Multi-modal single-cell profiling of sarcomas from archival tissue reveals mechanisms of resistance to immune checkpoint blockade

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Research summary overview:

Soft tissue sarcoma is a rare and heterogeneous malignancy of mesenchymal origin of which more than 60 subtypes have been described. The various subtypes of sarcoma can arise from muscle, adipose tissue, bone and cartilage, and each subtype exhibits different clinical and molecular features. This diversity is particularly important to consider when evaluating new therapeutic options. Most sarcomas have limited treatment options and are associated with poor outcomes in the advanced/metastatic setting. While immune checkpoint inhibitors (ICI) have changed the therapeutic landscape for many cancers, different sarcomas have variable response rates to ICIs, and few exhibit durable, clinically significant responses to anti-PD-1 checkpoint blockade.

SARC028 was a multi-cohort phase II study of the PD-1 inhibitor pembrolizumab in common subtypes of soft tissue sarcoma (Tawbi, Hussein et. al, *Lancet Oncology* 2017). Findings correlated with a limited but informative body of literature demonstrating that undifferentiated pleiomorphic sarcoma (UPS) exhibits a more inflamed tumor immune microenvironment with relatively higher mutational burden and higher infiltration by CD8+ T-cells as compared the other sarcoma subtypes included on SARC028. Unfortunately, as compared other cancers, relatively little is known about the immune microenvironments of the various sarcoma subtypes, and this has limited the development of novel immunotherapies for the treatment of this disease.

The underlying contribution of niche-specific immunity of different sarcoma subtypes and how spatial interactions among cancer cells and immunity determine responses to immunotherapy in sarcomas is poorly defined. To address these fundamental challenges, we aimed to use high-dimensional single cell RNA sequencing techniques to interrogate and compare the immune microenvironment in two sarcoma subtypes, undifferentiated pleiomorphic sarcoma (UPS) and intimal sarcoma (INS). Single-cell genomics is an enabling technology that may inform the molecular underpinnings of drug response and resistance in patient biopsies.

Methods:

Single-cell sequencing methods are difficult to implement in the study of rare diseases such as sarcomas due to specimen requirements and technical limitations. Here, we evolved novel methods that we recently reported in melanoma (Wang, Fan et al., *bioRxiv* 2022) which enable single-nucleus RNA, T cell receptor (snRNA/TCR-seq), and pool-matched whole-genome sequencing (WGS) from archival frozen sarcoma tissue. This enabled profiling of 75,716 cells and 788 matched TCR clonotypes from six patients with intimal sarcoma (INS) and

undifferentiated pleomorphic sarcoma (UPS), including two matched pair samples from pre/post-immune checkpoint inhibitor (ICI).

Results:

Our analysis revealed substantial transcriptional cancer cell heterogeneity driven by varying copy number alterations (CNAs). In one patient with INS with a complete response to ICI followed by an isolated recurrence, we identified a rare cancer cell clone defined by CNA (confirmed with WGS) and resulting transcriptional outputs that pre-existed and emerged during resistance. Furthermore, in a UPS patient with intrinsic resistance to ICI, we find adequate T cell clonal expansion, activation, and differentiation, suggesting appropriate T cell response to ICI dampened by intrinsic mechanisms of ICI resistance within the cancer cells. Non-negative matrix factorization (NMF) analysis identified cell states associated with either intrinsic or adaptive resistance to ICI that was distinct from resistance to doxorubicin. These observations are consistent with those previously reported from sequential biopsies obtained from KEYNOTE-001 in metastatic melanoma (Wang, Fan, et al., bioRxiv, 2022), which also revealed emergence of pre-existing populations of resistant clones defined by their underlying aneuploidy patterns.

Conclusions:

Together, these results demonstrate feasibility of implementing single-cell genomics from archival tissue to study sarcoma and propel our understanding of drug resistance. Conceptually, this work suggests that large-scale CNAs may allow us to identify cell sub-populations associated with ICI resistance in sarcoma and in other diseases.