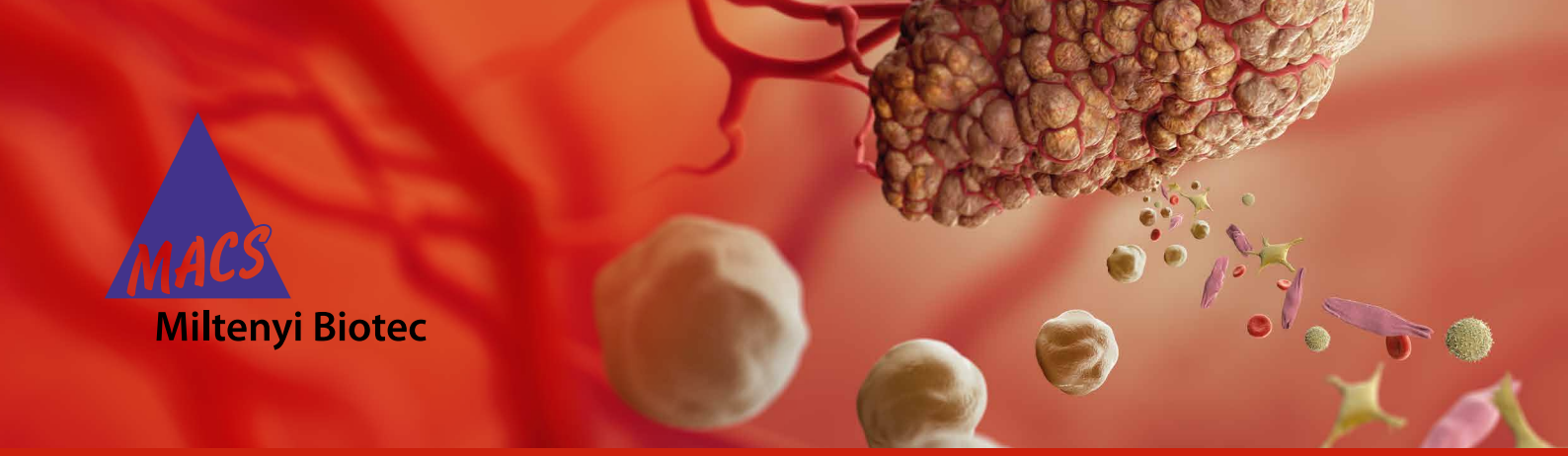

Isolation and analysis of tumor cells from human solid tumor tissue extracted by needle biopsy

Isolation and analysis of tumor cells from various human solid tumor tissues extracted by needle biopsy

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Background

Comprehensive molecular analysis of tumor tissue samples is often limited by the amount of tissue available as well as the intratumoral heterogeneity. Especially needle biopsies yield only small amounts of tissue, which in many cases do not suffice for dissociation and detailed flow cytometric analysis. Additionally, isolation of certain cell populations from biopsy samples for further analysis or cultivation has been extremely challenging, as most workflows and protocols require relatively high amounts of tissue and cells. Another major hurdle is the quality of the cellular material extracted by needle biopsy as this technique often leads to tissue preparations that do not appropriately represent the cellular composition of the patient tumor. Moreover, needle biopsies can lead to lesions within the tissue or the sample. Among the different biopsy methods and needles available, Spirotome™ Biopsy Needles (Bioncise) have several benefits for sample extraction from various tissues, including high yield[1,2]. This is of fundamental importance as a relatively large amount of tissue sample is required for optimal tissue dissociation and subsequent cell isolation, and eventually for representative and reliable results in molecular downstream analyses. Although Spirotome Biopsy Needles allow for higher tissue yields, the size of the incision is not larger compared to other biopsy methods. In addition, the design of the needle reduces mechanical stress and thus diminishes the occurrence of lesions within tissues.

This application note describes a complete workflow ultimately enabling the isolation and analysis of tumor cells from primary breast cancer samples as well as disseminated tumor cells from lymph nodes obtained by biopsy. The workflow includes i) tissue biopsy obtained with Spirotome Needles, ii) dissociation of the solid tumor tissue into single-cell suspensions using the gentleMACS™ Dissociator in combination with the Tumor Dissociation Kit, human and iii) specific isolation of tumor cells using the Tumor Cell Isolation Kit, human. The isolated cells are suitable for different downstream analyses, e.g., flow cytometry, cell culture, or molecular analyses.

