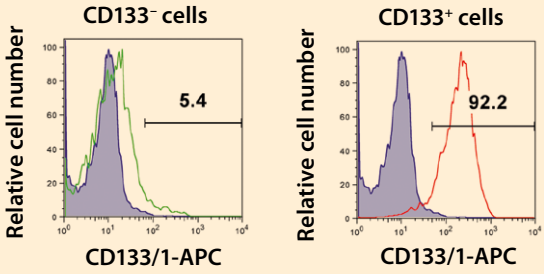

Figure 2: Validation of CD133$^+$ cell fractions obtained by column isolation. Tumor-forming cells are highly enriched in the CD133$^+$ cell fraction. Shown are studies with CD133$^-$ versus CD133$^+$ cells from eight pooled ovc316-X xenografts.

Conclusion

Isolation of CD133$^+$ cells from ovarian tumors can be accomplished with ease using the gentleMACS Dissociator and MACS MicroBeads.

References

1. Strauss, R. *et al.* (2011) Analysis of epithelial and mesenchymal markers in ovarian cancer reveals phenotypic heterogeneity and plasticity cancer. PLoS ONE 6(1): e16186.

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