Concurrent genotyping and transcriptomics of Desmoid Tumor single cells and nuclei to study disease trajectory and therapeutic vulnerability: Planning Project Update

Mushriq Al-Jazrawe[1*], Joelle Straehla[1,2], Candace Haddox[2], Dave Shulman[2,3], Aaron Thorner[4]

[1] Koch Institute for Integrative Cancer Research at MIT; [2] Dana-Farber Cancer Institute; [3] Harvard Medical School; [4] Center for Cancer Genomics, Dana-Farber Cancer Institute; * Corresponding author (mushriq@mit.edu)

Desmoid tumors display unpredictable clinical behavior and spontaneous regression in some cases, despite nearly uniform *CTNNB1* or *APC* mutations. The mechanisms underlying this variability remain unclear. We hypothesized that differences in cellular and transcriptional composition may distinguish progressing from non-progressing tumors. However, desmoid tumors pose unique challenges for single-cell analysis, as they lack copy number alterations that typically aid in identifying neoplastic cells. To address this, we performed single-nucleus RNA sequencing (snRNA-seq) on 15 samples (13 desmoid tumors, 2 matched muscle samples) from 12 patients with well-characterized clinical courses, including progression, stability, and regression. After quality control, we profiled over 100,000 nuclei across diverse cell types. Non-progressing tumors showed increased endothelial cell abundance and immune variability compared to progressing tumors. Expectedly, conventional classifiers broadly labeled fibroblastic cells as "stromal," masking tumor-specific populations. We therefore implemented parallel single-cell genotyping for *CTNNB1* mutations to identify neoplastic fibroblastic cells. Although the capture rate was low (~2%), mutant cells formed distinct transcriptional clusters, enabling focused differential expression analysis. This analysis revealed candidate genes associated with clinical behavior between *CTNNB1* mutant populations. Finally, to support the broader research community, we developed a first iteration of *SCInteract-DT*, an interactive publicly accessible portal for exploring the dataset, enabling users to visualize cell types, gene expression, and clinical annotations. These findings, if supported by analysis of additional samples, raise the possibility that tumor behavior could be predicted using histology or immunohistochemistry. They also implicate a possible role for stromal endothelial and immune cells in influencing tumor fate, although controlled studies are needed to rule out sampling bias. Together, these efforts represent the first step toward building a genotype-resolved, clinically annotated single-cell atlas of desmoid tumors to address a critical gap in understanding tumor identity and the biology of the disease's unpredictable clinical behavior.