Materials and methods

Materials for biopsy and tissue dissociation

- Spirotome Biopsy Needles (Bioncise): The Spirotome Device is a macrobiopsy instrument developed by Bioncise (www.bioncise.com) to offer high-quality biopsies to the medical community to facilitate research in molecular biology, personalized medicine, regenerative medicine, and tissue engineering. Sample size ranges from 50 (14 G) up to 300 mg (8 G). Clinical scientific studies endorse efficacy, safety, and comfort to the patient.[1–4] The system is provided ready-to-use in steam-sterilized pouches and available worldwide.
- Tumor Dissociation Kit, human (# 130-095-929)
- RPMI 1640
- gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-096-334)
- Benchtop centrifuge (300×g required)
- MACS® SmartStrainers (70 µm) (# 130-110-916)
- 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 (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

Materials for tumor cell isolation and flow cytometry

- Tumor Cell Isolation Kit, human (# 130-108-339)
- MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant VYB (# 130-096-116)
- Fluorochrome-conjugated anti-human REAfinity™ Recombinant Antibodies, e.g., CD31-FITC (clone REA730; # 130-110-668); CD44-VioBlue® (clone REA690; # 130-110-298); CD45-FITC (clone REA747; # 130-110-631); CD45-PE-Vio® 770 (clone: REA747; # 130-110-634); CD90-PE (clone REA897; # 130-114-860); CD235a (Glycophorin A)-FITC (clone REA175; # 130-100-266); CD235a (Glycophorin A)-VioBlue, (clone REA175; # 130-100-256); CD326 (EpCAM)-APC (clone REA764; # 130-111-000)

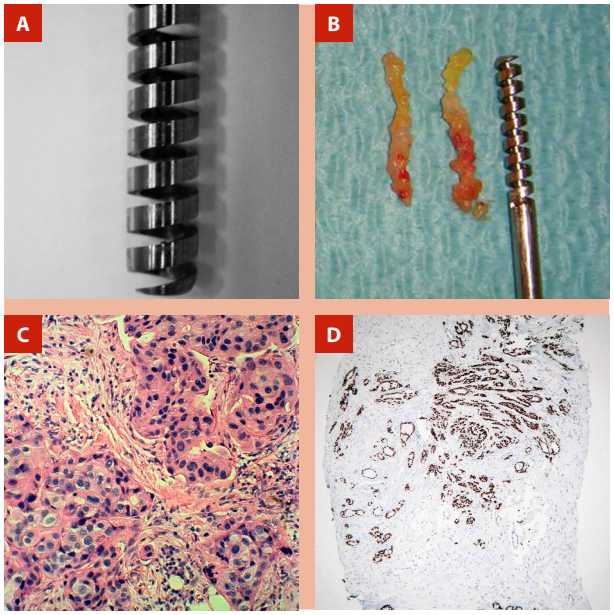

Figure 1: (A) Spirotome Biopsy Needle with a cutting helix that works in conjunction with a cutting cannula. (B) Extracted tissue samples. (C) Hematoxylin and eosin staining of tissue slices from a breast tumor for microscopic analysis. (D) Immunohistochemistry of the estrogen receptor in a breast tissue sample.

Tumor biopsies

Biopsies were performed with Spirotome® Needles (Bioncise). In this 10-gauge system the coaxial needle also serves as cutting needle. Examples of extracted tissue samples are depicted in figure 1.

Tumor dissociation and analysis

Tumor samples taken with Spirotome Needles were dissociated into single-cell suspensions, using the Tumor Dissociation Kit, human and the gentleMACS Octo Dissociator with Heaters. Notably, instead of 5 mL digest solution only 2.5 mL were used per sample. As the length of the extracted tissue samples slightly varied, they were cut into 3–5 pieces depending on their length. All other steps were carried out according to the protocol included in the kit. For flow cytometry, the cells were stained with fluorochrome-conjugated antibodies as specified in the respective figures, according to the protocols delivered with the antibodies.

