CCR5/*CCL5* gene expression in colorectal cancer (CRC): comprehensive profiling and clinical value

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Introduction

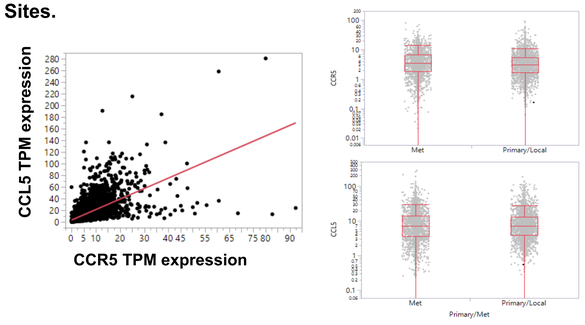
- Cytokine signaling plays a major role in modulating the tumor microenvironment (TME) promoting CRC progression.
- The C-C motif chemokine ligand 5 (CCL5), one of the three ligands that bind to the C-C motif chemokine receptor 5 (CCR5), and the CCR5 receptor itself have been found to be over expressed in CRC, and elevated levels of CCL5 are linked to a poorer clinical outcome.
- Signaling through CCR5 can enable tumor progression and metastasis through multiple mechanisms including cancer stem cell progression, increased angiogenesis, recruitment of immunosuppressive immune and stromal cells, and immunosuppressive polarization of macrophages within the TME.
- We previously reported that genetic polymorphisms in *CCL5* and *CCR5* genes were significantly associated with treatment outcomes in patients with mCRC receiving anti-angiogenic and anti-EGFR treatment.
- Here we aimed to characterize the molecular features associated with *CCR5*/*CCL5* expression in CRC and whether *CCR5*/*CCL5* levels could impact treatment outcome.

Methods

- A total of 7,604 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 *CCR5* and *CCL5* 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 and MCP counter. X^2/Fisher-Exact 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.

Results

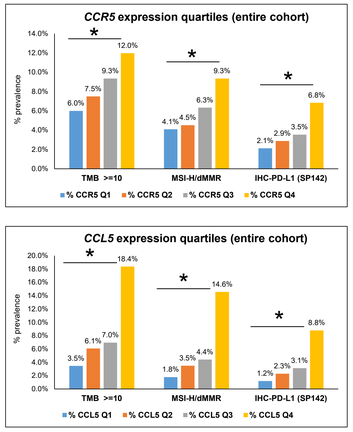
Figure 1. Correlation between *CCR5* and *CCL5* Expression in CRC and Expression Levels in Primary Tumors vs Metastatic Sites.



A linear correlation was observed between *CCR5* and *CCL5* expression ($P < 0.0001$). *CCR5* expression was higher in metastatic sites vs primary tumors (median TPM: 3.44 vs 3.05, $P < 0.001$), while no difference was found in *CCL5* expression levels (median TPM: 7.25 vs 7.16, $P = 1$).
No significant differences were observed in terms of patient demographics (age and gender) across *CCR5* and *CCL5* expression quartiles (data not shown).

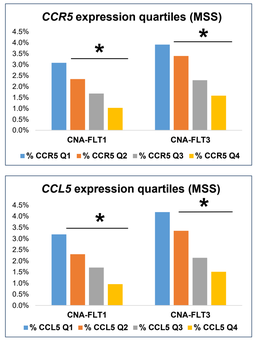
Figure 3. Association with Tumor Molecular Characteristics.

