Intratumoral heterogeneity in microsatellite instability status at single cell resolution

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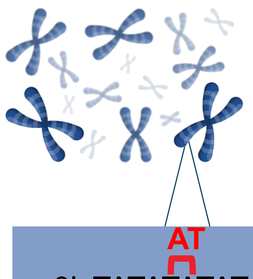
Background

Microsatellite instability (MSI)



- Accumulation of indels in repeating regions[1]
- Classified into high (MSI-H) or stable (MSS)
- Biomarker used to guide immune checkpoint blockade therapies[2]

3’ TATATATATATATATATAT 5’
5’ ATATATATATATATATATA 3’

Mismatch repair intact

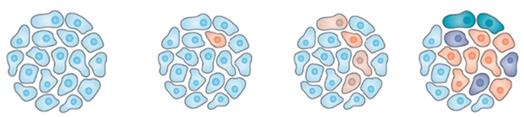
A defective mismatch repair pathway prevents removal of hairpin errors, and they become indels[3].

Mismatch repair deficient

5’ ATATATATATATATATA 3’
3’ TATATATATATATATAT 5’

5’ ATATATATATATATATATA 3’
3’ TATATATATATATATAT 5’

Intratumoral heterogeneity (ITH)



ITH describes the number of unique subclones in a tumor. It is critical to consider with respect to biomarker detection as it is known to complicate test interpretation and plays a role in primary treatment resistance.

Research question

Do only some tumor cells have MSI?

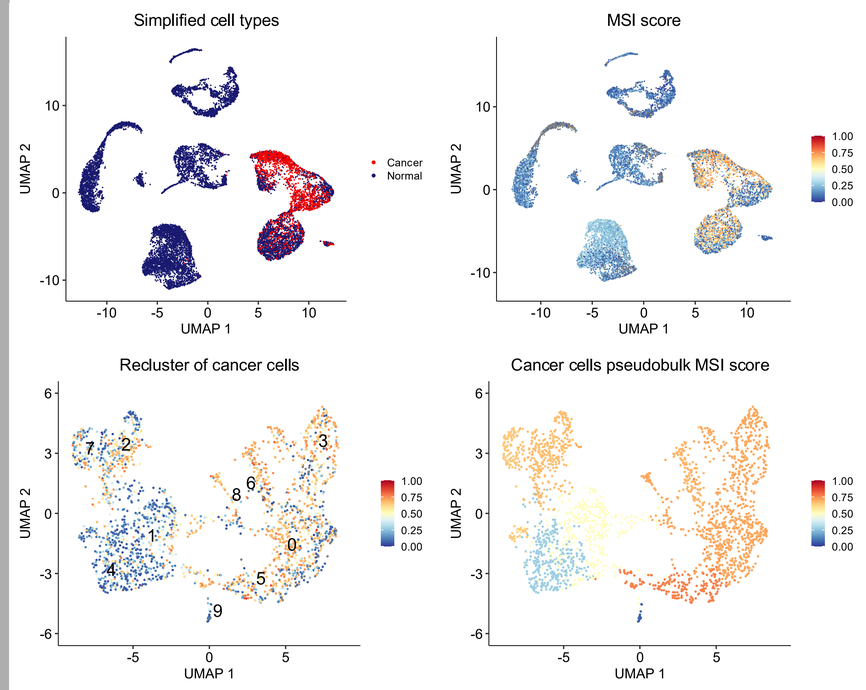
Current literature suggests MSI to be binary in nature (MSI-H or MSS); however, we hypothesize that tumors can consist of both MSI-H and MSS subclones. We justify testing this hypothesis because ITH is linked to poor biomarker interpretation and intrinsic treatment resistance, both of which are current issues when using MSI to guide a personalized treatment regime[4-6].

