

# Tumor dissociation methods reveal potential distinct fibroblast groups

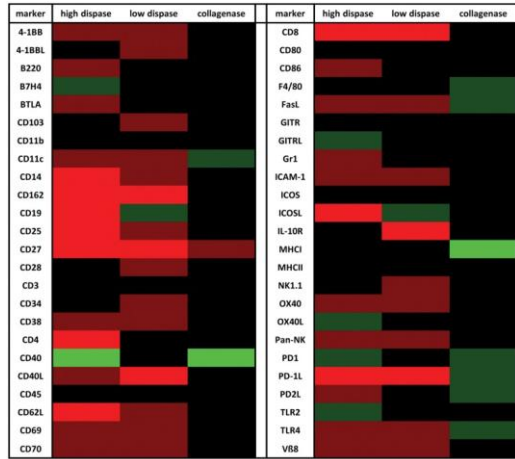
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Chen Chen, Bedi Nikita, Gentles Andrew, Plevritis Sylvia

Work supported by Grant #: NIH U54 CA209971

Modeling the Role of Lymph Node Metastases in Tumor-Mediated Immunosuppression.

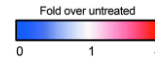


# Background: Enzymatic vs mechanical tissue dissociation causes changes in expression in multiple cell types

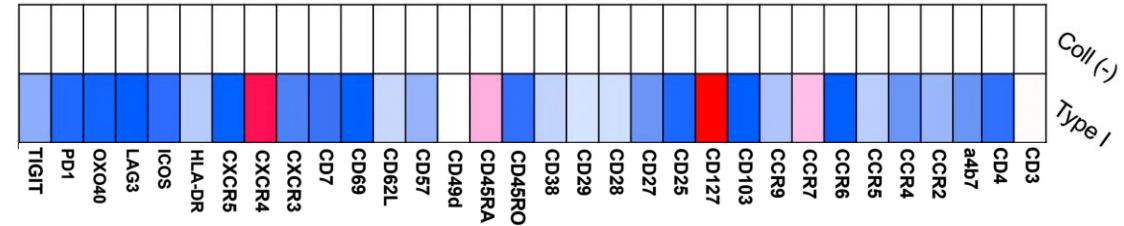


Flow cytometry surface analysis of splenocytes shows artificial protein expression using dispase or collagenase for tissue dissociation.

Autengruber A, et. al. **Impact of enzymatic tissue disintegration on the level of surface molecule expression and immune cell function** Eur J Microbiol Immunol (Bp) 2012

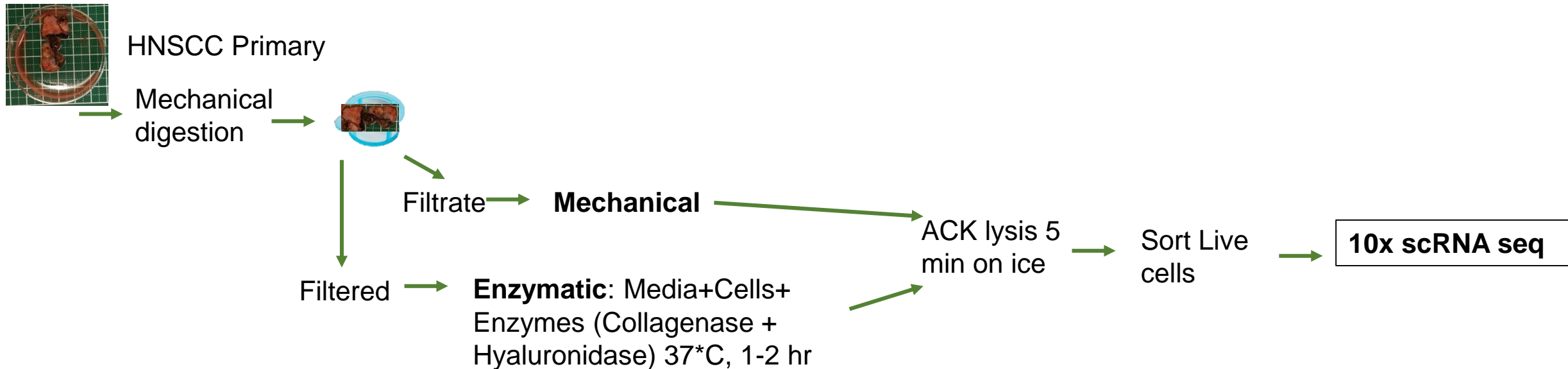


Type 1 collagenase shows increased protein expression of CCR7, CD127, CD45RA, and CXCR4 in lymphocytes of the gut using CyTOF



