Efficient derivation and expansion of tumor cell lines from primary and xenotransplanted ovarian and renal tumors

Permanent abstract number: 3714

David Agorku[1], Anne Langhammer[1], Franziska Zickgraf[2], Felix Geist[2], Elisa M. Noll[2], Christian Eisen[2], Andreas Bosio[1], Martin R. Sprick[2], Andreas Trumpp[2], and Olaf Hardt[1]
[1] Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
[2] Heidelberg Institute for Stem Cell Technology and Experimental Medicine, Heidelberg, Germany

Introduction

Cancer cell lines are widely used as *in vitro* models to study tumor biology and for efficacy testing of novel anti-cancer therapeutics. In the past, most of this work has been done using classical cell lines that have been cultured for decades. However, extensive *in vitro* propagation has been shown to i) lead to multiple genetic and epigenetic alterations in cancer cell lines, ii) reduce the cellular heterogeneity present in the parental tumor, and iii) tend to reduce tumor-initiating as well as multilineage differentiation capacity of propagated tumor cells. Consequently, "classical" tumor cell lines only insufficiently represent the characteristics of a tumor[1]. Therefore, *de novo* cell lines are derived from primary tumor biopsies and used at low passage numbers. However, this process has been shown to be inefficient for most tumor entities. In addition, most "homebrew" media include large amounts of largely undefined serum, which has been shown to exert a selective pressure. This ultimately leads to a reduction of clonal heterogeneity in primary tumor cell cultures and induces a differentiated state when used over multiple passages.

We developed advanced serum-free media for derivation and expansion of tumor cell cultures from pancreatic[2], ovarian, or renal tumors. Our new TumorMACS Media were optimized for each tumor entity with regard to formulation, stability, and usability. In this study, we used our tumor-specific media to generate primary cell cultures and lines from both primary tumors from patients as well as xenotransplanted renal and ovarian tumors. Properties of the generated cell cultures propagated in our TumorMACS Media closely resembled essential characteristics of the parental tumor, including expression of subtype-specific markers, cellular heterogeneity, genetic as well as epigenetic signatures, and tumorigenic potential.

Results

1 Standardized workflow for deriving primary cell lines from solid tumors

Derivation of primary cell cultures from solid tumor material involves multiple critical handling steps. To improve this complex procedure, we developed a workflow allowing for rapid and flexible tissue preparation for subsequent derivation of primary tumor cell cultures using our novel TumorMACS Media (fig. 1). The workflow covers sample storage, optimized tissue dissociation, isolation of tumor cells, flow cytometry analysis, and cultivation.

It enables highly standardized cell line derivation and still offers the opportunity to seed dissociated tumors and/or selected cell fractions such as isolated tumor cells or tumor subpopulations. Notably, TumorMACS Media support plating and expansion of both unsorted cells after tumor dissociation and isolated cells.

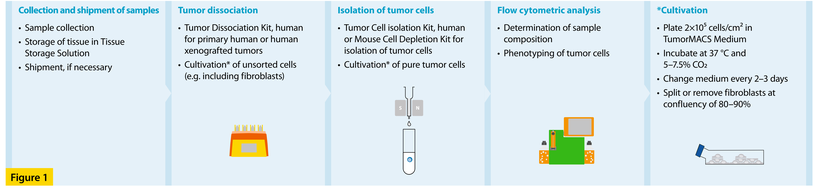


Figure 1

2 Renal tumor cell lines obtained from patient-derived xenograft tumors

We applied the presented workflow to prepare tumor cells from patient-derived xenografted renal cell carcinoma (RCC). The cells were cultivated and propagated *in vitro* using the Renal TumorMACS Medium. In contrast to widely used serum-containing homebrew media, our medium supported plating and expansion of tumor cells directly from tissue pieces (explant cultures, not shown), or directly after dissociation (not shown), as well as after tumor cell isolation (fig. 2). Notably, morphological features and cellular heterogeneity were preserved not only during initial passages but also upon propagation over many passages.

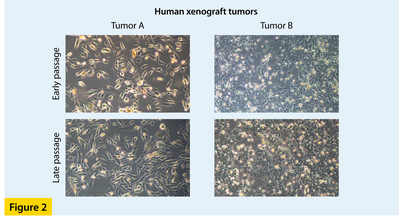


Figure 2

3 Tumor-derived RCC cell lines resemble initial tumors *in vivo*

As sophisticated studies such as biodistribution analyses can only partly be modeled *in vitro*, there is a huge demand for relevant *in vivo* models closely resembling a patient tumor. In the past, xenograft models were derived from "classical" tumor cell lines. However, these cell line–derived models only insufficiently reflect histological and functional features of primary human tumors due to the lack of histological and functional heterogeneity. In contrast, cell lines derived from primary tumors and propagated in Renal TumorMACS Medium retained their initial heterogeneity. These cell lines closely resembled the parental patient tumor in histological analyses, such as the clear-cell phenotype, upon xenotransplantation (fig. 3). Renal TumorMACS Medium thus provides a tool to generate easily accessible 2D *in vitro* models, which can also be used to set up xenograft models that closely and reliably resemble the initial patient tumor. Therefore, these models should provide a higher predictive power for *in vitro* as well as *in vivo* studies.

