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

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Introduction
Colorectal cancer (CRC) is a major cause of cancer-related deaths worldwide1. To study mechanisms underlying the disease, cell lines, spheroid culture systems and patient derived xenografts are used as clinical research models2,3. However, recent pioneering work has led to the development of organoids which are 3D cell clusters that mimic the native organ microstructures and are derived from self-organizing mammalian pluripotent or adult stem cells in vitro4,5. 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-spondin2), epidermal growth factor (EGF), bone morphogenetic protein (BMP)/transforming growth factor (TGF)-β inhibitors and a β1 integrin-activated kinase (MAP2K3) inhibitor6. Here, our major objective is to derive organoids 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. However, the genetic paradigm has been expanded to incorporate the disruption of epigenetic regulatory mechanisms7. A hallmark of many cancers is the redistriution of DNA methylation8. 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 instability8. CRCs also develop promoter methylation of specific genes, including a number of tumor suppressor genes (TSGs) such as CDKN2A, PTEN, SEPT9/10 or MLH19. 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 tumors9. CIMP high status, characterized by widespread cancer-specific hypomethylation of numerous promoter CpG island loci, generally correlates with poor survival in patients with metastatic CRC10,11.

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.

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.

Stable transfection of iRFP with electroporation
Expression of iRFP in organoids will allow convenient tracking of engraftment efficiency and metastases in xenografts.

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 literature12).

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 organoid cultures provides an advanced model system to study the role of the epigenome, especially DNA methylation, and its impact on tumor burden.

References