Development of a novel RNA-based microsatellite stable predictive response signature (MSS-PRS) to identify MSS colorectal cancer (CRC) patients with a microsatellite instability-high (MSI-H) molecular phenotype

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BACKGROUND

- MSI-H and mismatch repair deficient (dMMR) tumors are associated with favorable immune checkpoint inhibitor (ICI) responses (André et al., 2020), but up to 95% of CRC tumors are microsatellite stable (MSI-low; designated MSS hereafter)/proficient MMR (pMMR) leading to poorer prognosis and treatment outcomes than MSI-H/dMMR patients (Le et al., 2015, Ribic, et al., 2003).
- In MSS/pMMR metastatic CRC, multiple combination therapies are being investigated without biomarkers to guide therapy (Lizardo et al., 2020, Pecci et al., 2021).
- Therefore, we developed an RNA-based MSS predictive response signature (MSS-PRS) that selects tumors not identified with conventional MSI testing but have molecular characteristics consistent with microsatellite instability, making them a potential target for ICI.

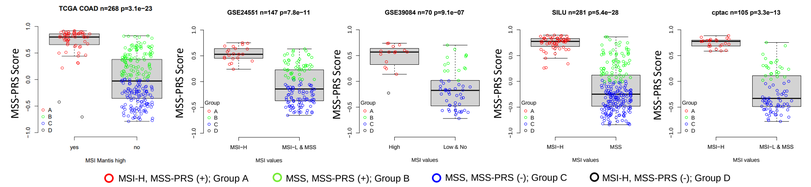
METHODS

- MSS-PRS was developed using the TCGA CRC cohort (COAD; n=268). Training labels were assigned using the MSI Mantis score and mutation status from the top 8 genes with reported MSI association (i.e., 'training label genes'; Li et al., 2020, Sorokin et al., 2021).
- "Activated" samples had both one or more of the training label gene mutations and an MSI Mantis score ≥ 0.4 (i.e., MSI-H), and "non-activated" samples were wild type for all training label genes and had an MSI Mantis score < 0.4. All other samples were designated "ambiguous" and excluded from training.
- Two thirds of the non-ambiguous samples were assigned to the classifier training set and all remaining samples were assigned to the test set. Using ClaNC software (Dabney, 2006) and cross-validation in the training set, a nearest centroid classifier was developed from a set of high mean, high variance candidate genes to select an optimal gene set to separate activated (PRS score > 0) and non-activated (PRS score < 0) groups. Classifier performance was evaluated in the test set as well as 4 additional separate CRC RNA-seq datasets accessed through GEO (GSE24551 and GSE39084) or cBioPortal (coad_silu_2022 and coad_cptac_2019).

RESULTS

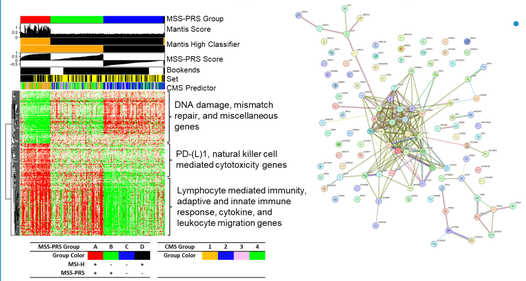
- The MSS-PRS contained 112 genes. Cross validation for its ability to correctly call MSI-H samples showed high agreement and exhibited comparable performance in both the TCGA COAD test and train set. Further, MSS-PRS produced a similar distribution in four additional CRC cohorts (**Figure 1**), validating its consistency in classifying colon adenocarcinoma samples.

Figure 1: Similar Distribution of MSS-PRS Scores between MSI-H and MSS Tumors Across Five Independent Colon Cancer RNA-seq Datasets



- The biological basis of the MSS-PRS was explored by dividing TCGA COAD into 4 groups defined by the intersection of MSI Mantis (MSI-H or MSS) and MSS-PRS (+ or -): Groups A, B, C, and D (**Figure 2**).
- Heat map display of the top 256 differentially expressed genes between Groups A, B, and C showed a clear distinction between Groups A and C, with Group B having an intermediate phenotype like Group A (**Figure 2, left panel**).

Figure 2: Differentially Expressed Genes Distinguish MSI-H/MSS-PRS (+) and MSS/MSS-PRS (-); MSS-PRS Signature Enriched with Immune Genes



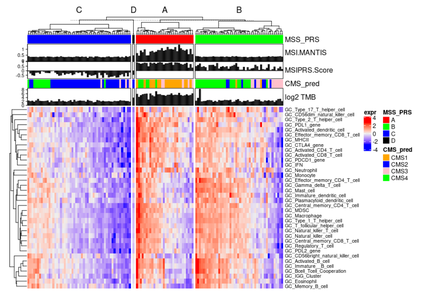
- Protein-protein interaction network mapping in String (Szklarczyk et al., 2023; https://string-db.org/) revealed a core set of immune genes related to chemotaxis, natural killer cells, and T-lymphocytes as well as several unlinked one-off genes (**Figure 2, right panel**).

