Generation of autologous tumor lysates using the gentleMACS™ Dissociator

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Background
Dendritic cells (DCs) present antigens (Ags) to naive and memory T lymphocytes. DCs that artificially present tumor Ags, either in the form of defined protein sequences or as antigenic material obtained from autologous tumor cells, are efficacious antitumor vaccines for mouse-transplanted tumors. Many DC-based clinical trials have been performed in cancer patients with evidence of increased immune responses and clinical activity, but efficacy is unfortunately lower than that observed in mouse models. A key difference might be that cancer patients with bulky disease present multiple immunosuppressive regulatory mechanisms, such as the augmentation of regulatory T cells.

Alfaro et al. tested the safety and biological activity of immunotherapy based on type 1 DC, that are powerful producers of IL-12, induce immune responses dominated by Th1 and cytotoxic T lymphocytes and are also potent activators of NK cells, in advanced cancer patients. The combination strategy was based on preclinical data that were developed in mouse models. Innovative elements include the stimuli used to induce type 1 DC maturation and a step in which the autologous tumor lysates are preheated to denature thermolabile proteases and immunosuppressive factors that otherwise reduce the suitability of tumor lysates for loading tumor Ags into DCs. This protocol describes the procedure to generate autologous tumor lysates using the gentleMACS™ Dissociator.

Materials and methods

Materials
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- Thermoblock and liquid nitrogen
- Lysis buffer
- (Optional) AIM-V® Medium, Liquid

Methods
1. Transfer 1–2 g of needle tumor biopsies or surgical samples into the gentleMACS C Tube containing 2 mL of lysis buffer.
2. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
3. Run twice the gentleMACS Program h_tumor_01.
4. Transfer tumor lysates to a microcentrifuge tube (Eppendorf® 2 mL Safe-Lock Tube) and freeze samples.
5. Treat tumor lysates by five rounds of thawing/freezing in liquid nitrogen and 10 Gy irradiation with a 5 minutes heating step at 100 °C using a thermoblock during the first thawing step.
6. Quantify the amount of protein in the sample using a Bradford assay.
7. Store tumor lysates at −20 °C.
8. Alternatively continue with DC loading (300×10⁶ cells) with 100–200 µg/mL protein during 2 hours at 37 °C in 5 mL of AIM-V Medium, Liquid.
Results

In summary, we developed a DC vaccination strategy that incorporated several novel elements. The treatment was feasible, well tolerated, and induced cellular immunity, as well as several relevant biological effects, including reduction of Tregs, high circulating concentrations of IL-12p70, and decreases in circulating tumor cells (CTC) and circulating endothelial cells (CEC). The decrease in the number of CEC and CTC supports that this strategy might be especially relevant in the setting of minimal residual disease.

Figure 1: Silver-stained SDS-PAGE analyses of tumor lysates obtained from CT26 mouse obtained following five freezing/thawing cycles with or without heating to 100 °C in the first thawing cycle. Overlay profiles show the differences in protein bands. Green and red arrows mark the highest differences between the electrophoresis profiles with and without heating to 100 °C. Bands enriched with heating are marked with red arrows, whereas those that predominate in the nonheated samples are marked with green arrows.

Conclusion

Generation of autologous tumor lysates can be accomplished with ease using the gentleMACS Dissociator.

References


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