Unlocking primary cell culture for more relevant *in vitro* models

Published Abstract Number: 56

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Introduction

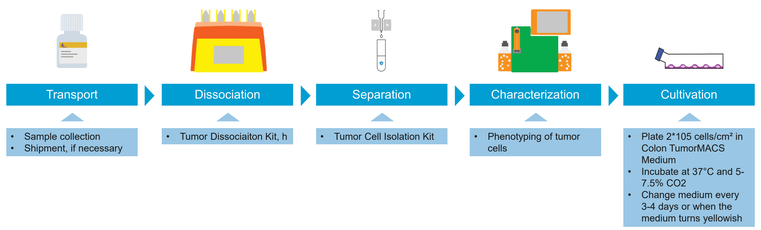
The development of effective cancer therapies requires a comprehensive understanding of the complex biology of tumor cells. Primary tumor cells, which are derived directly from patient tumors, provide a more valuable tool for investigating the mechanisms of tumorigenesis and testing potential treatments than traditional cell lines. This is due to the fact that the traditional method of growing primary tumor cells in serum-containing media can lead to changes of the cellular phenotype, gene expression pattern and heterogeneity, limiting the significance of downstream applications for translational purposes. Many cell lines have been cultured in serum-containing medium for decades, continuously accumulating genetic and epigenetic aberrations [1, 2]. Without a doubt, these cell lines have very little in common with the tumor they originate from, resulting in low biological and clinical relevance. Despite these drawbacks, cell lines are still the most frequent research tool when it comes to the use of *ex vivo* models. Yet, serum-free cultivation methods have become increasingly popular in recent years, due to the higher consistency, reproducibility, and biological relevance, more and more researchers are paying attention to which cells and culture medium to use [3, 4]. Although primary cell cultures certainly reflect cellular and genetic heterogeneity of the original tumor, finding a suitable medium that supports their growth and maintains genetic heterogeneity remains challenging.

Results

1 Workflow to derive primary cell cultures from solid tumors

The workflow of deriving primary cell cultures from solid tumor material involves several crucial handling steps which impact the success rate. We have set up a work flow which seamlessly covers all the critical steps from collecting the tumor material, optimized tissue dissociation, to cultivating primary tumor cells at the end. It guarantees a highly standardized approach to cell line derivation while still providing the option to cultivate dissociated tumors and/or selected cell populations, such as isolated tumor cells or subpopulations thereof. Notably, the Colon TumorMACS™ Medium supports the growth and expansion of both unsorted cells following tumor disintegration as well as isolated tumor cells. The cultured cells can be characterized and expanded for further studies or used in drug screening assays.

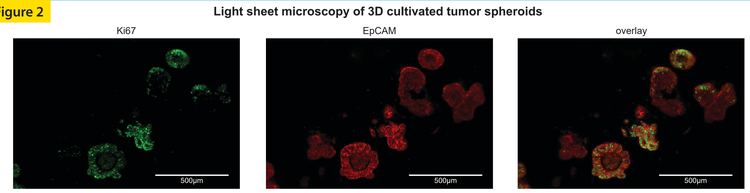
Figure 1



2 Colon adenocarcinoma cell lines derived from primary patient tumors

To this end, we developed serum-free cell culture media to cultivate primary epithelial tumor cells of pancreatic, renal, and ovarian origin. Notably, generating *in vitro* models with high biological relevance was successful when using our media during the derivation and expansion of tumor cell lines from primary and xenotransplanted tumors. Here, we present another serum-free cell culture media supporting the *in vitro* growth of primary colon adenocarcinoma. Using this optimized medium, we had a 42% success rate in establishing a primary cell culture for a given tumor sample, higher than for any other media tested in our hands. Next to 2D cell culture, Colon TumorMACS™ Medium supports 3D tumoroid formation, when cells are embedded in a supportive matrix. To perform light sheet microscopy using the UltraMicroscope Blaze™, tumoroids were cleared using the MACS® Clearing Kit. A cross-section of cleared tumoroids is depicted in Figure 3, showing a typical dysplastic tumoroid structure. Proliferating epithelial tumor cells (EpCAM$^+$/Ki67$^+$) can be found throughout different areas of the tumoroid.

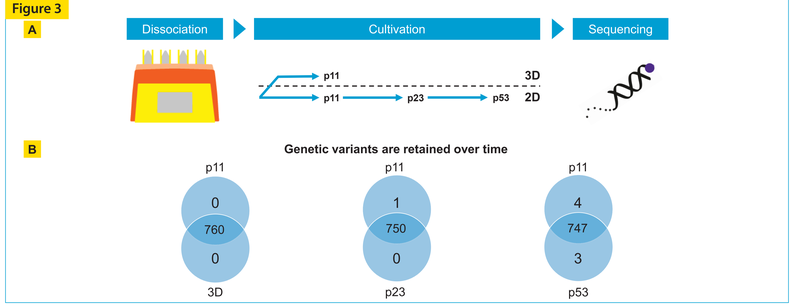
Figure 2



3 Genetic stability of *in vitro* propagated primary tumor cell lines

To test the genetic stability of *in vitro* propagated primary tumor cells, we used the QIAseq TMB Panel. Targeted sequencing of 486 tumor-associated genes revealed that more than 99% of the detected genetic variants in an early passage (p11) were also present after extended time in culture (p53). The mutations detected in unique variants are mostly T or A mismatches. Even though slight differences in between early and late passages exist, most unique variants have a frequency below 10%. Unique p11 variants showed mutations in RPA2, ABL1, CD276, and KDM6A, whereas unique p53 variants showed mutations in NOTCH2, MRE11, and CREBBP. No difference was detected among cells cultured in 2D versus 3D.