Figure 3: Cluster Analysis of Common Oncogenic Mutations in TCGA COAD by MSS-PRS and CMS

- Some gene alterations (e.g., BRAF) were more prevalent in MSI-H/MSS-PRS (+) tumors (Group A) whereas others (e.g., TP53, APC) were more frequent in MSS (Groups B and C) regardless of MSS-PRS status (**Figure 3**).
- High TMB values were predominantly associated with MSI-H tumors.
- The Consensus Molecular Subtype 1 (CMS1) accounted for about two-thirds of MSI-H tumors whereas CMS2 was most prevalent in MSS Group C and about half the tumors of MSS Group B were CMS4 and the remainder was mixed.
- The mutation status and TMB of MSS Group B and C tumors were similar at the aggregate level, but notable differences were observed for CMS classification and KRAS and BRAF alterations. Group D, MSI-H/MSS-PRS (-), could not be characterized because it only contained 2 tumors.

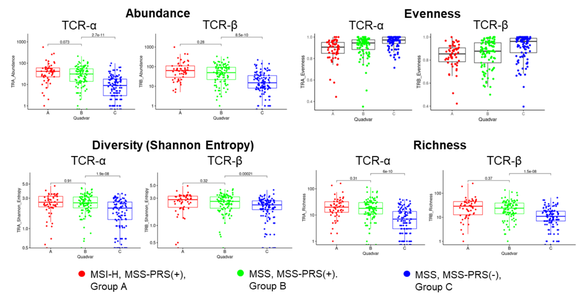
Figure 4: MMR Gene Alterations More Common in MSI-H/MSS-PRS (+) than MSS Tumors and Immune Expression Profiles Separate MSS-PRS (+) and MSS-PRS (-)

Group	MSI and MSS-PRS Status	Total, n	MMR alt+ (%)	MMR alt– (%)
A	MSI-H, MSS-PRS(+)	56	27 (48)	29 (52)
B	MSS, MSS-PRS(+)	98	12 (12)	86 (88)
C	MSS, MSS-PRS(-)	112	5 (4)	107 (96)
D	MSI-H, MSS-PRS(-)	2	0 (0)	2 (100)



- MMR genes (MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, PMS2) were examined in cBioPortal to address whether MSS tumors harbored MMR alterations (**Figure 4, left panel**). Of the MMR genes, only MLH1 was included as a training label for MSS-PRS.
- As expected, Group A (MSI-H/MSS-PRS+) carried the most MMR alterations with 48% of tumors having at least one alteration. While Group B (MSS/MSS-PRS+) tumors had more MMR alterations, 12%, than Group C (MSS/MSS-PRS-), 4%, these differences did not explain the difference in molecular phenotype between these two MSS groups for the majority of tumors (**Figure 4, left panel**)
- In contrast, gene expression-based immune profiles in MSS tumors called activated (Group B) were markedly more like MSI-H tumors (Group A) compared to MSS tumors called non-activated (Group C; **Figure 4,right panel**).
- Examination of T cell receptor (TCR) α/β repertoires confirmed differences in adaptive immunity between MSS-PRS+ (Groups A and B) and MSS-PRS- (Group C; **Figure 5**). TCR clonal expansion is an epigenomic event, thus the differences in TCR repertoire offer an independent indication that the immune molecular state of Groups A and B are more similar to each other than to Group C.

Figure 5: T-cell Receptor (TCR) Repertoires Independently Confirm Immune Differences Between MSS-PRS (+) and MSS-PRS (-)



SUMMARY AND CONCLUSIONS

- Herein we described the development of the novel MSS-PRS that selects tumors not identified with conventional MSI testing (e.g., MSI-H/dMMR) but have molecular characteristics consistent with microsatellite instability, thus making them a potential target for ICI.
- Based upon these initial findings, further development of the MSS-PRS and its clinical validation as a tool to select patients with MSS tumors who may benefit from ICI-containing treatment regimens is warranted.
- Efforts are ongoing to develop the MSS-PRS as a novel RNA-based diagnostic test and further evaluate it in both retrospective and prospective clinical studies. Additionally, to expand the potential utility of MSS-PRS further, a liquid biopsy analog of this signature is under development.

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