Development and characterization of 3-D tumor organoids as a preclinical model for colorectal cancer

L. Tran[1,2], R. Sheibani-Tezerji[1], T. Dillinger[1,2], M. Kuroll[1,2], V. S Atanasova[1], A. Tiefenbacher[1,2,3], H. Schachner[3], S. Schulz[3], W. Pulverer[4], A. J. Deloria[5], R. Haindl[5], W. Drexler[5], K. Wöran[3], J. Stift[3], M. Bergmann[6], H. Dolznig[7], G. Egger[1,2,3]

[1]Ludwig Boltzmann Institute Applied Diagnostics, Vienna, Austria. [2]Medical University of Vienna, Vienna, Austria. [3]Department of Pathology, Clinical Institute of Pathology, Medical University Vienna, Vienna, Austria. [4] AIT Austrian Institute of Technology, Vienna, Austria. [5]Center for Medical Physics and Biomedical Engineering, Medical University Vienna, Vienna, Austria [6]Department of General Surgery, Medical University Vienna, Vienna, Austria [7]Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria.

Introduction

Colorectal cancer (CRC) is a major cause of cancer-related deaths worldwide[1]. To study mechanisms underlying the disease, cell lines, spheroid culture systems and patient derived xenografts are used as clinical research models[2,3]. However, recent pioneering work has led to the development of organoids which are 3-D cell clusters that mimic the native organ microstructures and are derived from self-organizing mammalian pluripotent or adult stem cells *in vitro*[4,5]. They are embedded into an extracellular matrix (e.g. Matrigel, Corning) and are overlaid with medium containing inhibitors and essential growth factors important for self-renewal. This includes Wnt activators (Wnt3A and R-spondin1), epidermal growth factor (EGF), bone morphogenetic protein (BMP)/transforming growth factor (TGF)-R1 inhibitors and a p38 mitogen–activated kinase (MAPK) inhibitor[5]. Here, our major objective is to derive organoid cultures from tumorigenic and adjacent healthy tissue obtained from the same patient diagnosed with colorectal cancer. This approach enables the evaluation of the disease state while controlling for potentially confounding factors in the healthy specific genetic background.

Traditionally, cancer has been viewed as a disease driven by the accumulation of genetic mutations[6]. However, the genetic paradigm has been expanded to incorporate the disruption of epigenetic regulatory mechanisms[7]. A hallmark of many cancers is the redistribution of DNA methylation[8]. In CRC, global hypomethylation has been described and tumor tissue shows 10-40% lower levels of absolute methylation compared with normal colonic tissue. This is primarily due to loss of methylation within repetitive elements such as long interspersed nuclear element-1 (LINE-1) and ALU, and is thought to contribute to CRC initiation by enhancing genomic instability[7,8]. CRCs also develop promoter methylation of specific genes, including a number of tumor suppressor genes (TSGs) such as *CDKN2A, PTEN, SEPTIN9* or *MLH1*[9,10]. Promoter DNA methylation profiling showed that primary colorectal tumors can be classified into 4 subgroups: CIMP high, CIMP low, and two non-CIMP clusters that are associated with different anatomical location of the primary tumor[11]. CIMP high status, characterized by widespread cancer-specific hypermethylation of numerous promoter CpG island loci, generally correlates with poor survival in patients with metastatic CRC[9,11].

Results

Organoid culture

Tumor organoids can be derived and expanded after 7 days. They can be passaged and cultured for more than 6 months. Cultures are stored as cryostocks in liquid nitrogen. So far 25 organoid lines have been established.

