Depletion of mouse cells from human tumor xenografts significantly reduces bias in molecular analysis and improves culture of target cells

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Introduction

Human tumor xenografts represent the gold standard method for research areas like drug discovery, cancer stem cell biology, and metastasis prediction. Xenografts can be derived from primary human tumor material, serially transplanted tumor tissue, or cultured cells. When compared to *in vitro* cell culture models, human tumor xenografts show a higher validity for most assays[1].
During the growth phase *in vivo*, xenografted tissue is vascularized and infiltrated by cells of mouse origin, including heterogeneous lymphocyte subpopulations, fibroblasts, and endothelial cells. The level of infiltration is highly dependent on multiple factors like tumor subtype, growth rate, and region of transplantation. However, even when these factors are kept constant, the amount and composition of infiltrating mouse cells are highly variable, which makes accurate molecular downstream analyses difficult. The contaminating mouse cells lead to i) cross-hybridization of mouse-derived molecules to human probes on microarrays and ii) a significant reduction of sensitivity caused by measurement of mouse signals during next-generation sequencing or proteome analysis[2]. In addition, the culture of human tumor cells is frequently hampered by murine fibroblasts overgrowing the target cells.

Results

1 Establishing an antibody cocktail recognizing all mouse cells across multiple tissues

A screening was performed to determine a combination of antibodies recognizing all mouse cells across multiple organs, including skin, lung, brain, kidney, and skeletal muscle, all of which represent target tissues for xenotransplantation. A crucial point when using cell surface epitopes for screening is to use a gentle procedure and pure enzymes for tissue dissociation to avoid degradation of the target molecules. In this study, all tissues were dissociated in a fully automated way by using the gentleMACS™ Octo Dissociator with Heaters and the respective tissue dissociation kits, which are optimized for epitope preservation. Combinations of antibodies recognizing, e.g., mouse CD45 and MHC class I epitopes have already been used to deplete mouse cells after xenotransplatation[3]. However, using these marker combinations, only a subset of mouse cells could be detected in all dissociated tissues analyzed, including skin (A) and lung (B), even after lysis of red blood cells. In contrast, the pan-mouse cocktail (Anti-Mouse-APC) resulting from our screening recognized all cells of mouse origin, including red blood cells, irrespective of the tissue of origin (A, B, and data not shown).

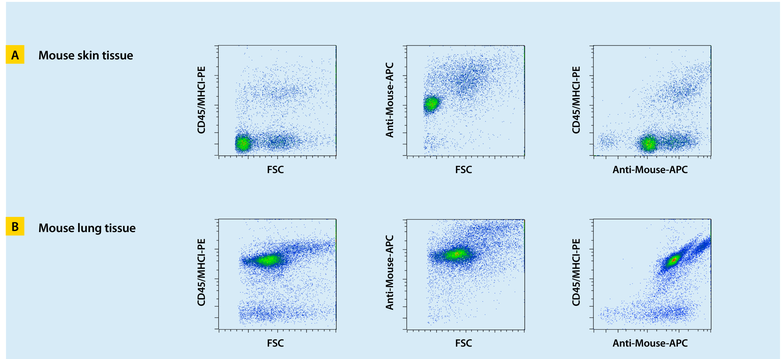

Figure 1

2 Reliable and fast depletion of mouse cells

Upon determining the best antibody combination, conjugates of these antibodies with superparamagnetic nanoparticles (MicroBeads) were generated. The conjugates were used to develop an optimized protocol for the depletion of mouse cells from human tumor xenografts by magnetic separation. It was possible to eliminate >99% of the contaminating mouse cells in less than 20 min, even when the frequency of contaminating cells was around 80%. Cell fractions were labeled with the pan-mouse antibody cocktail and an antibody against human CD326 (EpCAM).

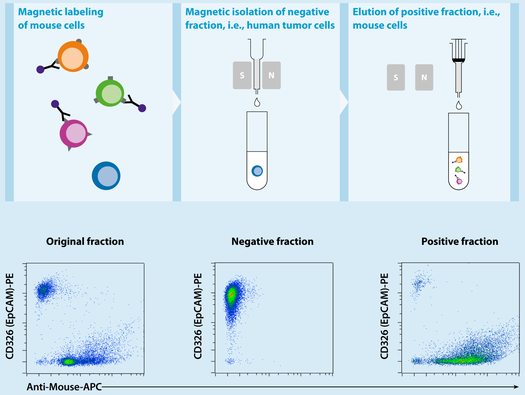

Figure 2

3 Depletion of mouse cells improves downstream culture of human tumor cells

The culture of human tumor cells from xenografts is frequently hampered by mouse fibroblasts because the fibroblasts attach and expand more efficiently, thereby overgrowing the target cells. Even when the target cells attach and grow well, *in vitro* cell culture assays (e.g. drug cytotoxicity testing) are problematic since mathematical correction for effects originating from contaminating mouse cells is impossible in most cases. Upon magnetic separation, the negative (B) and positive fractions (C) were cultured for three days, fixed, and stained for a mouse-specific fibroblast marker (vimentin) and the human-specific tumor marker CD326 (EpCAM). The unseparated original fraction served as a control (A). Even after three days of culture, a nearly pure population of human tumor cells was observed in the negative fraction (B). Only a minor portion of target cells was lost to the positive fraction (C).

