Tumor organoid and immune cells co-cultures to study myeloid cells-targeting immunotherapy

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HUB ORGANOIDS

Abstract 2696

Introduction

Cancer immunotherapy is one of the main pillars of oncology treatment, due to the clinical success of therapies aimed at enhancing anti-tumoral T-cell responses. Nonetheless, many patients do not benefit from these approaches, highlighting the need to develop novel immunomodulatory strategies. Exploiting the anti-tumor capacity of innate immune cells is a promising approach that can overcome specific limitations of T cell-targeting therapies. Still, promising therapeutic developments face hurdles in translating preclinical findings into treatment since conventional 2D cancer models hold low clinical predictive value. HUB developed an innovative alternative, building on the discovery that adult stem cells proliferate and organize into three-dimensional organotypic structures. Patient-derived organoids (PDO) are generated from healthy and diseased tissues and recapitulate complex characteristics of the original parental tissue, including molecular heterogeneity and morphological and functional traits. To assess the efficacy of therapies that engage innate immune cells, we developed an assay in which organoids derived from colorectal (CRC) or breast cancer patients were co-cultured with PBMC-derived macrophages or monocytes to evaluate the activity and cytotoxic potential of exogenously activated myeloid cells. First, we used this assay to assess whether our PBMC-organoid co-culture platform is suited to detect monocyte-mediated effects. Lipopolysaccharide (LPS) plus IFN-γ were used to activate monocytes in the co-culture assays and cytokine secretion, monocyte activation, and organoid viability were analyzed. Moreover, we established a co-culture with macrophages and organoid cells and tested the ability of macrophages to engulf human CRC organoid cells in response to anti-CD47 antibody treatment.

Figure 1| Schematic representation of PBMC-PDO experimental setup

CRC or breast PDOs were co-cultured with allogenic PBMC. For imaging, PDOs were labeled with Nucblue before seeding. After 24h, the supernatant was collected for ELISA and co-culture dissociated in single cells for FACS analysis.

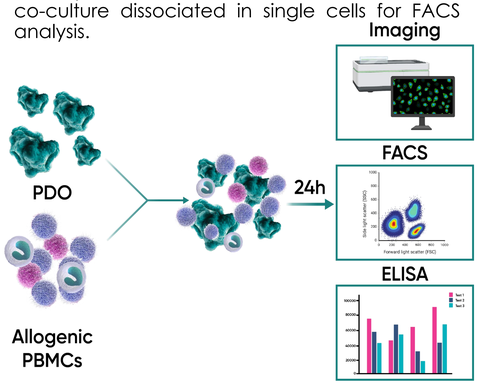


Figure 2| Increased activation of monocytes and tumor organoid apoptosis in PBMC-PDO co-culture upon LPS+IFN-g stimulation

A) CXCL10/IP-10 secretion in the co-culture supernatant. The supernatant was collected 24h after the start of the co-culture and CXCL10/IP-10 was measured by ELISA. **B)** Expression of the indicated activation markers on monocytes in the co-culture, measured by flow cytometry 24h after the start of the co-culture. PD-L1 and CD64 median fluorescence intensity (MFI) on $CD11b^{+}CD45^{+}$ cells is shown. **C)** Quantification of caspase activity induction in organoids. Live imaging of PDO-PBMC co-cultures stained with Incucyte Caspase3/7 green dye was performed and background-corrected intensity of Alexa 488 fluorescence within organoid area 24 h after plating is shown. Technical replicates of one representative experiment are shown.

