Characterization of Necroptosis Activators in Colorectal Cancer

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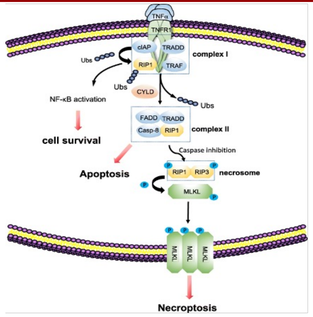
Introduction

- Necroptosis is a pro-inflammatory form of programmed cell death triggered by cellular stresses and extrinsic cytokines.
- It is a potential therapeutic target for immunotherapy (IO) in colorectal cancer (CRC).
- Here, we present comprehensive clinical and molecular characterization of key necroptosis regulators in CRC.

Methods

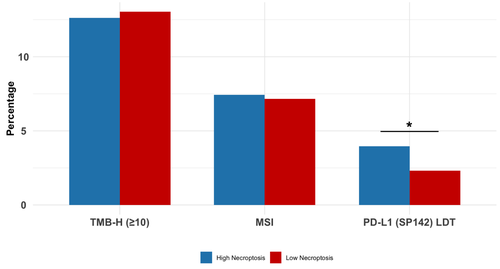
- 24,257 samples of CRC tested at Caris Life Sciences (Phoenix, AZ) with WTS (Illumina NovaSeq), NextGen DNA sequencing (NextSeq, 592 Genes and NovaSEQ, WES), and PD-L1 expression (LDT SP142; TPS ≥5%) were analyzed.
- Expression of activators in the necroptosis pathway (*RIPK3*, *MLKL*, *CYLD*, and *ZBP1*) was categorized as either high (H) or low (L) using median expression as the cutoff.
- CRCs with high expression of all four activators were considered to have high propensity for necroptosis activity (NA-H, N=4595) compared to tumors with low expression in all activators (NA-L, N = 4946).
- RNA deconvolution analysis with QuantiSEQ estimated immune cell infiltration in the tumor microenvironment (TME).
- Differences in overall survival (OS) were analyzed from insurance claims data and calculated from treatment initiation using Kaplan-Meier estimates.
- Statistical significance was set as a P-value adjusted for multiple comparisons (*q*<0.05).

Necroptosis Pathway



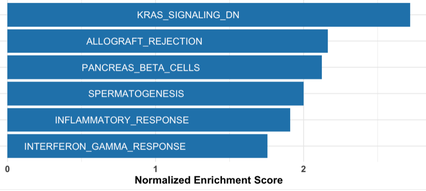
Results

Figure 1: Immune-Related Markers.



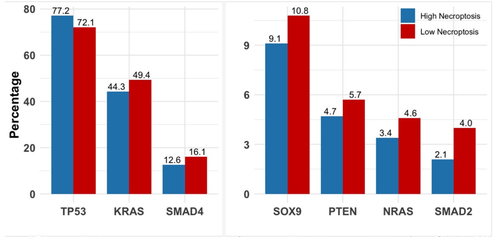
NA-H was associated with increased PD-L1 positivity, immune cell infiltration, and T-cell inflamed score (all *q*<0.001).

Figure 2: Gene Set Enrichment Analysis with NA-H.