Tumor cell isolation

Single-cell suspensions were magnetically labeled using the Non-Tumor Cell Depletion Cocktails contained in the Tumor Cell Isolation Kit, human, according to the protocol included in the kit. These cocktails label non-tumor cells and thus enable the isolation of unlabeled tumor cells.

Tumor cell culture

Tumor cells isolated from breast cancer samples were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine and Penicillin/Streptomycin for 3 weeks.

Results

Analysis of tissue sample obtained with Spirotome™ Biopsy Needles

A sample of a submandibular lymph node from a patient with known metastatic malignant melanoma was dissociated and analyzed for the presence of tumor cells (fig. 2). Besides high amounts of leukocytes (approx. 58%), erythrocytes, endothelial cells (approx. 2%), and fibroblasts (approx. 5%), a major cell population positive for CD44, but negative for CD31 (endothelial cells), CD90 (fibroblasts), leukocytes (CD45), or CD235a (GlyA; erythrocytes), was identified (fig. 2F, upper left quadrant). This population represents tumor cells present in the lymph node.

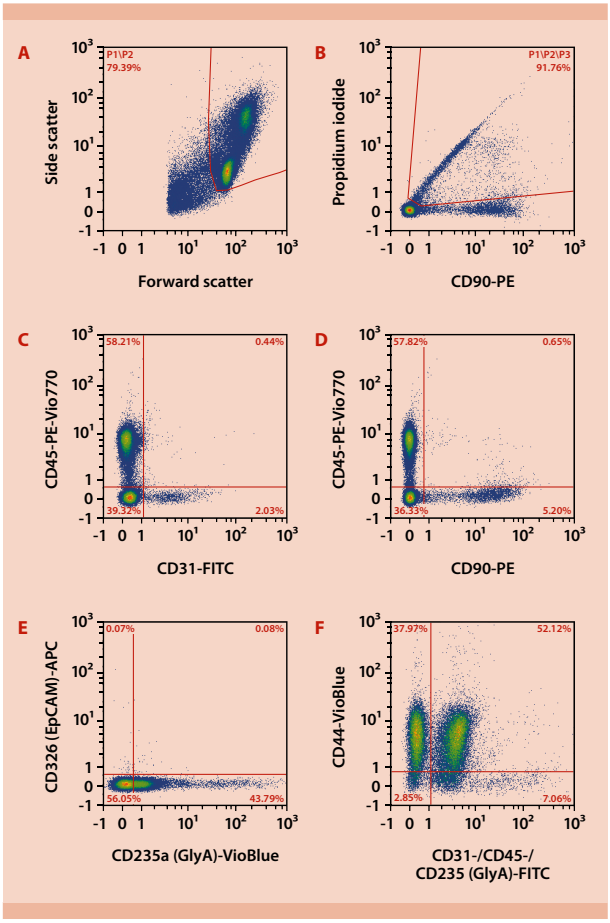

Figure 2: Flow cytometric analysis of dissociated submandibular lymph node from a patient with known metastatic malignant melanoma. First, debris was excluded from the analysis (A), then dead cells were excluded by using propidium iodide staining (B) and finally the cellular composition of the sample was assessed (C–F) by using CD45, CD31, CD90, CD235a (GlyA), CD44, and CD326 (EpCAM) antibodies.