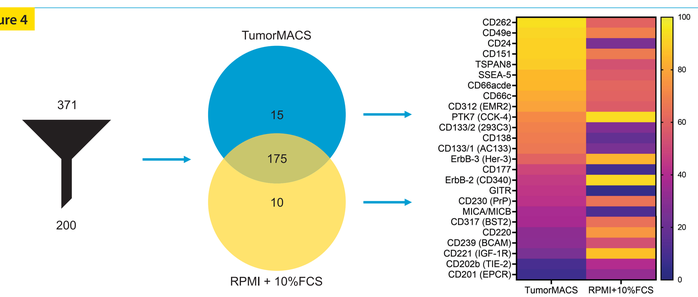
Figure 3



4 The impact of medium on the expression of surface markers

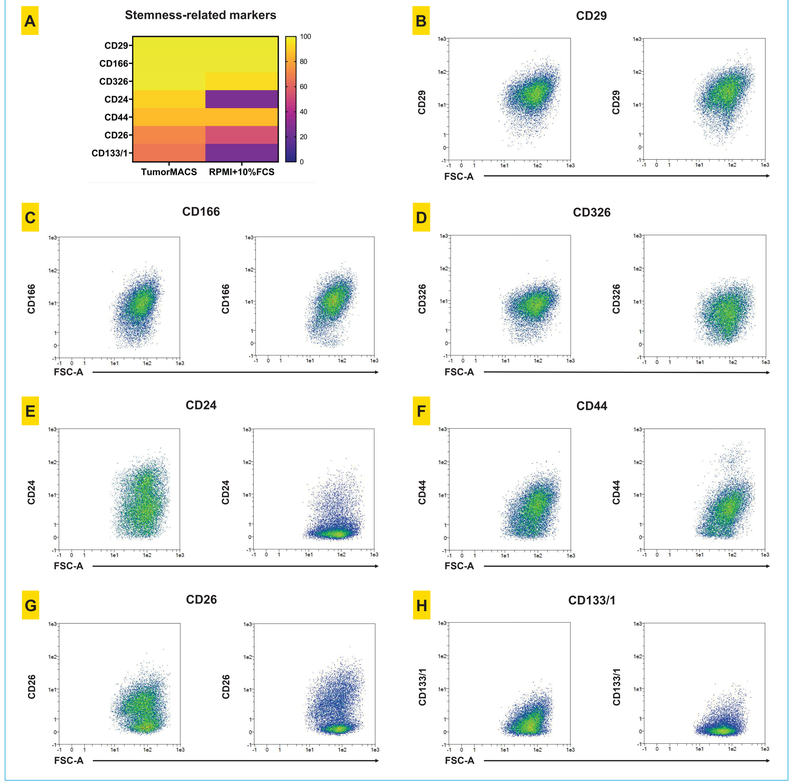
A primary tumor cell line, derived according to the workflow described above, using Colon TumorMACS™ Medium was cultivated for a total of 8 passages in RPMI + 10% FCS. In parallel, we continued growing cells in Colon TumorMACS™. A MACS Marker Screen was performed to analyze the expression of 371 human surface markers. In total 200 markers were expressed above background and considered for further analysis. The mean difference of frequency expression plus one standard deviation was used as a threshold to select the markers with the highest differences. 25 markers, of which 15 were higher expressed when cells were cultivated with Colon TumorMACS™ and 10 when RPMI + 10% FCS was the cell culture media, were showing absolute differences of 25-65% and relative differences of up to 4.4-fold.

Figure 4



Furthermore, we selected seven markers that are known stemness-related markers for colon cancer [5]. Whereas comparable frequencies are obtained for CD29, CD44, CD166, and CD326, the frequencies for CD26, CD133/1 and CD24 differences were 18%, 42% and 65% higher, respectively. It seems that cells cultured in Colon TumorMACS™ Media do more closely resemble cancer stem cells, in comparison to cells cultured in RPMI + 10%FCS.

Figure 5



Conclusion

In summary, our new serum-free medium enables the efficient derivation and expansion of primary tumor and xenograft-derived colon adenocarcinoma cell cultures in 2D and 3D, yet retaining their genetic identity. Furthermore, using Colon TumorMACS™ seems to promote the growth of cancer stem cells which are of major interest and relevance for the development of anti-cancer therapies and retaining their tumor initiating capacity for downstream *in vivo* experiments.

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