Abstract # 601

Comprehensive –omic analysis of 152 CRC patients allows greater subclassification than CMS or sidedness alone

CONTRIBUTING RESEARCHERS

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BACKGROUND

Despite relatively high TMB in CRC, immune checkpoint inhibition (ICI) **response is lower** than in similarly mutated tissues such as melanoma (ORR 10-20% vs. 20-50%). MSI-status can be used to pre-select likely-responders, however **MSI is rare**. There is a **need to further guide ICI candidacy** in CRC. Four transcriptomic-based **CRC consensus molecular subtypes** (CMS) have been described with ***ad hoc* clinical associations**. We sought to **confirm these subtypes in proteomic assays and their clinical associations**.

METHODS

- 152 CRC tumors from the National Cancer Centre Singapore were available for analysis
- Tumor/normal-paired DNAseq (WGS or WES) and deep RNAseq was performed
- MSI-status was determined by both PCR and WGS/WES profiles
- CMS types, checkpoint expression, and immune-infiltration deconvolution were calculated upon RNAseq data
- Significant enrichment for MSI, immune status, CMS types, and clinical covariates was analyzed
- Mass-spec based global proteomics was successfully performed on 143/152 samples. Consensus between RNAseq and global proteomics was confirmed by correlation significance analysis
- A CMS-like clustering of proteomic data was identified by analyzing homogeneity of candidate clusterings with CMS types

RESULTS

Figure 1. **RNAseq-based inferred immune activity clusters into hot and cold tumors**

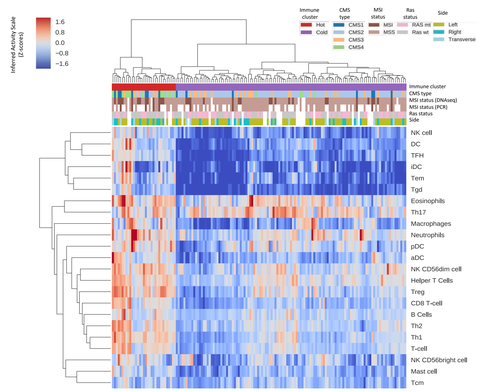


Figure 2. **Significant associations between DNA/RNA biomarkers and clinical covariates**

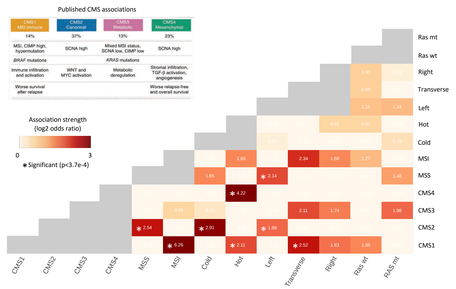
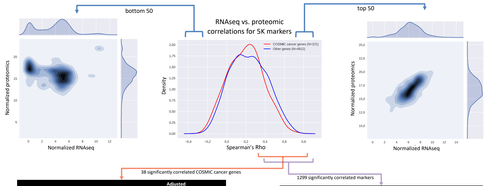


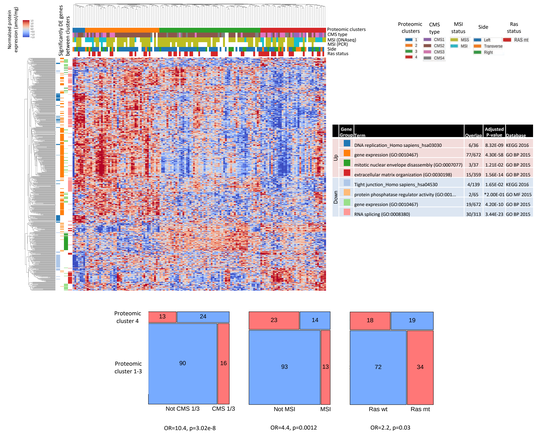
Figure 3. **Global proteomics as a secondary expression profile**



Term	Overlap	Adjusted P-value	Database
MHC class II protein complex binding (GO:0023026)	3/16	0.000101	GO MF 2015
MHC protein complex binding (GO:0023024)	3/16	0.000101	GO MF 2015
kinase binding (GO:0019900)	8/487	0.000191	GO MF 2015
protein kinase binding (GO:0019901)	7/414	0.000311	GO MF 2015
cell adhesion molecule binding (GO:0050839)	5/198	0.000311	GO MF 2015
phosphatase binding (GO:0019902)	4/108	0.000327	GO MF 2015
hormone receptor binding (GO:0051427)	4/140	0.000621	GO MF 2015
steroid hormone receptor binding (GO:0035258)	3/57	0.000658	GO MF 2015

Term	Overlap	Adjusted P-value	Database
Metabolic pathways_Homo sapiens_hsa01100	290/1193	8.85E-60	KEGG 2016
cellular amino acid metabolic process (GO:0006...	121/421	8.90E-43	GO BP 2015
tubulin binding (GO:0048487)	76/298	3.30E-26	GO MF 2015
coenzyme binding (GO:0050662)	66/288	1.88E-21	GO MF 2015
alpha-amino acid metabolic process (GO:1901605)	45/173	1.88E-21	GO BP 2015
Carbon metabolism_Homo sapiens_hsa01200	47/213	3.35E-24	KEGG 2016

Figure 4. **Proteomics-based CMS-like clustering finds orthogonal associations**



Gene Ontology Term	Overlap	Adjusted P-value	Database
DNA replication_Homo sapiens_hsa03030	4/36	8.32E-09	KEGG 2016
gene expression (GO:0010467)	77/672	4.30E-58	GO BP 2015
mitotic nuclear envelope disassembly (GO:0007077)	3/37	1.21E-02	GO BP 2015
extracellular matrix organization (GO:0030198)	15/359	1.56E-14	GO BP 2015
Tight junction_Homo sapiens_hsa04530	4/139	1.65E-02	KEGG 2016
protein phosphatase regulator activity (GO:001...	2/65	*2.00E-01	GO MF 2015
gene expression (GO:0010467)	19/672	4.20E-10	GO BP 2015
RNA splicing (GO:0008380)	30/313	3.44E-23	GO BP 2015

KEY FINDINGS

- Clustering of immune-expression deconvolution bifurcated into **hot and cold tumors**
- DNAseq-based MSI and PCR-based MSI were statistically equivalent
- **3075/5135 genes were significantly correlated between RNAseq and global proteomic assays** (1299 after multiple test correction). The most correlated genes within COSMIC cancer-related genes were **enriched for MHC binding processes**
- Significant association was found between **CMS1, MSI, transverse sides, and being immune hot**. Conversely, **CMS2 was found to be significantly MSS, left-sided, and immune cold**.
- A semi-supervised **clustering of global proteomic data** significantly recapitulated some CMS subtypes, but **grouped CMS1 (MSI enriched) & CMS3 (Ras mt enriched) subtypes**. Genes driving this association were significantly enriched for ECM organization.

CONCLUSIONS:

CMS1 tumors are the best candidates for ICI therapy. CMS3 co-clusters with CMS1 in ECM genes within proteomic data, warranting further research of CMS3 ICI outcomes

1. Kim, Y. J., et al. "Data-independent acquisition mass spectrometry to quantify protein levels in FFPE tumor biopsies for molecular diagnostics." *Journal of proteome research* (2018).
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3. Bindea, Gabriela, et al. "Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer." *Immunity* 39.4 (2013): 782-795.
1. Guinney, Justin, et al. "The consensus molecular subtypes of colorectal cancer." *Nature medicine* 21.11 (2015): 1350.

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