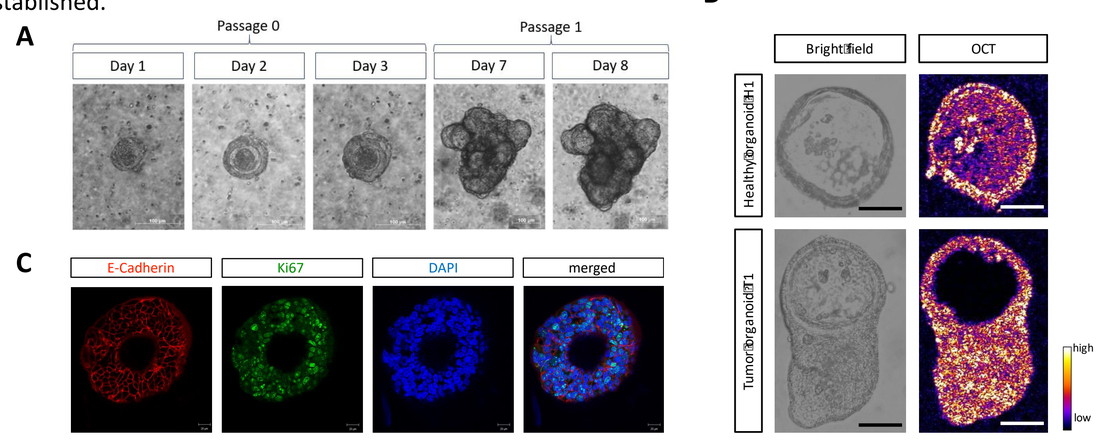

Figure 1. **A** Time course culture of human colon adenocarcinoma cells. **B** Optical Coherence Tomography/Microscopy pictures of healthy adjacent tissue and tumor organoids derived from the same patient. *En face* single cross section in axial direction. Scale bar: 100 µm. **C** Immunoflouresence staining. CRC organoid highly express the proliferation marker Ki67 (green). E-cadherin (red), DAPI (blue).

Stable transfection of iRFP with electroporation

Expression of iRFP in organoids will allow convenient tracking of engraftment efficiency and metastases in xenografts.

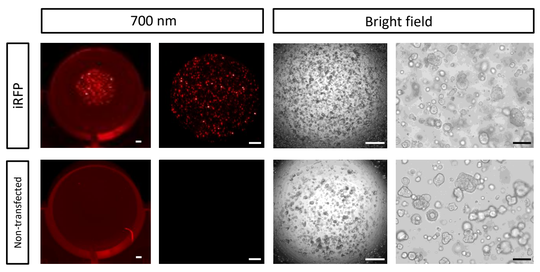

Figure 3. Tumor organoids stably express iRFP (excitation max. 690 nm and emission max. 713 nm) upon electroporation with piRFP vector. Selection with G418 (0.3 mg/ml). The organoids will be used for the establishment of xenografts in immunodeficient mice (in cooperation with **Oncotest, Charles River**).
White scale bars: 500 µm, black scale bars: 100 µm.

Histology & microsatellite stability profiling

The expression pattern of the MSI panel (proteins important for DNA mismatch repair) in the organoids is similar to the pattern observed in the primary tumor tissue.

