

## Organoid culture as a model to study cellular microenvironment

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An organoid culture is a three dimensional (3D) cell culture that resembles an organ. Recently, organoid cultures were established from different organs such as small intestine, colon, stomach and pancreas; however those organoid cultures are exclusively composed of epithelial cells, thus missing the stromal niche. Here we aimed at the: 1) establishment of the organoid co-culture system to study stromal-epithelial cross-talk in the intestinal niche, 2) investigation whether fibroblasts could contribute to the early stages of tumorigenesis.

The niche was reconstructed by combining murine small intestinal crypts together with mesenchymal cells in 3D culture system. As mesenchymal cells the following cells were used: intestinal fibroblasts, carcinoma associated fibroblasts (CAFs), fetal esophageal fibroblasts and cardia fibroblasts. Mesenchymal-epithelial cross-talk was analyzed by phase contrast microscopy, Ki-67 and PAS staining, microarray analysis, mass spectrometry, and inhibitor studies.

In the co-culture, ~50% of crypts were growing as spheroids that were positive for Ki-67. Surprisingly, no differences between CAFs and other mesenchymal cells could be observed. By morphology and decreased number of PAS positive cells, the spheroids resembled intestinal organoids from *Apc*<sup>+1638N</sup> mouse tumors. Transcriptomic analysis of crypts from the co-culture further confirmed that fibroblasts induce proliferation and do not promote differentiation in the crypts *ex vivo*. Experiments with different composition of culture medium, showed that spheroid formation was independent of R-Spondin. Moreover, pre-treatment of fibroblasts with IWP-2 (inhibitor of Wnt secretion) did not abrogate the spheroid formation. Indirect co-culture and conditioned media experiments revealed that spheroid induction was mediated by soluble factors. Mass spectrometric analysis of the secretome from the co-culture suggested the involvement of extracellular matrix-receptor pathway and focal adhesion pathway.

Taken together, our studies demonstrate that the microenvironment shapes intestinal crypts in a similar way as cell-autonomous genetic alterations and point out to the potential role of fibroblasts during tumor initiation.

### Biography:

The research interests of Agnieszka Pastula include tumor microenvironment and three dimensional cell biology. Agnieszka Pastula completed her Master degree in biology with honors at the Jagiellonian University in Cracow in 2009. She gained experience in research by doing multiple internships and working as a research assistant in Poland, the Netherlands, and Germany. Since 2011 she has been doing doctorate at the Klinikum rechts der Isar TUM, where she received the Forschungsförderung der Dr.-Ing. Leonard Lorenz-Stiftung and Laura Bassi Award. She is a reviewer of the Bio-Protocol, and a member of the American Association for Cancer Research and Deutsche Krebsgesellschaft.