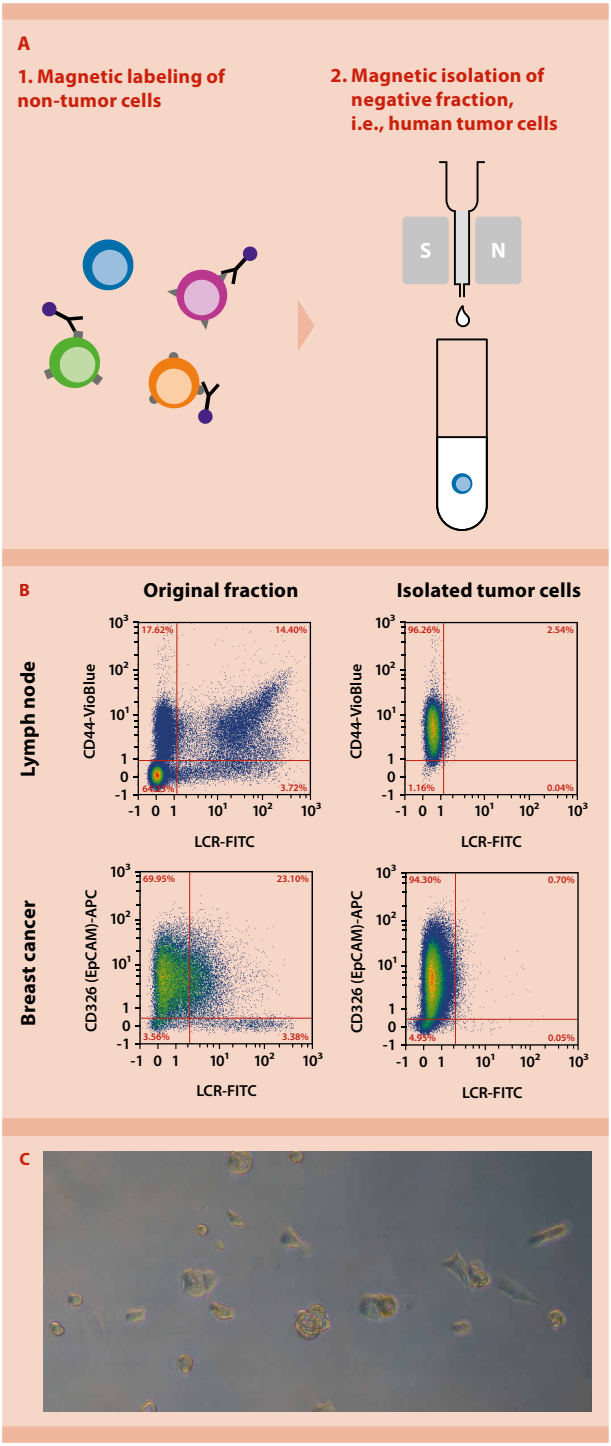

Figure 3: Principle of the Tumor Cell Isolation Kit, human (A). The density plots display non-tumor cells indirectly labeled with Labeling Check Reagent (LCR; # 130-095-226) vs. $CD44^+$ or $CD326\ (EpCAM)^+$ tumor cells (B). Shown are the original fraction prior to cell isolation (B, left panel) and isolated tumor cells (B, right panel), for a lymph node sample from a patient with known metastatic malignant melanoma (B, upper panel) and a human breast cancer sample (B, lower panel). Cells isolated from the breast cancer sample were cultivated for 3 weeks (C).

Isolation of tumor cells from tissue samples obtained with Spirotome™ Biopsy Needles

Tissue samples were obtained using Spirotome™ Biopsy Needles from i) a submandibular lymph node (0.12 g) from a patient with known metastatic malignant melanoma and ii) a primary breast tumor (0.21 g). Tissues were dissociated and tumor cells were then isolated based on MACS® Technology (fig. 3A). The sample from the breast cancer biopsy mainly consisted of tumor cells expressing EpCAM (approx. 70%; fig. 3B, lower panel), whereas the lymph node sample contained only few CD44-positive tumor cells (approx. 17%; fig. 3B, left panel). From both samples, tumor cells were isolated to a purity of >95%, regardless of the starting frequency of tumor cells.

Conclusion

- Biopsies performed with Spirotome Needles yielded cell numbers that were sufficient for downstream flow cytometry analysis, MACS® Technology–based cell isolation, and even cultivation of isolated tumor cells.
- Despite the use of small biopsy samples (0.06–0.21 g), tissue dissociation based on the gentleMACS™ Octo Dissociator with Heaters in combination with the Tumor Dissociation Kit, human resulted in single-cell suspensions with high viability.
- Moreover, the Tumor Cell Isolation Kit, human yielded highly pure tumor cells even from samples with low total cell numbers and low starting frequencies of tumor cells as highlighted by the results from the lymph node sample.

References

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