ABSTRACT #: 572

The Prognostic Role of Microsatellite Status, Tumor Mutational Burden, and Protein Expression in Colorectal Cancer

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BACKGROUND

- In colorectal cancer (CRC), several biomarkers have been translated to patient care, including RAS, BRAF mutations, MSI and CIMP status
- Comprehensive molecular profiling of CRC can inform treatment decisions by identifying patient subgroups with varying risks of death.
- Microsatellite instability (MSI) is prognostic in CRC and is used to select patients for immunotherapy.
- High tumor mutational burden (TMB) is associated with genomic instability and response to checkpoint inhibitor therapy. It is also a positive prognostic marker in melanoma.
- We used mass spectrometry-based proteomic to characterize proteomic differences in CRC, in order to understand patient prognosis and clinical outcome

METHODS

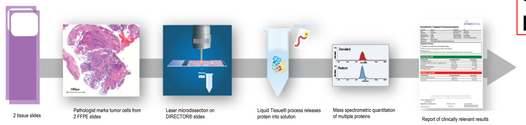

Figure 1. In archived clinical samples of CRC, 76 proteins were analyzed using mass spectrometry. MSI was measured by whole genome sequencing; unstable loci were quantified in tumor and normal samples. Cutoffs were derived via ROC analysis: high TMB was defined as >4.5 somatic mutations per megabase; p16 as ≥ 108 amol/ug. Patients were grouped by microsatellite status (MSI vs. microsatellite stable [MSS]), TMB (high vs. low), and p16 protein expression level. Survival curves were compared with the Mantel-Cox log-rank test. Global proteomic profiling was performed in 30 CRC samples.

RESULTS

Relationship between overall survival and microsatellite status, TMB, p16 in CRC patients

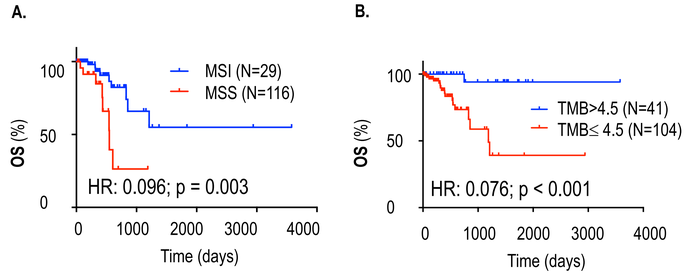

Figure 2. A. Patients with MSI tumors had longer overall survival (OS) than patients with MSS tumors (HR: 0.096; p = 0.003). **B.** Patients with high TMB had longer OS than those with low TMB (HR: 0.076; p < 0.001).

Quantitative proteomic analysis of CRC is an emerging high-throughput method to collect large amounts of molecular data that linked to tumor phenotype and outcome. We have assessed the prognostic value of targeted biomarkers in the TMB-low and MSS populations of CRC.

Among the patients with worst outcome, we found that p16 expression characterized a subset of patients with longer survival.

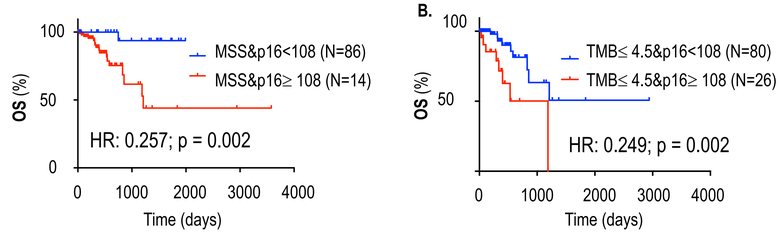

Figure 3. (A, B). High p16 protein expression (≥ 108 amol/ug) was prognostic of poor survival (HR: 2.874; p = 0.019) in the all population. Among patients with MSS tumors or low TMB, those with low p16 levels had longer OS than patients with high p16 (HR: 0.257; p = 0.002 and HR: 0.249; p = 0.002, for MSS and low TMB, respectively).

Proteomic characterization of CMS status using DIA-mass spectrometry

There is an urgent and important need for a detailed characterization of the proteomic differences underlying CMS phenotypes, in order to understand how these differences may impact therapeutic decisions.

Figure 4. Ultimate 3000 UHPLC system coupled to Q-Exactive HF (Thermo Fisher Scientific)

- LC-MS/MS: Data Independent Acquisition (DIA) was performed to identify and quantify proteins.
- A total of 3757 proteins were identified including known proteins over-expressed in CRC.
- DIA data analysis was performed using Spectronaut™ Pulsar (Biognosys, Schlieren, Switzerland) software,
- Normalized DIA-readouts of detected proteins were used for unsupervised hierarchical clustering (Ward's method).

Global proteomic profiling identified protein expressoin-driven subgroups

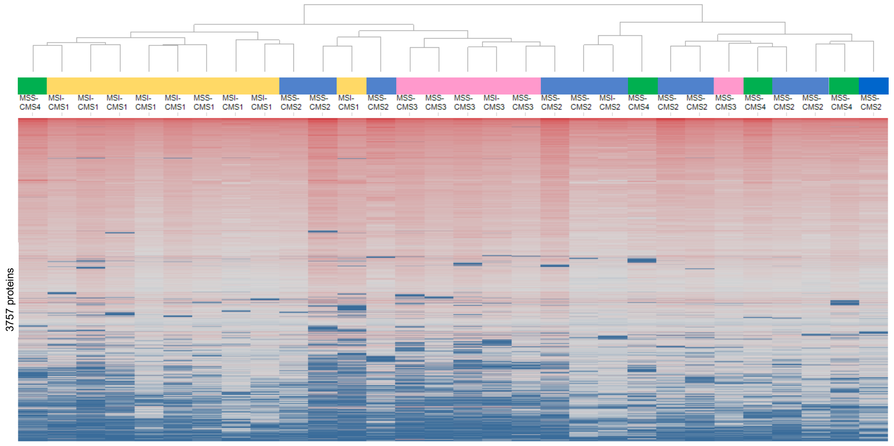

Figure 5. 30 CRC samples (10 MSI, 20 MSS) of varying stages (I-III) and consensus molecular subtypes (CMS) were analyzed by DIA-based LC-MS. Unsupervised hierarchical clustering using Log2-transformed protein expression levels of 3757 proteins. Color codes: **CMS1**, **CMS2**, **CMS3**, **CMS4**

CONCLUSIONS

- In patients with MSS or low TMB, p16 expression below 108amol/ug characterized a subset of these poor prognosis patients with longer survival.
- Based on unsupervised classification of whole proteome data from 30 stage I-IV CRC patients, a protein expression classification was developed that correlated with three of the intrinsic subtypes from CMS classification
- These protein expression subtypes will be validated in CRC patients by assessing prognosis and benefit from chemotherapy.
- Molecular profiling of CRC may identify patient subgroups with a relatively poor prognosis who could benefit from personalized therapy.
- Our approach combining quantitative proteomic and genomic analysis may accurately identify patients most likely to respond to different types of therapy.