Clinical and Molecular Characterization of FAP and SPP1 in Colorectal Cancer (CRC), CALGB (Alliance)/SWOG 80405 and Real-world Data

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Background

- *FAP* and *SPP1* contribute to immune modulation in the CRC tumor microenvironment (TME).
- FAP is expressed by cancer associated fibroblasts, aiding in tissue remodeling and tumor invasion.
- SPP1 is an integrin-binding protein expressed by tumor associated macrophages that promotes tumor growth, adhesion, and metastasis.
- We present a clinical and molecular characterization of *FAP* and *SPP1* in CRC.

Methods

- 24,257 CRC samples tested at Caris Life Sciences (Phoenix, AZ) with WTS (Illumina NovaSeq), NextGen DNA sequencing (NextSeq, 592 genes and NovaSEQ, WES), and PD-L1 expression (LDT SP142; TPS ≥ 5%) were analyzed.
- RNA deconvolution analysis with QuantiSEQ estimated cell infiltration in the tumor microenvironment (TME).
- In the Caris cohort, overall survival (OS) was evaluated from treatment initiation using insurance claims data.
- Gene set enrichment analysis (GSEA) using the Hallmark 50 gene sets was performed based on WTS data to assess significantly enriched pathways according to *AXL* expression.
- Data from the phase III CALGB/SWOG 80405 trial on 433 metastatic CRC (mCRC) patients treated with bevacizumab (Bev, n = 226) or cetuximab (Cet, n = 207) in combination with first-line chemotherapy were also evaluated.
- RNA isolated from FFPE tumor samples were sequenced with HiSeq 2500 (Illumina).
- OS and progression-free survival (PFS) were compared categorically by gene expression by tertiles into high (T3), medium (T2), and low (T1).
- Hazard ratios (HR) and 95% confidence intervals (CI) were estimated from Cox proportional hazard model, adjusting for age, sex, ethnicity, ECOG PS, tumor sidedness, number of metastatic sites, KRAS, BRAF, MSI status, treatment with targeted therapy (Bev vs Cet), and chemotherapy (FOLFOX vs FOLFIRI).

Acknowledgments

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Results

Figure 1: Immune-Related Markers.

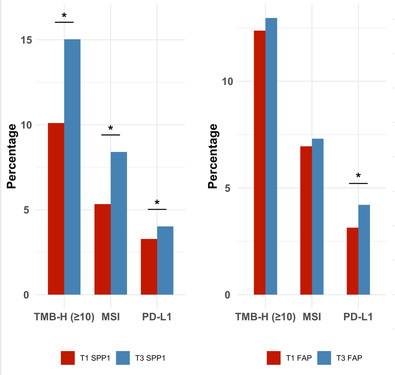
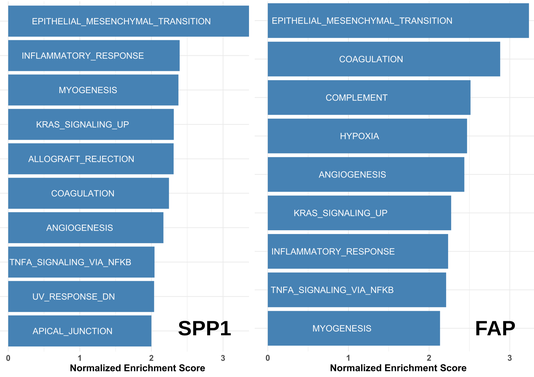
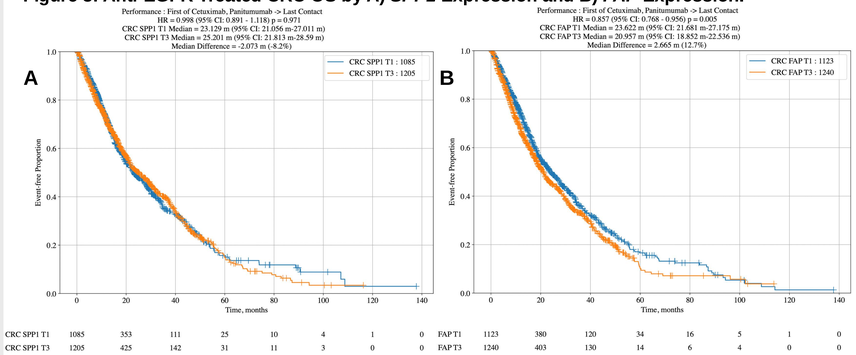


Figure 2: Gene Set Enrichment Analysis.



