DEFB1 gene expression and the molecular landscape of colorectal cancer (CRC)

Jae Ho Lo[1], Francesca Battaglin[1], Yasmine Baca[2], Joanne Xiu[2], Pavel Brodskiy[2], Sandra Algaze[1], Priya Jayachandran[1], Hiroyuki Arai[1], Wu Zhang[1], Benjamin A. Weinberg[3], Rachna Shroff, Davendra P.S. Sohal, Emil Lou[4], Anthony F. Shields[5], Richard M. Goldberg[6], John L. Marshall[3], W. Michael Korn[2], Shivani Soni[1], Heinz-Josef Lenz[1]

1 Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA. 2 Caris Life Sciences, Phoenix, AZ, USA. 3 Ruesch Center for The Cure of Gastrointestinal Cancers, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA. 4 Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, Minnesota, USA. 5 Department of Oncology, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA. 6 West Virginia University Cancer Institute, Morgantown, WV, USA

Keck School of Medicine of USC

CARIS PRECISION ONCOLOGY ALLIANCE

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fbattagl@usc.edu

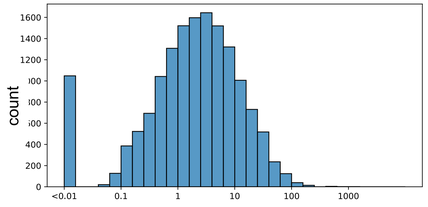
Introduction

- Defensins are antimicrobial peptides that play important roles in innate immune response.
- Deregulation of beta-defensin-1 (*DEFB1*) gene expression has been implicated in several cancers.
- We previously showed that single nucleotide polymorphisms in *DEFB1* are associated with clinical outcomes in patients with metastatic CRC.
- Here, we aimed to further characterize the molecular features associated with *DEFB1* gene expression in CRC.

Methods

- A total of 14,416 CRC tested at Caris Life Sciences (Phoenix, AZ) with NextGen Sequencing on DNA (Illumina Next Seq, 592 genes, or Illumina NovaSeq, WES) and RNA (Illumina NovaSeq, WTS) were analyzed.
- Top quartile transcripts per million (TPMs) for *DEFB1* expression were considered high (Q4) while bottom quartile low (Q1) expression.
- Consensus molecular subtypes (CMS) were assessed using RNAseq. Cell infiltration (CI) in the tumor microenvironment (TME) was estimated by QuantiSEQ.
- X^2/Fisher-Exact tests were used for comparison and significance was determined as *P*-value adjusted for multiple comparison (Q < 0.05).
- Real-world overall survival information was obtained from insurance claims data and Kaplan-Meier estimates were calculated for molecularly defined patients.

Distribution and Demographic



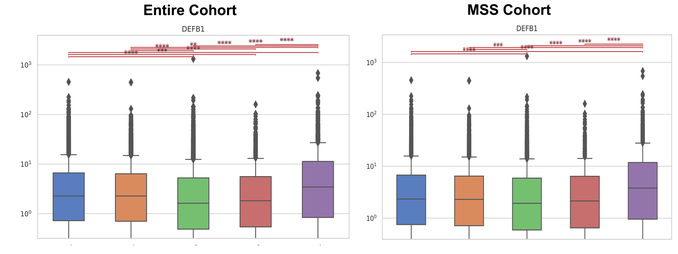
DEFB1 expression (TPM)

All	*DEFB1* Q1	*DEFB1* Q4
Count (N)	3857	3856
Median Age (range)	63.0 (14 - >89)	63.0 (17 - >89)
Male	53.0%	56.0%
Female	47.0%	44.0%

MSS	*DEFB1* Q1	*DEFB1* Q4
Count (N)	3405	3688
Median Age (range)	62.0 (14 - >89)	63.0 (17 - >89)
Male	54.6%	56.2%
Female	45.4%	43.8%

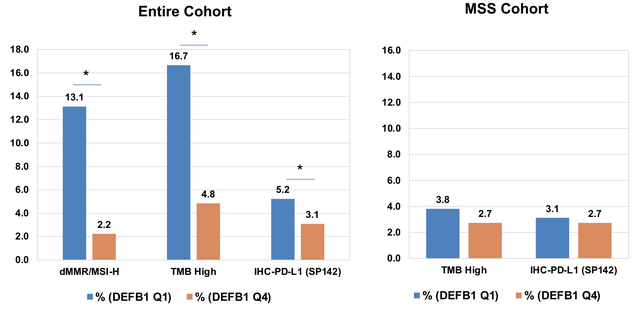
Results

Figure 1. Correlation between *DEFB1* Expression and Primary Tumor Side.



DEFB1 expression was highest in left-sided and rectal tumors and lowest in right-sided tumors.
In the MSS cohort, *DEFB1* expression was highest in CMS2 and lowest in CMS3 (2.84 vs 1.67 median TPM, Q < 0.05) [data not shown].

Figure 2. Association of tumor *DEFB1* Expression with Immune Markers.

