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Isolation and separation

Tumor-infiltrating lymphocytes

Isolation of lymphocytes from head and neck tumor samples

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Background

Head and neck squamous cell carcinoma (HNSCC) is a cancer entity that serves as a model of inflammation-associated carcinogenesis and tumor progression. Surgery, chemotherapy, and radiation are currently the major options for HNSCC treatment. All three induce local or systemic inflammation triggered by tissue injury and cancer cell death.
Accumulating evidence indicates that tumor cells release damage-associated molecular patterns (DAMPs) to impair tumor-directed immune activity which induces tolerance in order to foster tumor-escape mechanisms.
An interesting member of the DAMP family is the evolutionarily conserved nuclear protein, high mobility group box 1 (HMGB1). HMGB1 is present in the nucleus and cytoplasm of nearly all cell types and acts as a danger signal by active secretion from living inflammatory cells, or as an inflammatory mediator by passive release from necrotic or stressed cells.

This note describes the procedure used by Wild *et al.*[1] to isolate and separate tumor-infiltrating lymphocytes using the gentleMACS™ Dissociator in combination with the Tumor Dissociation Kit.

Materials and methods

Materials

- Tumor Dissociation Kit, human
- RPMI 1640
- 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)

Methods

1. Prepare enzyme mix of the Tumor Dissociation Kit, human by adding 100 µL of Enzyme H, 500 µL of Enzyme R, and 25 µL of Enzyme A to 4.4 mL of RPMI 1640.
2. Cut biopsies in small pieces of 2–4 mm.
3. Transfer the tissue into the gentleMACS 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 termination of the program, 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 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.
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 20 mL of RPMI 1640 and centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.
13. Resuspend cells in appropriate buffer for flow cytometry.

Results

The data of the present study suggest a role for tumor-derived HMGB1 in the interaction with regulatory T cells (Tregs) in patients with HNSCC and provide evidence for a novel role of HMGB1 in Treg-mediated tumor escape.

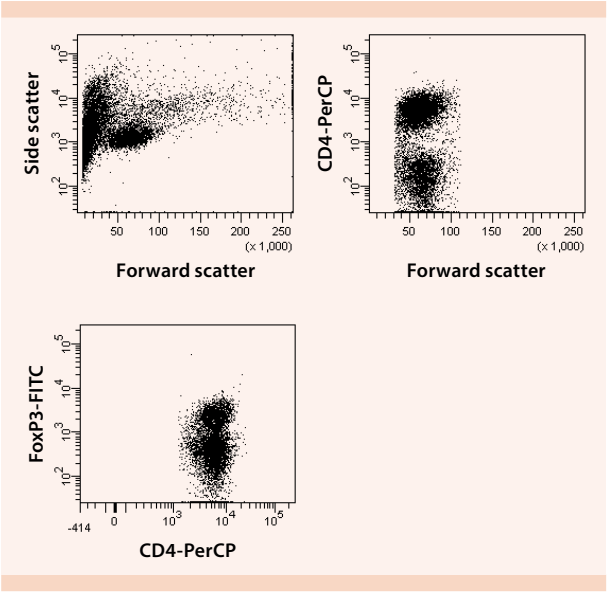

Figure 1: Tumor-infiltrating lymphocytes from a HNSCC patient.

Conclusion

Isolation and separation of tumor-infiltrating lymphocytes from head and neck tumor samples can be accomplished with ease using the gentleMACS™ Dissociator.

Reference

1. Wild, C. *et al.* (2012) HMGB1 is overexpressed in tumor cells and promotes activity of regulatory T cells in patients with head and neck cancer. Oral Oncology 48: 409–416.

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