Dissociation of head and neck tumor samples for the analysis of tumor-infiltrating lymphocytes

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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 dissociate head and neck tumor tissue with the gentleMACS™ Dissociator and the Tumor Dissociation Kit for subsequent analysis of tumor-infiltrating lymphocytes.

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 Solution 1, 500 µL of Solution 2, and 25 µL of Solution 3 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 tissue dissociation process based on the gentleMACS™ Dissociator and the Tumor Dissociation Kit, human preserves cell surface epitopes so that subpopulations of tumor-infiltrating lymphocytes (TILs) from HNSCC samples can be easily identified by flow cytometry. The data shown here demonstrate the presence of CD4+FoxP3+ regulatory T (Treg) cells as part of the TIL population. The data of the present study suggest a role for tumor-derived HMGB1 in the interaction with Treg cells in patients with HNSCC and provide evidence for a novel role of HMGB1 in Treg cell–mediated tumor escape.

Conclusion

Dissociation of head and neck tumor tissue samples can be accomplished with ease using the gentleMACS Dissociator. The resulting single-cell suspensions are appropriate for the detailed analysis of TILs including Treg cells.

Reference


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