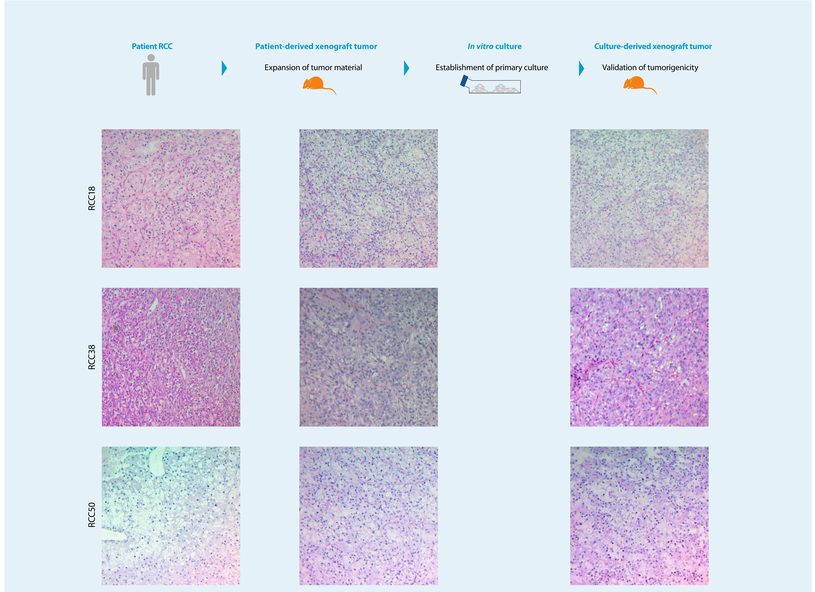


Figure 3

4 Ovarian TumorMACS Medium allows for derivation of primary cell lines that retain initial heterogeneity

For derivation of primary ovarian cancer cell lines, we used the same workflow as presented before. Ovarian TumorMACS Medium supported plating and expansion of ovarian cancer cells from pieces in explant cultures (fig. 4A), directly after dissociation, or after isolation of tumor cells (not shown). Importantly, in contrast to classical cell lines, the cell lines derived and propagated in Ovarian TumorMACS Medium retained their initial heterogeneity (fig. 4B; OC: ovarian cancer). Thus, upon xenotransplantation these cell lines closely resembled the parental patient tumor in histological analyses while tumors derived from the classical cell line OVCAR-3 showed rather homogeneous tumors (fig. 4B).

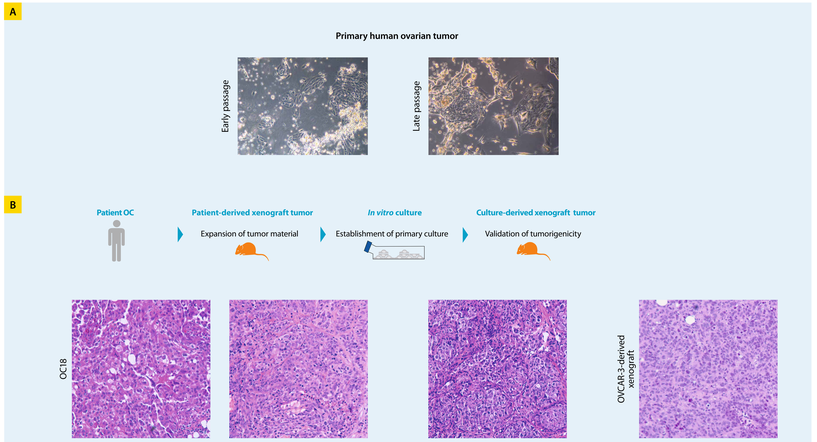


Figure 4

5 Cancer stem cell marker expression pattern is preserved after xenotransplantation of primary ovarian cancer cell lines

Multiple markers have been suggested for the identification of cancer-initiating or cancer stem–like cells. For ovarian cancer, a widely used marker combination is CD24 vs. CD44. We analyzed the expression of CD24 and CD44 in patient-derived xenograft tumors, in the derived cell lines, and in tumors derived from primary cell lines propagated in Ovarian TumorMACS Medium (fig. 5). Interestingly, although we observed a shift of marker expression during *in vitro* propagation, the culture-derived xenograft tumors closely resembled the expression pattern found in the parental (xenograft) tumor. Accordingly, our *de novo* generated primary cell lines retain the heterogeneity found within patient tumors.

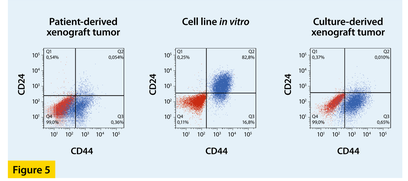


Figure 5

Conclusion

- We developed various tumor type–specific TumorMACS Media, allowing for the efficient derivation of cell cultures from primary and xenotransplanted tumors.
- Stable cell lines could be derived from most of these primary cultures.
- The newly derived tumor cell cultures and lines were shown to be more representative of the highly complex and heterogeneous disease characteristics. This was demonstrated for renal and ovarian tumors by the morphological and phenotypic heterogeneity as well as the preserved tumor–initiating capacity and genetic stability (not shown) of the cell cultures and lines.
- Due to these features, cell cultures and lines that were generated based on this novel workflow will provide improved *in vitro* models for cancer research and drug screening in the future.

References
1. Gillet, J.P. *et al.* (2011) Proc. Natl. Acad. Sci. USA. 108: 18708–18713.
2. Noll, E.M. *et al.* (2016) Nat. Med. 22: 278–287.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS and the MACS logo are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide.
Copyright © 2019 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.