

Transcriptomic analysis of a 3D engineered cancer model recapitulating stage-dependent heterogeneity in colorectal PDX tumors

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Colorectal cancer (CRC) is the third-most leading cause of cancer-related deaths in the United States. To advance the understanding of CRC tumor progression, models which mimic the tumor microenvironment (TME) and have translatable study outcomes are urgently needed. CRC patient-derived xenografts (PDXs) are promising tools for their ability to recapitulate tumor heterogeneity and key patient tumor characteristics, such as molecular characteristics. However, as *in vivo* models, CRC PDXs are costly and low-throughput, which leads to a need for equivalent *in vitro* models. To address this need, we previously established an *in vitro* model using a tissue engineering toolset with CRC PDX cells. However, it is unclear whether tissue engineering has the capacity to maintain patient- and/or cancer stage-specific tumor heterogeneity. To address this gap, we employed three PDX tumor lines, originated from stage II, III-B, and IV CRC tumors, in the formation of 3D engineered CRC PDX (3D-eCRC-PDX) tissues and performed an in-depth comparison between the 3D-eCRC-PDX tissues and the original CRC-PDX tumors. To form the tissues, CRC-PDX tumors were expanded *in vivo* and dissociated. The isolated cells were encapsulated within poly(ethylene glycol)-fibrinogen hydrogels and remained viable and proliferative post encapsulation over the course of 29 days in culture. To gain molecular insight into the maintenance of PDX line stage heterogeneity, we performed a transcriptomic analysis using RNA seq to determine the extent to which there were similarities and differences between the CRC-PDX tumors and the 3D-eCRC-PDX tissues. We observed the greatest correspondence in overlapping differentially expressed human genes, gene ontology, and Hallmark gene set enrichment between the 3D-eCRC-PDX tissues and CRC-PDX tumors in the stage II PDX line, while the least correspondence was observed in the stage IV PDX line. The Hallmark gene set enrichment from murine mapped RNA seq transcripts was PDX line-specific which suggested that the stromal component of the 3D-eCRC-PDX tissues was maintained in a PDX line-dependent manner.

Consistent with our transcriptomic analysis, we observed that tumor cell subpopulations, including human proliferative (B2M⁺Ki67⁺) and CK20⁺ cells, remained constant for up to 15 days in culture even though the number of cells in the 3D-eCRC-PDX tissues from all three CRC stages increased over time. Yet, tumor cell subpopulation differences in the stage IV 3D-eCRC-PDX tissues were observed starting at 22 days in culture. Overall, our results demonstrate a strong

correlation between our *in vitro* 3D-eCRC-PDX models and the originating *in vivo* CRC-PDX tumors, providing evidence that these engineered tissues may be capable of mimicking patient- and/or cancer stage-specific heterogeneity.