SINGLE-CELL PROTEOGENOMICS (CITE-SEQ) ANALYSIS OF CGAS-STING PATHWAY ACTIVATION ALONE AND IN COMBINATION WITH NIVOLUMAB USING A PATIENT-DERIVED 3D EX VIVO TUMOROID PLATFORM

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Background The tumor immune microenvironment comprises a heterogeneous collection of adaptive and innate immune cells that play a critical role in immune evasion and response to immunotherapeutic agents. cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway results in activation of various immune cells promoting innate immunity in addition to senescence of cancer cells. However, the mechanisms involved in response and resistance to cGAS-STING pathway activation is not well understood. Using Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq), we explored immunological heterogeneity of tumor microenvironment in colorectal cancer and analyzed transcriptional and compositional changes of the immune landscape in response to cGAS-STING pathway activation alone and in combination with a PD-1 inhibitor nivolumab.

Methods All human tumor samples were obtained with proper patient consent and IRB approval. Fresh patient tumor tissue was processed to generate uniform sized live 3D tumoroids measuring 150 µm in size. Treatment groups included a STING agonist, ADU-S100, alone or in combination with nivolumab. Here, we applied multi-modal CITE-seq profiling using the 10X Genomics platform to interrogate cellular responses to ex vivo treatment. Culture supernatants were collected for multiplex analysis of cytokine release in media. Additionally, flow cytometry was used to assess the activation profile of resident immune cells.

Results Multimodal analysis of transcriptomes or proteomics at the single-cell level provided an unprecedented view of cellular diversity and enabled better understanding of how activation of STING pathway alone and in combination with nivolumab affects the TME in colorectal cancer. Flow cytometric analysis of immune cell populations isolated from 3D tumoroids demonstrated treatment mediated activation of tumor resident T-cells and changes in the innate immune cells, which coincided with marked changes in pro-and anti-inflammatory cytokine profiles determined by multiplex analysis.

Conclusions These results demonstrate that the 3D-EXplore ex vivo tumoroid model provides a unique platform to assess the efficacy of immunotherapeutic agents and to develop novel therapeutic combinations. Furthermore, implementation of this platform in the clinical studies may also allow identifying clinically relevant biomarkers to enable the most effective treatment strategies for individual patients.

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