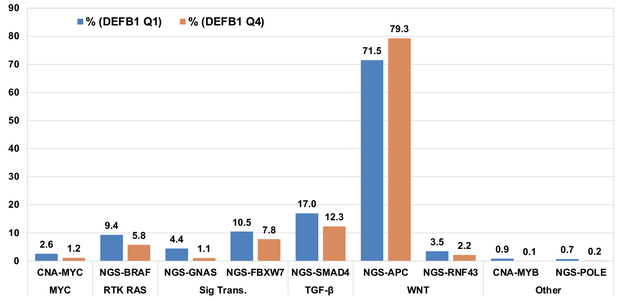


* Q < 0.01; PDL-1 cutoff 2+ 5%

Overall, when compared to low expression, high *DEFB1* was negatively associated with high TMB (≥ 10 Mut/Mb) (4.8% vs 17%), dMMR/MSI-H (2.2% vs 13.1%), and PD-L1 expression (3.1% vs 5.2%) (all Q < 0.05).
This trend held true for TMB-H in the MSS cohort.

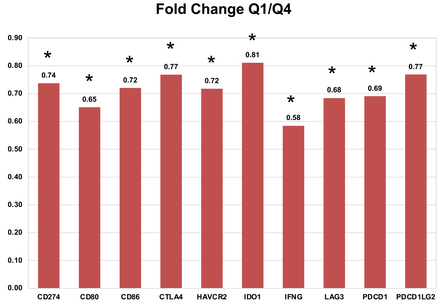
Figure 3. Association with Tumor Molecular Characteristics.

A. Mutations and CNA (MSS Cohort, significant results).



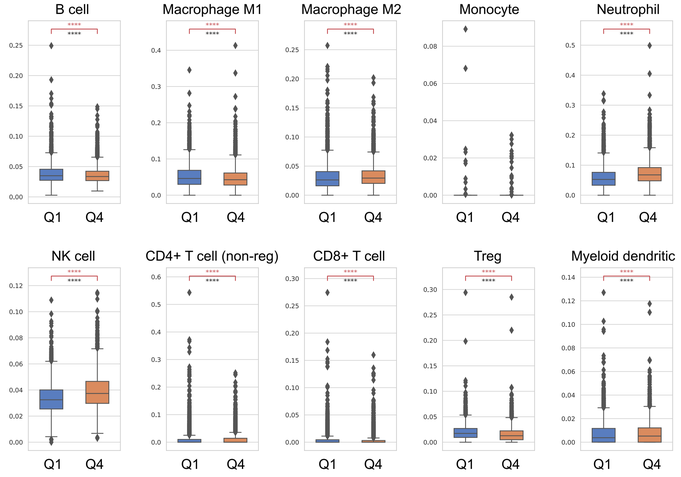
In the MSS cohort, *APC* mutations were more frequent in *DEFB1* high tumors (79% vs 72%) while *BRAF* (5.8% vs 9.4%), *GNAS* (1.1% vs 4.4%), *FBXW7* (7.8% vs 10.5%), *SMAD4* (12.3% vs 17%), *RNF43* (2.2% vs 3.5%) and *POLE* (0.2% vs 0.7%) mutations as well as *MYC* (1.2% vs 2.6%) and *MYB* amplifications (0.1% vs 0.9%) were less frequent in *DEFB1* high (all Q < 0.05).

B. Immune-related Gene Expression (MSS Cohort).



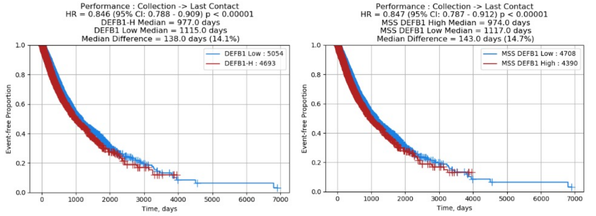
Overall, high *DEFB1* (Q4) was associated with higher expression of immune checkpoint genes *CD274*, *CD80*, *CD86*, *HAVCR2*, *LAG3*, *PDCD1* and *PDCD1LG2* (Fold Change/FC: 1.27-1.56) but lower *IDO1* (FC: 0.89) (all Q < 0.05).
Similar results were confirmed in MSS tumors only, but IDO1 was now positively associated with *DEFB1* high (FC: 1.23).

Figure 4. TME Cell Infiltration According to *DEFB1* Expression in MSS Tumors.



Higher neutrophils, NK cells, M2 macrophages, CD4+ T cells and myeloid dendritic cells but lower M1 macrophages, Tregs and CD8+ T cells in the TME were significantly associated with high *DEFB1*; both in the overall and MSS cohorts (Q < 0.001).

Figure 5. Association between *DEFB1* Expression and Patient Outcomes.



Patients with *DEFB1* tumor expression level above the median had worse OS compared to those below the median both in the overall cohort (HR: 1.18, 95% CI: 1.10-1.27) and in MSS tumors (HR: 1.18, 95% CI: 1.10-1.27).

CONCLUSIONS

Our data show a distinct molecular landscape, including mutational profiles, CMS, immune biomarkers, and TME cell infiltration associated with *DEF1B* gene expression in CRC.

These findings suggest a key role for *DEF1B* in modulating anti-tumor immunity and TME.