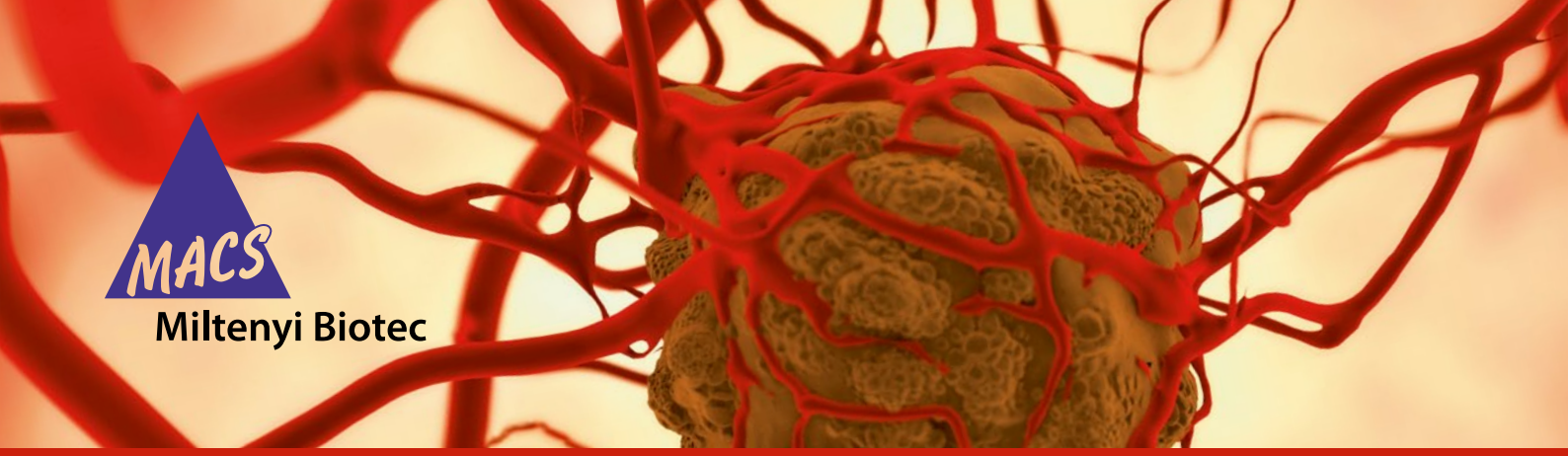

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Isolation and separation

Depletion of mouse cells

Depletion of mouse cells from dissociated patient derived xenograft tumors using the gentleMACS™ Octo Dissociator with Heaters, followed by magnetic cell separation

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Background

Patient derived xenografts (PDXs) are a very useful mouse model for drug development studies because they have been shown to provide a faithful representation of the patient's original tumor both immunohistochemically and genetically as well as in terms of response to common therapeutics. They also offer a real representation of the heterogeneity of tumors and they maintain at least some aspects of the human microenvironment for weeks with the complete substitution with murine stroma occurring only after 2–3 passages in mouse[1]. However, after more than three passages in mouse, these models provide tumor samples which are "contaminated" by a variable amount of murine stromal cells. DNA analysis, i.e NGS on tumor DNA before and after treatments, should be performed on DNA samples as purified as possible from murine contaminations to obtain reliable results.

This note describes the standard procedure used by Moro *et al.*[1] to isolate and to separate mouse cells from xenograft tumors using the gentleMACS Octo Dissociator with Heaters and the Mouse Cell Depletion Kit.

Materials and methods

Materials

- Antibiotic-containing buffer: Prepare a solution containing phosphate-buffered saline (PBS) 1×, 200 U/mL penicillin, and 200 µg/mL streptomycin.
- gentleMACS Octo Dissociator with Heaters
- Tumor Dissociation Kit, human

Additional requirements for separation

- Mouse Cell Depletion Kit
- autoMACS® Pro Separator

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

Methods

PDX engraftment

Samples of primary non-small-cell lung cancer (NSCLC) have been obtained from patients undergoing surgical resection.

1. Cut samples into small pieces of 25–30 mm^3 in antibiotic-containing buffer.
2. Implant subcutaneously in the flank region of 4 to 6 weeks old female nude or SCID anesthetized mice.
   Note: Animals are anesthetized with an intraperitoneal injection of ketamine/xylazine/saline mixture (20 : 2.5 : 77.5 v/v/v) at a dose of 10 mL/kg body weight. Fragments are then implanted using a trocar gauge and mice are maintained in rooms with constant temperature and humidity.

Mouse cell depletion

PDX samples have been dissociated using the Tumor Dissociation Kit, human in combination with the gentleMACS Octo Dissociator with Heaters according to the protocol. Cell suspensions have been magnetic labeled with the Mouse Cell Depletion Kit and separated using the **Depletes** program on the autoMACS Pro Separator according to the protocol.