Methods & Data

- 134 samples from 49 individuals (49 MSI-H, 18 MSS, and two unknown) downloaded from SRA/GSE/EGA
- 10X single-cell sequencing data generated with either a 3’ or 5’ reagent kit
- Computational pipeline: Cell Ranger, Seurat, MSIsensor-RNA, and InferCNV
- Subclones were identified based on CNVs and ITH quantified with one-way ANOVA test on the MSI scores in each cluster (reported as an F-statistic)

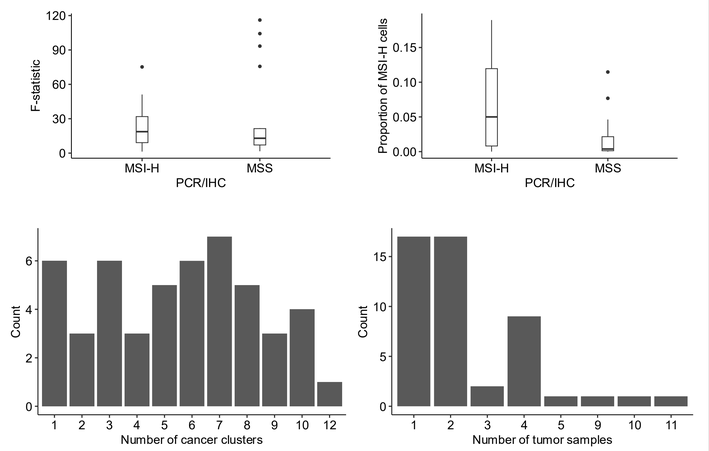
Result 1

Evidence of heterogeneity in MSI status in one MSS individual



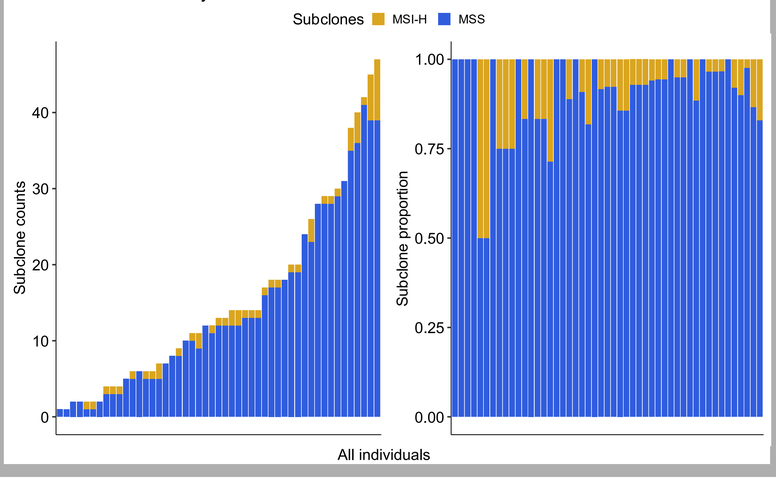
Result 2

Many individuals have high ITH (F > 25); also shown are summary statistic distributions



Result 3

Nearly all individuals have MSI-H and MSS subclones



Discussion & Conclusions

We found evidence of ITH in MSI using a novel computational pipeline. We demonstrate this within one individual (Result 1) and across all others in the study (Results 2,3). **Importantly,** ITH is currently not considered when testing for MSI, but our results suggest there could be a need for a multi-sample and multi-regional test instead of the single-sample one used in practice.

Nearly all individuals had a mixture of MSI-H and MSS subclones (Result 3). These results could be complicated by class imbalance as MSI-H cells are more difficult to identify than MSS cells. However, the presence of both subclones (sometimes at a .5 proportion) **could explain issues** with intrinsic treatment resistance in MSI-H patients as treatment regimes that target MSI-H tumors would only be effective against MSI-H subclones leaving behind a population of MSS cells.

Limitations & Future Directions

As we only measured heterogeneity in a small group of individuals from a single sequencing type, further studies are warranted to determine the frequency of heterogeneity in this biomarker at the population level. Our results would also be complemented by a clinical study to determine if the presence of MSI-H and MSS subclones impact treatment efficacy or detection.

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