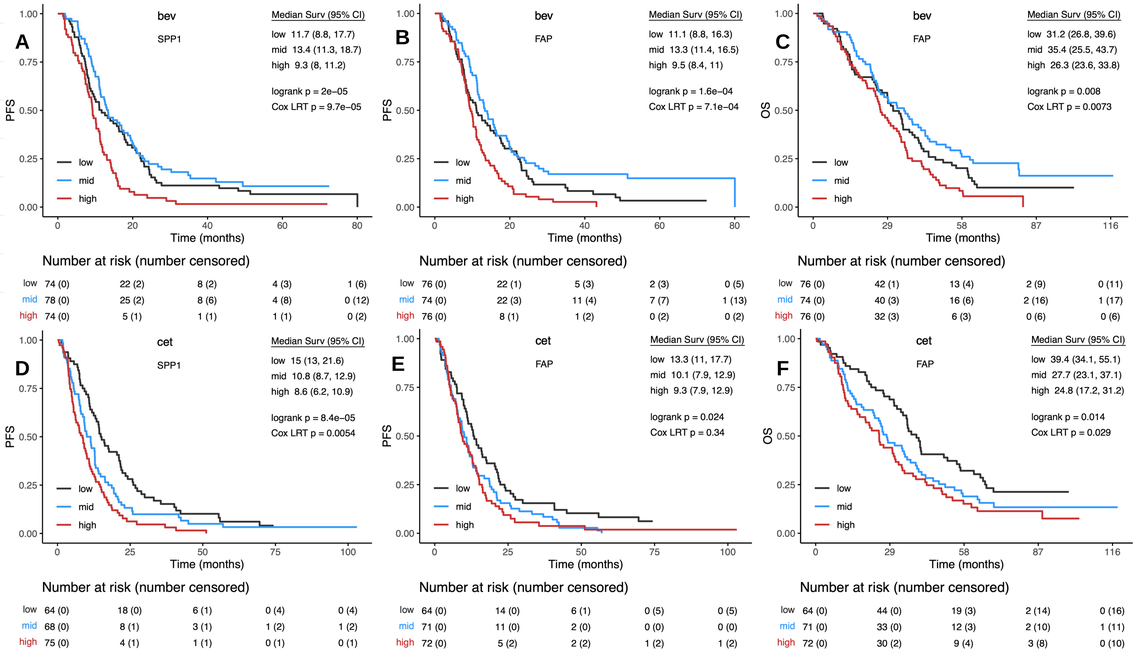
FAP-T3 and *SPP1*-T3 had increased PD-L1 positivity (q<0.05), while *SPP1*-T3 also demonstrated increased MSI-H (8.4% vs 5.3%) and TMB-H(>10 mt/mb, 15.0% vs 10.1%) status relative *SPP1*-T1 (all q<0.001) (Figure 1). T3 of both genes of correlated with increased M1/M2 macrophages, NK cells and T cell inflamed score (q<0.001); dendritic cells and neutrophils were increased in *SPP1*-T3 and *FAP*-T1 tumors (q<0.05). *FAP*-T3 and *SPP1*-T3 had increased pathway activation of epithelial-mesenchymal transition (EMT), inflammatory response, TNF-a signaling, angiogenesis and *KRAS* signaling (all $q < .005$, Figure 2).

Figure 3: Anti-EGFR Treated CRC OS by A) *SPP1* Expression and B) *FAP* Expression.



In Caris cohorts, *SPP1* did not correlate with OS in Cet/panitumumab treated CRC(Figure 3A), but *FAP*-T3 demonstrated worse OS in (T3: 23.6 vs T1: 21.0 months [mo], $P = .005$; HR 0.85, 95% CI [0.77-0.95]) (Figure 3B).

Figure 4: CALGB/80405 Trial mCRC Treatment Subgroups: Bevacizumab-treated Patient A) PFS by *SPP1* Expression B) PFS by *FAP* Expression B) OS by *FAP* Expression. Cetuximab-treated Patient D) PFS by *SPP1* Expression *E*) PFS by *FAP* Expression *F*) OS by *FAP* Expression..



In 80405, FAP-T3 showed shorter PFS (T3: 9.5 vs T2: 11.5 vs T1: 12.6 mo, T3 vs T1 (reference) adjusted HR 1.27 [1.07-1.51]) and OS (25.2 vs 29.4 vs 35.5 mo, adjusted HR 1.31 [1.10-1.57]). Similarly, SPP1-T3 demonstrated worse PFS (9.0 vs 12.7 vs 14.0 mo, adjusted HR 1.29 [1.12-1.48]) and OS (20.9 vs 34.0 vs 36.3 mo, HR 1.24 [1.07-1.44], $P < 0.001$). Treatment interaction tests noted FAP-T1 CRC benefited from Cet over Bev with respect to PFS ($P = 0.003$) and OS ($P = 0.044$), while SPP1-T1 tumors demonstrated a PFS benefit with Cet ($P = 0.009$). OS and PFS treatment subgroups for FAP and PFS treatment groups for SPP1 are shown (Figure 4).

Conclusions

- Our results indicate that increased FAP and SPP1 expression is associated with immune cell infiltration, EMT, and inflammatory signaling.
- FAP and SPP1 expression may be prognostic and predictive of targeted therapy.
- These data support the evaluation of FAP and SPP1 as predictive markers and therapeutic targets in CRC.