Results

Mouse cell depletion – PDX

Three PDX models have been treated with the Mouse Cell Depletion Kit after disaggregation of the tumor sample with the Tumor Dissociation Kit, human. Cells were analyzed to determine the murine fraction before and after the mouse cell depletion by flow cytometric analysis (murine HLA). In all tested models the amount of murine cells after

depletion was reduced to less than 1% (fig. 1), in particular for PDX187 murine fraction was depleted from 23.3% to 0.12%; for PDX323 from 5.33% to 0.16% and for PDX258 from 8.18% to 0.24% (fig. 1 A, B).

Mouse cell depletion – Lung

Lungs of PDX bearing mice have been treated with the Mouse Cell Depletion Kit after disaggregation of the tumor sample with the Tumor Dissociation Kit, human. The very rare cells disseminated from the subcutaneous PDX are difficult to identify by flow cytometric analysis (fig. 2). After mouse cell depletion the number of murine cells was highly reduced, making the identification of rare disseminated cells much easier. Furthermore, the depletion of murine lung cells from the samples obtained by the dissociation of poorly disseminated lungs can facilitate culturing of disseminated cells from these samples.

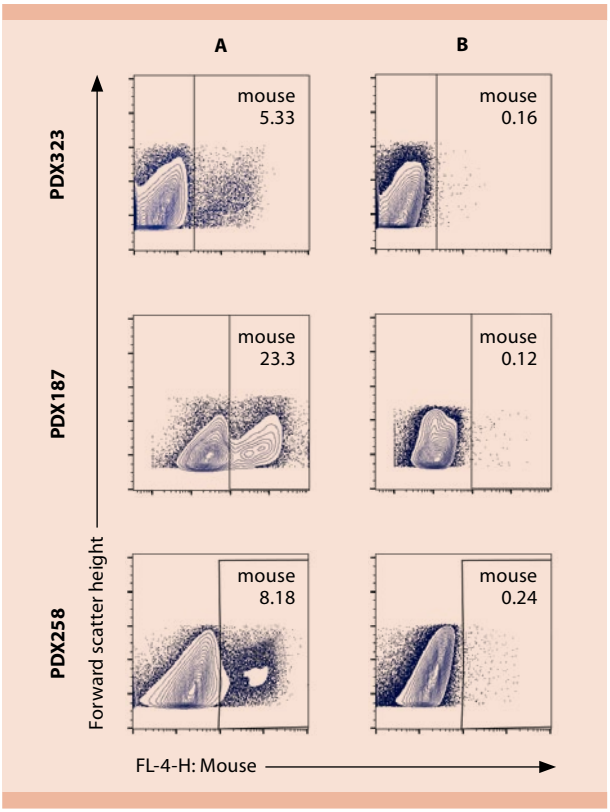

Figure 1: Depletion of murine stromal cells from three PDX models using the Mouse Depletion Kit. PDX maintain the stromal structure of the tumor of origin, recruiting murine stromal cells (A). After depletion with the Mouse Depletion Kit, the amount of murine cells was dramatically reduced (less than 0.25%) for all tested models (B).

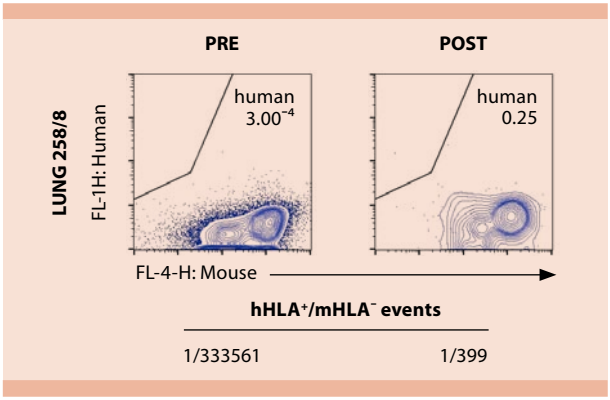

Figure 2: Depletion of murine cells from lungs of PDX carrying mice using the Mouse Depletion Kit. PDX are able, with very low frequency, to disseminate to lungs. After depletion with the Mouse Depletion Kit, the amount of murine cells was dramatically reduced making the identification of the rare disseminated cells easier and clearer.

Conclusion

The presented data show that the Mouse Cell Depletion Kit is suitable for separating mouse cells from human NSCLC tumor cells in PDX models. The depletion of the murine cells is almost complete and more effective than a CD326 (EpCAM)–dependent enrichment of human tumor cells. Moreover, the Mouse Cell Depletion Kit has proven useful in identifying very rare lung disseminated cells from subcutaneous PDX.

References

1. Moro, M. *et al.* (2012) Patient-derived xenografts of non small cell lung cancer: resurgence of an old model for investigation of modern concepts of tailored therapy and cancer stem cells. J. Biomed. Biotechnol. 2012: 568567.

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