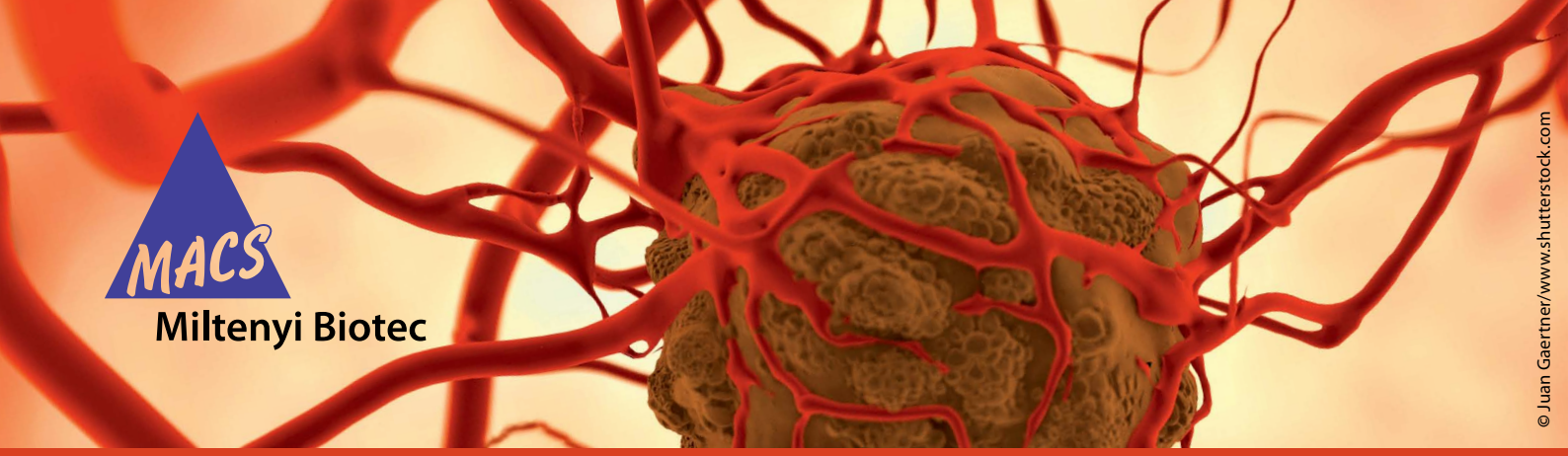

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Isolation, separation, and analysis

Breast cancer stem cells

Isolation of cancer stem cells from primary human breast tumors using the gentleMACS Dissociator™ and magnetic cell separation

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Background

There is a great body of evidence that stem cell–like cells exist in tumors/neoplasms and that they promote tumor initiation, progression, and metastasis. Their resistance against many drugs is of particular interest, since common therapies may not be suitable to eradicate CSCs (cancer stem cells) and may even foster an expansion of the CSC pool. Hence, CSCs surviving chemotherapy may often be responsible for a tumor relapse. It is thought that CSCs can be generated by transformation of adult stem cells as well as by epithelial-to-mesenchymal transition and that they express a certain subset of cell surface markers. In breast cancer, cells expressing high levels of CD44 and low levels of CD24 have been shown to have stem-like activities.[1] Hardt *et al.*[2] performed next generation sequencing- and microarray-based gene expression profiling of $CD44^+$/$CD24^-$/$CD45^-$ breast CSCs isolated from primary $ER\alpha^+$ breast cancer. Due to the fact that the CSC population forms a minor fraction in the tumor, the expression profile of the bulk tumor may mask the expression profile of the CSC population. Therefore, dissociation of human tumor tissue and isolation of the CSC population was required to get insight into the transcriptome of these cells.

This note describes the standard procedure used by Hardt *et al.*[2] to isolate and to separate cancer stem cells from primary human breast tumor using the gentleMACS™ Dissociator and MACS® MicroBeads to allow gene expression profiling of separated cells afterwards.

Materials and methods

Materials

- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- MACSmix™ Tube Rotator in combination with an incubator at 37 °C
- Centrifuge
- Cell strainer (mesh size 70 µm)
- Digest solution
- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution 1:20 with autoMACS® Rinsing Solution. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

Additional requirements for separation and analysis

- CD24 MicroBead Kit, human
- CD44 MicroBeads, human
- CD45 MicroBeads, human
- MACSQuant® Analyzer, MACSQuant Analyzer 10, or MACSQuant VYB
- µMACS™ SuperAmp™ Kit

For a detailed protocol, please refer to the respective data sheet.

Methods

1. Cut tumors into small pieces of 2–4 mm.
2. Transfer the tissue into the gentleMACS C Tube containing 5 mL of digest solution.
3. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
4. Run the gentleMACS Program **h_tumor_01**.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator.
6. Incubate sample for 30 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 **h_tumor_01**.

