Efficient derivation and expansion of tumor cell cultures from primary and xenotransplanted pancreatic tumors

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Introduction

Cancer cell lines are used to study tumor biology *in vitro* and as models for testing novel anti-cancer therapeutics. To date, the vast majority of studies in this field was done using established cell lines that were cultured for decades. Upon extensive *in vitro* propagation, cancer cell lines i) acquire multiple genetic and epigenetic alterations, ii) lose the heterogeneity present in the parental tumor, and iii) tend to lack tumor-initiating as well as multi-lineage differentiation capacity. Consequently, "classical" tumor cell lines only insufficiently represent the characteristics of a tumor[1]. To generate improved models for cancer research, novel cell lines are derived from primary tissues and used at low passage numbers. However, this process is very inefficient for most tumor entities. In addition, most of the media used include large amounts of mostly undefined serum, which has been shown to exert a selective pressure leading to a reduction of clonal heterogeneity in primary tumor cell cultures. Moreover, serum drives primary tumor cells to a more differentiated state when used over multiple passages.

We have developed an advanced medium for derivation and expansion of tumor cell cultures from pancreatic tumors[2]. The new Pancreas TumorMACS Medium has been optimized with regard to formulation, stability, and usability. In this study, we used this medium in the context of an optimized tumor processing workflow to generate primary cell cultures and lines from both primary patient tumors as well as xenotransplanted tumors. Properties of the generated cell cultures 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 Pancreas TumorMACS Medium (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, Pancreas TumorMACS Medium supports plating and expansion of both unsorted cells after tumor dissociation and isolated cells.

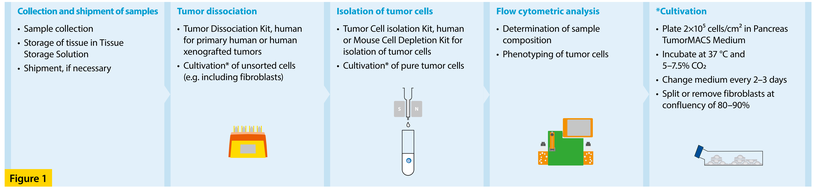


Figure 1

2 Pancreatic tumor cell lines derived from primary patient and xenotransplanted tumors

We applied the presented workflow to prepare tumor cells from human pancreatic primary and xenografted tumors. The cells were cultivated and propagated *in vitro* using Pancreas TumorMACS Medium. In contrast to standard media for propagation of established pancreatic tumor cell lines, our medium supported plating and expansion of pancreatic tumor cells both directly after dissociation (fig. 2A–C) and after tumor cell isolation (fig. 2D). Importantly, morphological heterogeneity was preserved not only during initial passages but also in late passages when cell lines were derived from these cultures. Accordingly, the medium supported cell expansion both in adherent (fig. 2A) and suspension cultures (fig. 2B). This is of particular importance, as our data show that for some specimens even epithelial tumor cells grow better in a suspension culture of spheroid-like structures (fig 2B).

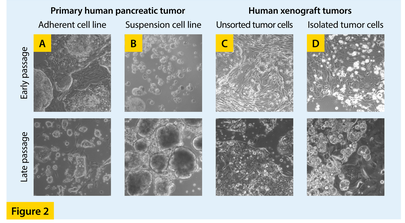


Figure 2

3 Tumor-derived cell cultures exhibit phenotypic heterogeneity *in vitro*

"Classical" tumor cell lines were propagated and cultivated in standard media over the course of decades. They have been used as *in vitro* models of (pancreatic) cancers in efficacy and toxicity studies for novel therapeutic approaches.[1] These studies have demonstrated the poor predictability of such models, leading to high failure rates of *in vitro*–approved hits at preclinical and clinical stages[1]. Flow cytometry analysis of established pancreatic tumor cell lines (fig. 3A–C) indicated that they have a homogeneous phenotype, as cells were either entirely positive for a certain marker of interest or not. Thus, these *in vitro* models lack the capacity to mirror the heterogeneity of patient tumors. In contrast, cell cultures generated in our Pancreas TumorMACS Medium were heterogeneous, harboring subpopulations for several markers (fig. 3D–E). Consequently, the *de novo* generated cell cultures and stable lines derived from these primary cultures more closely resemble the heterogeneity found within patient tumors.

