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 proteomics to characterize proteomic differences in CRC, in order to understand patient prognosis and clinical outcome.

METHODS

- 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 ≥ 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

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

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 ≥ 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.

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).

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)

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 MSI 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 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.