8. After termination of the program, detach C Tube from the gentleMACS Dissociator and incubate sample for 30 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
9. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **h_Tumor_01.**
10. Resuspend sample and apply the cell suspension to a cell strainer, mesh size 70 µm, placed on a 50 mL tube.
11. Centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant in PEB buffer for magnetic cell separation or flow cytometry using the MACSQuant Analyzer. For details on the sorting procedure, please refer to Hardt *et al.*[2]

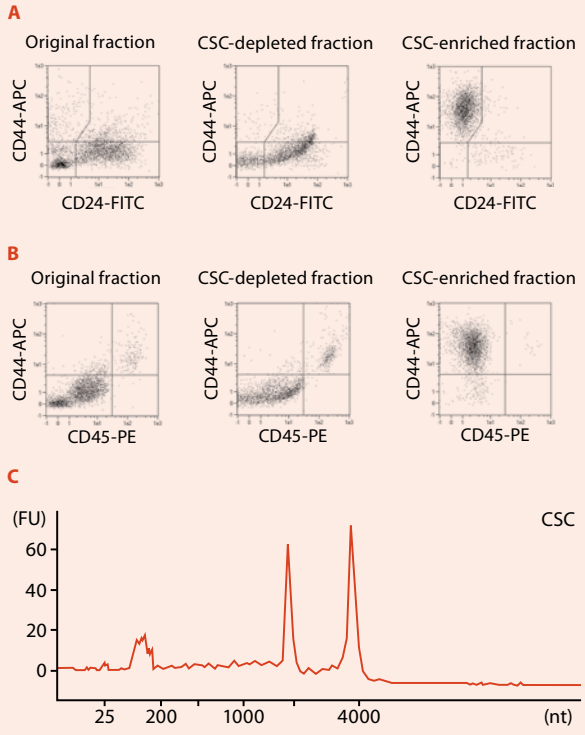

Figure 1: Flow cytometric phenotyping of cells before and after MACS-based cell sorting and analysis of RNA quality
Before and after MACS-based cell sorting all fractions were analyzed for CD44-, CD24-, and CD45-expressing cells by flow cytometry using the MACSQuant Analyzer. It could be shown that CD44$^+$/CD24$^-$/CD45$^-$ breast cancer cells from an invasive ductal carcinoma were isolated at a purity of 94% and efficiently depleted from the bulk fraction (A,B). Four to six thousand cells were used for flow analysis, dependent on the availability of material. Debris was excluded by forward scatter/side scatter (FSC/SSC) gating; dead cells were excluded from the analysis by gating off propidium iodide–positive (PI$^+$) events. Analysis of RNA integrity after dissociation and sorting showed no signs of degradation (C). Abbreviations: FU = fluorescence units; nt = nucleotide length.

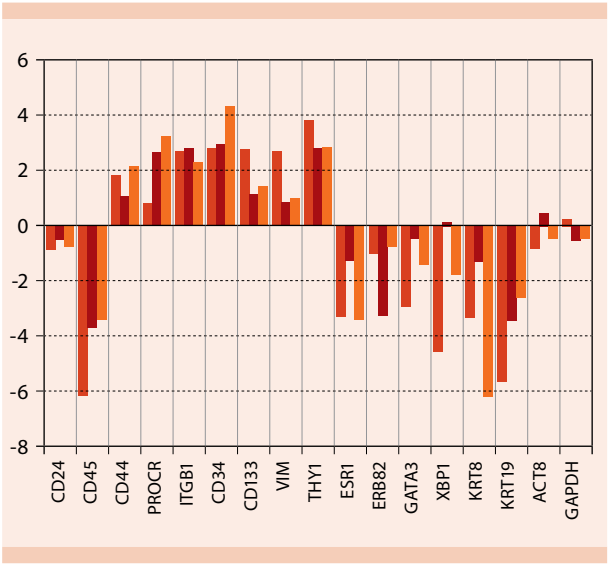

Figure 2: Reproducibility of NGS
Three replica (three different cDNAs from the same RNA) were analyzed by NGS for a number of selected genes. Each bar represents the log2 expression ratio of the CD44$^+$/CD24$^-$/CD45$^-$ fraction relative to the control fraction for one replicate of NGS mRNA quantification.

Results

In this study, Hardt *et al.* performed next generation sequencing- and microarray-based gene expression profiling of CD44$^+$/CD24$^-$/CD45$^-$ breast CSCs isolated from primary ERα$^+$ breast cancer. Besides overexpressing genes involved in maintenance of stemness, the CSCs showed higher levels of genes that drive the PI3K pathway. This suggests that, in CSCs of ERα$^+$ breast cancer, the PI3K pathway that is involved in endocrine resistance is hyperactive.

Conclusion

Isolation of cancer stem cells from primary human breast tumor can be accomplished with ease using the gentleMACS Dissociator in combination with magnetic cell separation.

References

1. Al-Hajj, M. *et al.* (2003) Prospective identification of tumorigenic breast cancer cells. PNAS 100: 3983–3988.
2. Hardt, O. *et al.* (2012) Highly sensitive profiling of CD44$^+$/CD24$^-$ breast cancer stem cells by combining global mRNA amplification and next generation sequencing: evidence for a hyperactive PI3K pathway. Cancer Lett. 325: 165–174.

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