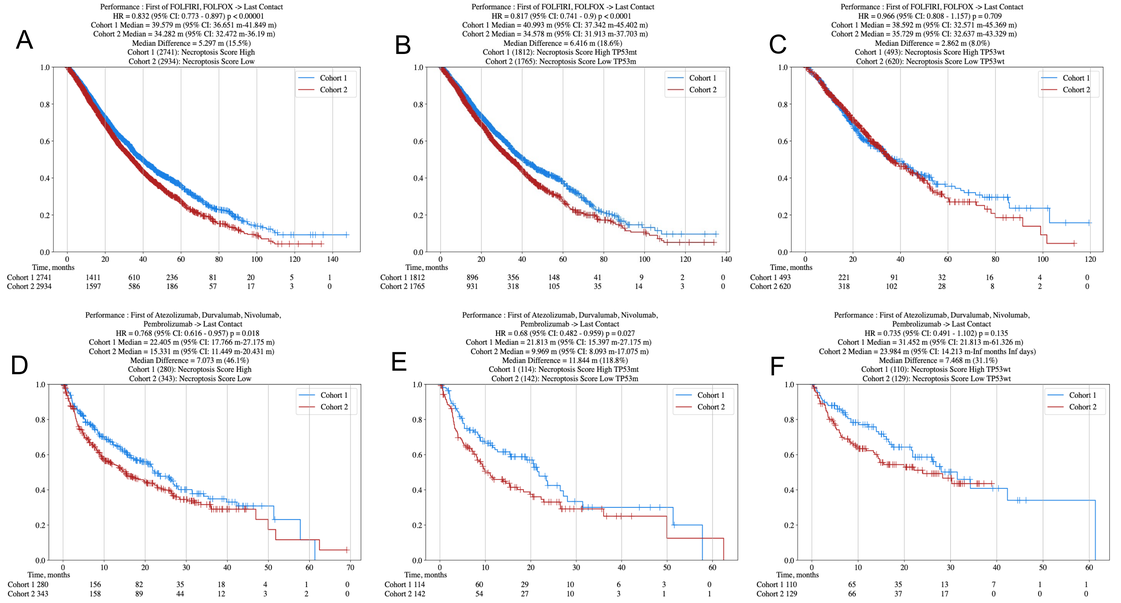
NA-H was associated with activation of allograft rejection, inflammatory/interferon gamma response, pancreatic beta cell function, and downregulation of KRAS signaling pathways (all *q*<0.05).

Figure 3: Tumor Molecular Alterations..



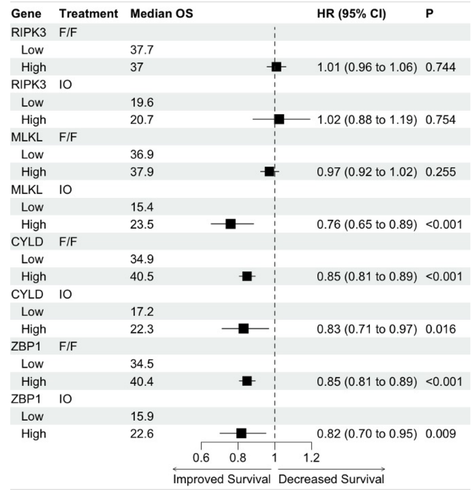
TP53 mutations were more frequent in NA-H, while *SMAD2*, *SMAD4*, *PTEN*, *SOX9*, *KRAS*, and *NRAS* mutations were associated with NA-L (all *q*<0.01).

Figure 4: Overall Survival for CRC Treated with FOLFOX/FOLFIRI in A) Total Cohort B) TP53 Mutant C) TP53 Wildtype and D) Immunotherapy Treated All Cohort E) TP53 Mutant F) TP53 Wildtype.



NA-H showed significantly improved OS in FOLFOX/FOLFIRI (F/F) (NA-L: 34.3 vs NA-H: 39.6 months, *P* < 0.001; HR = 0.83, 95% CI 0.77 – 0.90) and those treated with IO (15.3 vs 22.4 months, *P* = 0.018; HR = 0.77, 95% CI 0.62– 0.96) treated CRC patients. The benefit remained in *TP53*-mutated CRC treated with F/F (34.6 vs 41.0 months, *P* < 0.001; HR = 0.82, 95% CI 0.74-0.90) and IO (10.0 vs 21.8 months, *P* = 0.027; HR = 0.68, 95% CI 0.48-0.96) but not in the *TP53*-wildtype subgroup.

Figure 5: Forrest Plot of Overall Survival by Individual Genes and Treatments.



Improved OS was observed in Immunotherapy treated CRC with increased expression of *CYLD*, *ZBP1*, and *MLKL*. Increased expression of *CYLD* and *ZBP1* improved OS in FOLFOX/FOLFIRI treated CRC(Figure 5). The benefit remained in *TP53*-mutated CRC but not in the *TP53* wildtype subgroups (data not shown).

CONCLUSIONS

- **This is the largest molecular and clinical characterization of necroptotic genes in CRC.**
- **Our data shows that activation of the necroptosis pathway is associated with immune cell infiltration/pathway activation, PD-L1 expression, and improved OS on FOLFOX/FOLFIRI and Immunotherapy. This benefit is more pronounced in *TP53*-mutated CRC, suggesting possible novel therapeutic targets.**