COMPLEX PRIMARY ORGANOID CULTURES TO DISSECT IMMUNOGENIC EFFECTS OF THERAPY ON MACROPHAGES IN A PRECISION MEDICINE-LIKE APPROACH

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BACKGROUND
Primary co-cultures of colorectal cancer (CRC) organoids with various immune components are emerging as models for probing immunological effects of novel and established cancer treatments. Tumor associated macrophages (TAM) play a central role as regulators, directing responses of other immune cell types in the microenvironment. Cancer associated fibroblasts (CAF) were shown to polarize macrophages. Therefore, we aimed to set up a complex primary co-culture assay consisting of primary organoids, CAFs and TAMs illuminating phenotypic and functional changes of TAM under therapy in CRC patients.

MATERIALS AND METHODS
A living biobank of primary CRC organoids and CAF was established. Organotypic co-cultures of monocytes derived from healthy volunteers and organoids were set up in presence and absence of patient matched CAF. Flow-cytometry-based phagocytosis assays were established to assess functional capacity of monocytes to phagocyte CRC organoid cells. Model treatments included oxaliplatin, 5-FU and two oncolytic influenza A virus prototypes.

RESULTS
CAF presence was necessary for monocytes to develop a TAM phenotype upon three days of CRC organoid co-culture, defined by macrophage-like motility within the gel matrix and enhanced expression of TAM associated phenotypic markers CD163 and CD206. Treatment of complex organoids with oxaliplatin, 5-FU or oncolytic virus treatment re-polarized macrophages towards a pro-inflammatory phenotype with respect to marker expression. The magnitude of the potential re-programming was patient dependent. Phagocytosis of cancer cells from intact organoids could be modeled upon treatment. Presence of CAF enhanced phagocytosis of cancer cells. Phagocytosis upon oxaliplatin treatment was abrogated when CRC cell death was inhibited, indicating the observed effect consisted of clearance of dead cells. Phagocytosis under viral treatment was not significantly altered by inhibition of cell death, indicating an immunogenic effect.

CONCLUSIONS
CAF appear necessary to model the TAM phenotype and their responses to treatment in primary-CRC-organoid-based organotypic assays and functional phagocytosis assays. These systems allow to assess response to therapy on the myeloid compartment in primary organoid cultures using a precision medicine approach.

REFERENCES

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