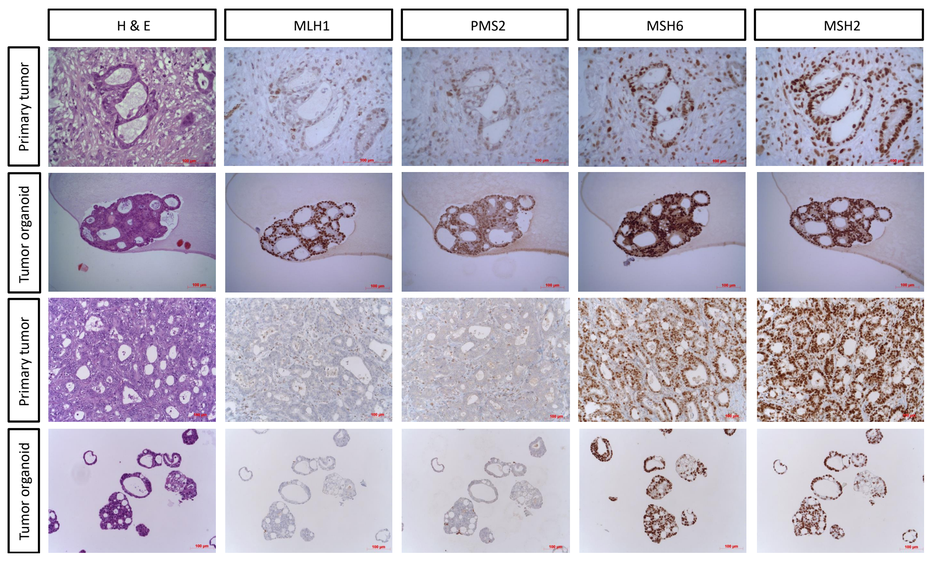

Figure 2. Immunohistochemistry stainings. H&E and stainings of DNA mismatch repair proteins of primary tumor tissue compared to tumor organoids of the same CRC patient. Comparison of a MSS and MSI case, respectively. The staining was compared to expression levels in adjacent healthy tissue (not shown).

Treatment of organoids with Decitabine

5-aza-2'-deoxycytidine (5-aza-dC) is a strong inducer of DNA demethylation. It is an analogue of cytosine, that when incorporated into DNA, irreversibly binds the methyltransferase enzymes as they attempt to methylate the cytosine analogue. This depletion of methyltransferase in the cell results in passive demethylation, which is known to reactivate epigenetically silenced genes.

Organoids treated with the hypomethylating drug Decitabine show an IC50 = 1.56 µM (in line with concentrations stated in the literature[12]).

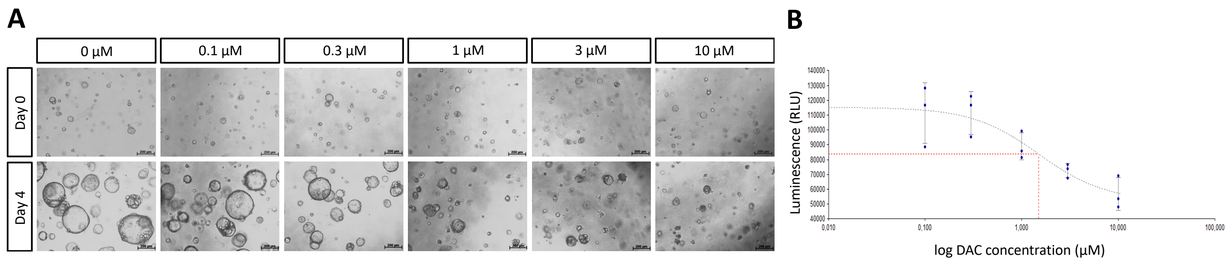

Figure 5. Effect of Decitabine on organoid growth and dose response curve of organoids to Decitabine. IC50 = 1.56 µM. Tested concentrations were: 10 µM, 3 µM, 1 µM, 0.3 µM and 0.1 µM. Luminescence was measured (normalized to Blank Matrigel samples; Cell Titer Glo 3D, Promega). Measurement was done in triplicates.

Conclusion & Outlook

For translational research, organoids provide the possibility of high throughput analysis of samples from individual patients bridging the gap between basic research and precision medicine. A biobank of human organoids presents a platform for biomarker testing, as well as drug or small molecule screening. Further, organoid cultures provide an advanced model system to study the role of the epigenome, especially DNA methylation, and its impact on tumor burden.

References

[1]Annual report World Health Organization 2015. www.who.int [2]Ohta, Y. and Sato Y. Frontiers in medicine, 2014. [3]Clevers, H., Cell, 2016. [4]Huch, M. and Koo, B.-K., Development, 2015. [5]Sato, T., et al., Gastroenterology, 2011. [6]Hanahan, D. and Weinberg, R.A., Cell, 2011. [7]Sahnane, N., et al., Clin Epigenetics, 2015. [8]Hinoue, T., et al., Genome Research, 2012. [9]Vogelstein, B., et al., Science, 2013. [10]Tse, J.W.T., et al., Trends Cancer, 2017. [11]Cancer Genome Atlas Network, Nature, 2012. [12]Karahoca, M. and Momparler R.L., Clin Epigenetics, 2013.