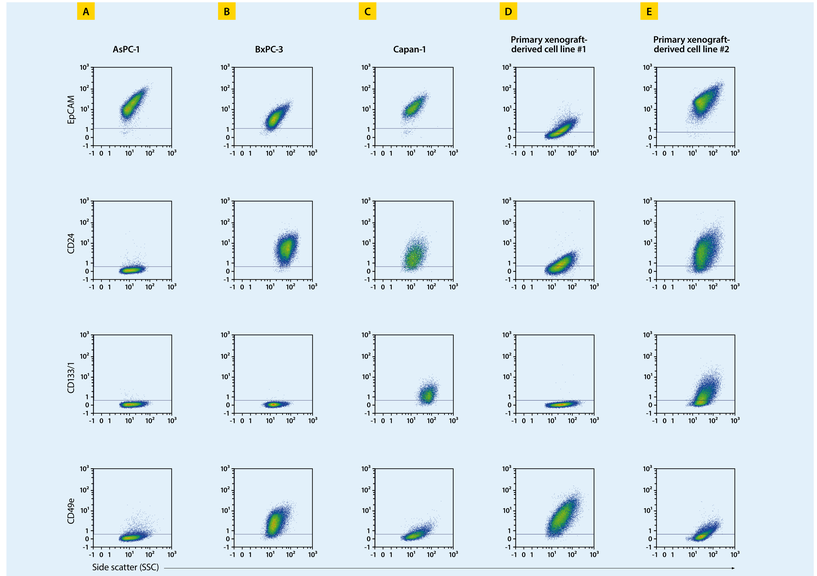


Figure 3

4 Tumor-derived cell lines resemble initial tumors *in vivo*

Sophisticated studies such as biodistribution analyses can only partly be modeled *in vitro*. Accordingly, there is a huge demand for relevant *in vivo* models closely resembling a patient tumor. For this purpose, xenograft models have been derived from "classical" pancreas tumor cell lines. However, these cell line–derived xenografts failed to reflect the histological and functional features of primary human tumors since these cell lines showed a homogeneous histology (fig. 4A). In contrast, cell lines derived from primary tumors and propagated in Pancreas TumorMACS Medium retained their initial heterogeneity. After xenotransplantation, these cell lines closely resembled the parental patient tumor in histological analyses (fig. 4B). Pancreas 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. Thus, these models should provide a higher predictive power for *in vitro* as well as *in vivo* studies.

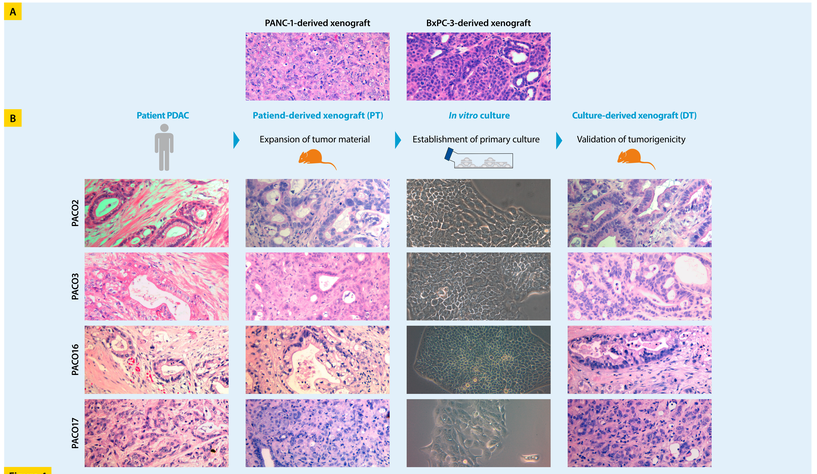


Figure 4

5 Derived tumor cell lines are stable upon *in vitro* propagation and xenotransplantation

Long-term propagation of tumor cell lines *in vitro* harbors the risk of inducing bias in gene expression profiles, thus resulting in the divergence from parental tumors. To test for the genetic stability of cell lines derived and propagated in Pancreas TumorMACS Medium, gene expression profiles of early (passage 5) and late (passage 15) passages of the respective cell lines were analyzed (fig. 5A). High correlation coefficients clearly demonstrated that the global gene expression profile was maintained upon long-term cultivation in our medium. Likewise, when cell line–derived, xenotransplanted tumors were compared to the initial patient tumors, the high level of correlation again demonstrated the preservation of genetic features (fig. 5B).

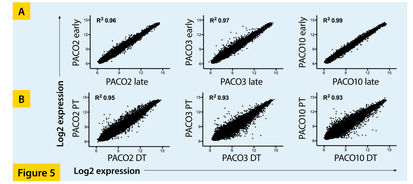


Figure 5

Conclusion

- Pancreas TumorMACS Medium allows for the efficient derivation of cell cultures from primary and xenotransplanted pancreatic tumors.
- Stable cell lines can be derived from a substantial fraction of these primary cultures.
- The newly derived pancreatic tumor cell cultures and lines were shown to be more representative of the highly complex and heterogeneous disease characteristics. This was demonstrated by the morphological and phenotypic heterogeneity as well as the preserved tumor–initiating capacity and genetic stability of the cell cultures and lines.
- Due to these features, cell cultures and lines derived using 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.

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