Trapezar M. et al, **An optimized and validated method for isolation and characterization of lymphocytes from HIV+ human gut biopsies** AIDS Res and Hum Retro 2017

## Methods: Tissue dissociation in primary tissue from head and neck cancer patients

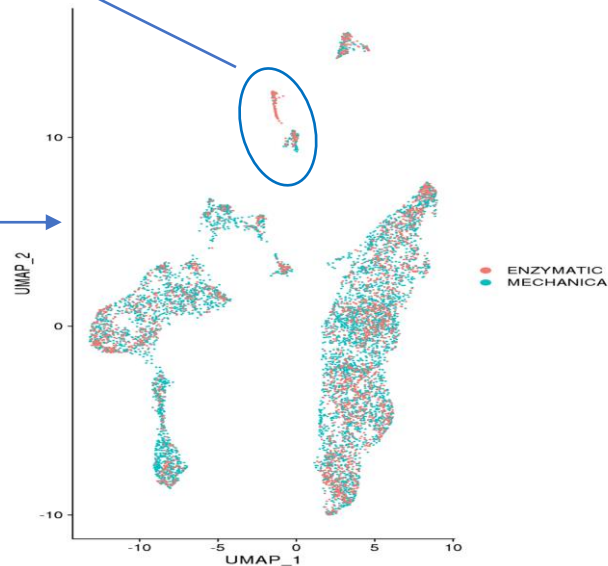
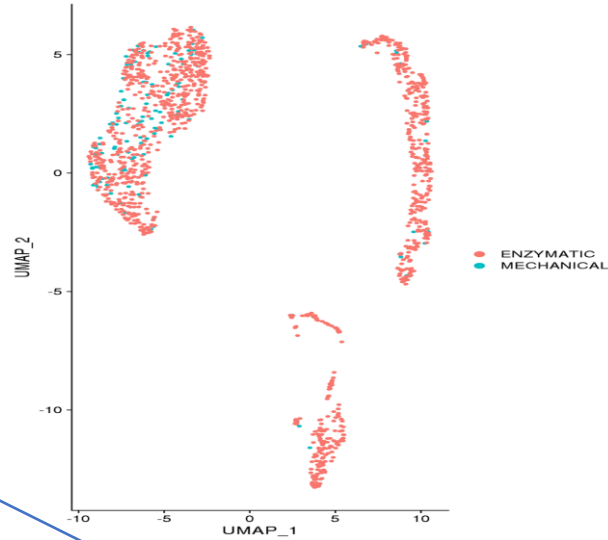




Distinct gene expression between enzymatic and mechanical digestion that could potentially reflect differences in subpopulations from digestion release or effects from enzyme digestion

Two distinctive subpopulations of fibroblasts

Similar cell capture between enzymatic and mechanical still shows enriched fibroblast population in the enzymatic



Different dissociation methods lead to unique cell-type specific transcriptional changes in fibroblasts

Enzymatic digestion is associated with increased cell death and cell stress genomic changes in all four cell subsets

Pathways upregulated in cluster 1:  
 MAPK signaling pathway  
 Apoptosis signaling pathway  
 Regulation of HSF1-mediated heat shock response  
 ATF-2 transcription factor (DNA damage response)

Pathways upregulated in cluster 2:  
 RNA polymerase II transcription  
 JAK-STAT molecular variation  
 IL-6 signaling  
 IL-8 and CXCR1 mediated signaling events  
 Role of erbb2 in signal transduction and oncology  
 MAPK1 activation