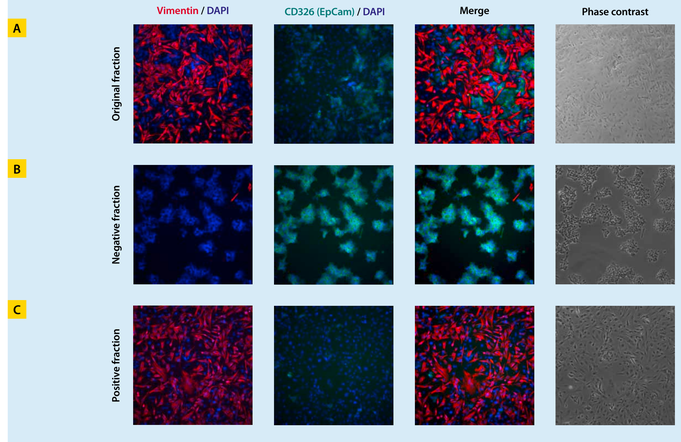

Figure 3

4 Depletion of mouse cells from xenografts is a prerequisite for downstream isolation of tumor subpopulations like cancer stem cells

The isolation of pure tumor subpopulations, such as cancer stem cells (CSCs), is a prerequisite to analyze the cells' functional and molecular characteristics, e.g., by microarray-based expression profiling or next generation sequencing. However, after a subpopulation has been isolated from the human cell fraction, mouse cells still contaminate the negative fraction, which leads to biased results in all major types of downstream analysis. To circumvent this drawback, a separation strategy was developed that yields human cell populations free from contaminating mouse cells. To isolate CSCs from human breast cancer xenograft tumors, for example, tumor tissue was dissociated using the gentleMACS Octo Dissociator with Heaters in combination with the Tumor Dissociation Kit, human, followed by removal of mouse cells in a single step (A). Subsequently, a second sort was performed using the CSC marker, resulting in pure human CSC and non-CSC subpopulations (B).

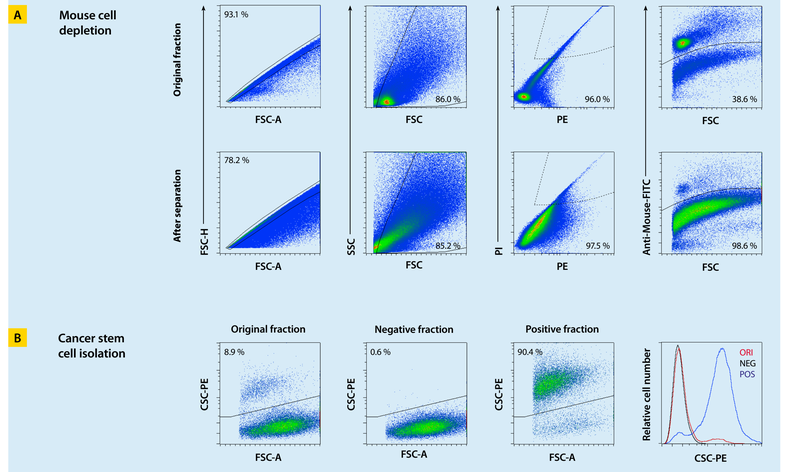

Figure 4

Conclusion

- We have developed an easy and fast (<20 min) cell separation method, which allows for accurate downstream analysis of human tumor xenografts, avoiding bias caused by contamination with mouse cells.
- The contaminating mouse cells are specifically labeled prior to their depletion from the dissociated xenograft tissue. Labeling of the human cells is not required. Therefore, the procedure can be used for the isolation of all kinds of xenografted human material without the need for a positive marker expressed on the human cells.

References
1. Rubio-Viqueira B and Hidalgo, M. (2009) Clin. Pharmacol. Ther. 85: 217–221.
2. Wong, S.Q. *et al.* (2013) Sci. Rep. 3: 3494. DOI: 10.1038/srep03494.
3. Zhang, C.C. *et al.* (2013) Stem Cells Transl. Med. 2: 233–242.

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