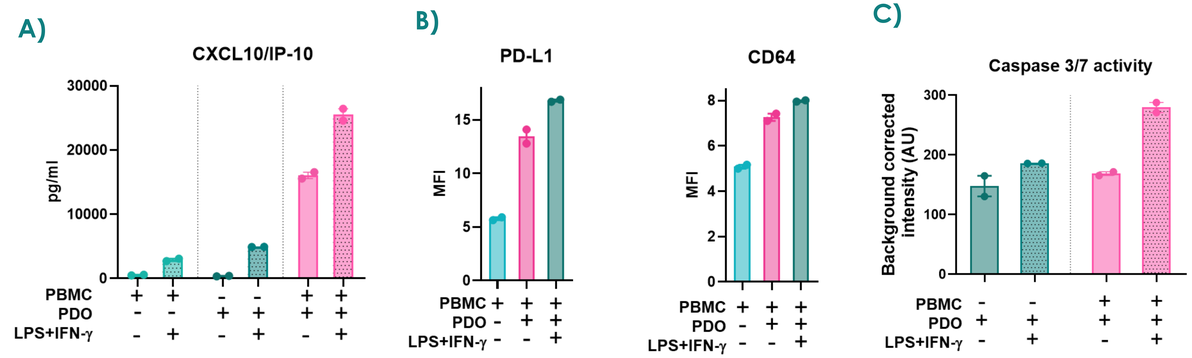


Figure 3| Schematic representation of Phagocytosis (ADCP) assay

CRC PDOs were dissociated in single cells and cultured with allogenic macrophages generated from PBMC-derived monocytes. For flow cytometry analysis, organoid cells were labeled with Calcein AM before seeding. The co-culture was analyzed 2h after seeding. An aCD11b-PeVio770 antibody was used to identify the macrophages. For time-lapse imaging, organoid cells were labeled with Hoechst blue and macrophages with Cell Tracker Green. Images were recorded for a period of 2 hours.

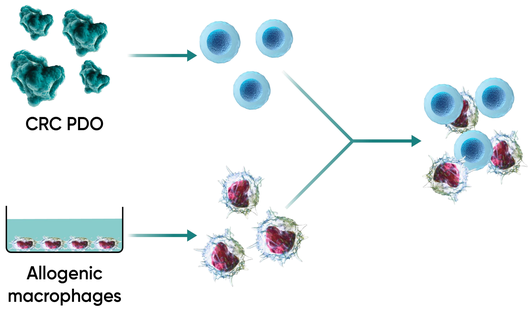
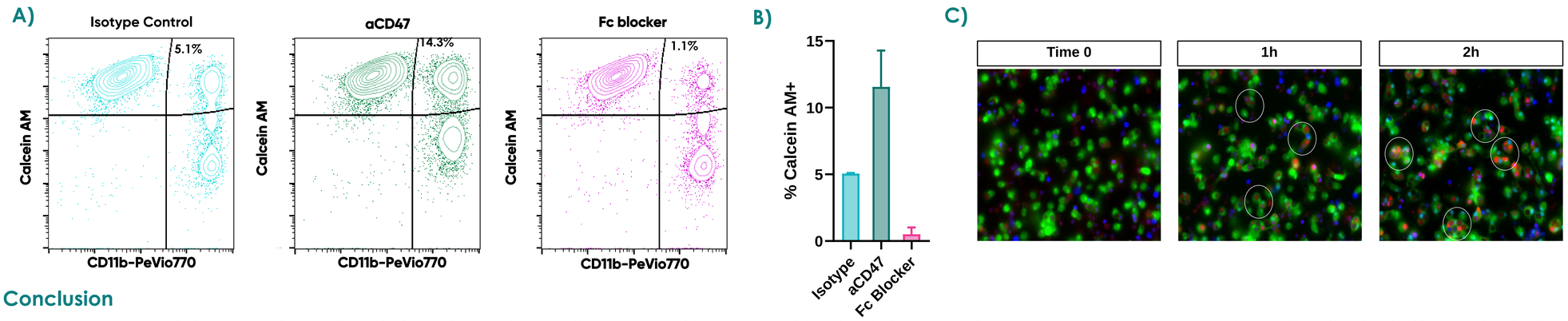


Figure 4| Increased tumor organoids phagocytosis by macrophages upon aCD47 antibody treatment

A) Representative flow cytometry plots of macrophage and PDO co-culture upon treatment with isotype control antibody, anti-CD47 antibody (B6H12), or Fc blocker (Human TruStain FcX, Biolegend), 2 h after start of co-culture. Numbers in plots indicate the frequency of Calcein $AM^{+}CD11b^{+}$ cells on total cells. **B)** Quantification of phagocyted organoid cells. The mean + SEM of 2 technical replicates of one representative experiment are shown. **C)** Representative fields of co-culture images at time 0,1 and 2h after the addition of aCD47 antibody. Circles highlight examples of macrophages (in green) engulfing organoid cells (in blue). IncuCyte pHrodo Red Cell Labeling Dye (Sartorius) was used to visualize phagocyted organoid cells.



Conclusion

In summary, our organoid-immune cell co-culture platform allows us to study the response of tumor organoids to innate immune cells such as monocytes and macrophages. Thus, it holds significant value for the preclinical development of immunotherapies based on innate immune cells, such as pattern-recognition receptor (PRR) ligands, myeloid cells-targeting monoclonal antibodies, or small molecule inhibitors.