A. Immune Markers



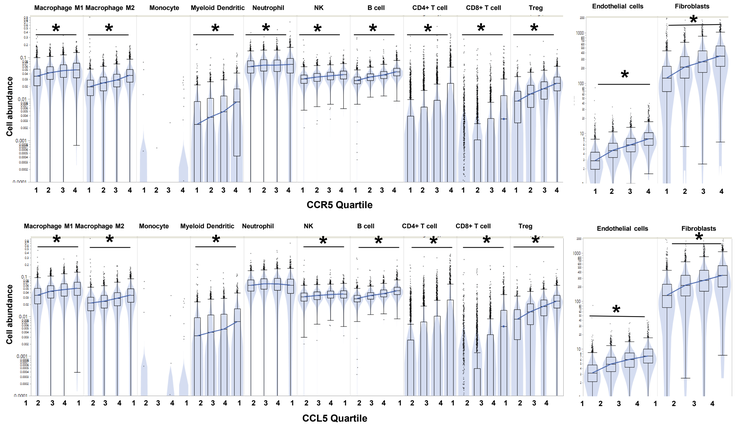
* Q < 0.01; PDL-1 cutoff 2+ 5%

B. Mutations and CNA



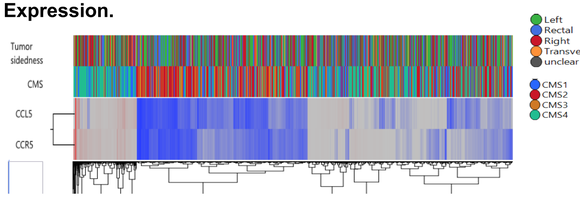
CCR5 and *CCL5* TPMs were associated with higher TMB (≥ 10 Mut/Mb), deficiency in mismatch repair (MMR) and PD-L1 (Q < 0.001). Similar patterns were observed in MMR proficient tumors (data not shown).
CCR5 and *CCL5* TPMs were negatively associated with *APC* mutations (data not shown) and *FLT1*/*FLT3* copy number alterations (CNA) in microsatellite stable (MSS) tumors (Q < 0.01).

Figure 4. TME Cell Infiltration According to *CCR5* and *CCL5* Expression in MSS Tumors.

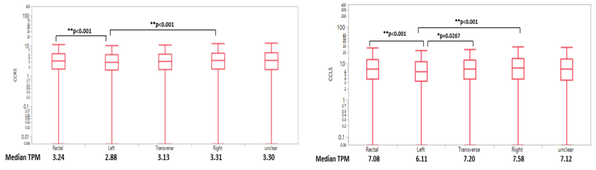


High *CCR5* and *CCL5* were associated with higher immune cell infiltration (including M1 and M2 macrophages, myeloid dendritic cells, B cells, NK cells, CD4+ and CD8+ Tcells, and T regulatory cells), endothelial cells and cancer associated fibroblasts (CAFs) in the TME in MSS tumors (* Q < 0.001).
Relative abundance of infiltrating immune cells suggested a shift towards a more immunosuppressive TME in high *CCR5* and *CCL5* tumors related to changes in the balance of M1/M2 macrophages and Tregs ratio.

Figure 2. Clustering of CMS Subtypes and Primary Tumor Side According to *CCR5* and *CCL5* Tumor Expression.



CCR5 and *CCL5* median expression in right-sided and rectal CRC tumors were significantly higher than left-sided tumors ($P < 0.001$).



CCR5 and *CCL5* expression showed a strong positive correlation with CMS1 and CMS 4 and a negative association with CMS 2 and CMS 3 ($P < 0.0001$, Q1 vs Q4), regardless of MSI status.

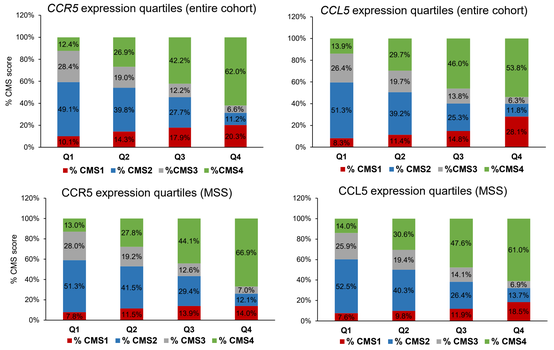
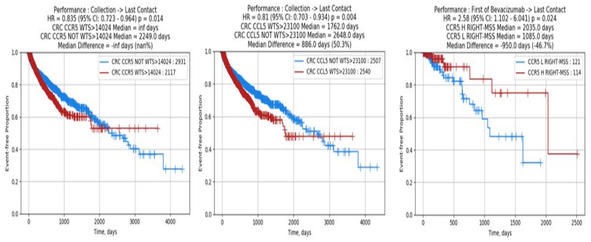


Figure 5. Association between *CCR5* and *CCL5* Expression and Patient Outcomes.



High *CCR5* and *CCL5* expression were associated with poor prognosis (HR 0.83; 95%CI, 0.72-0.96, $P = 0.014$ and HR 0.81; 95%CI, 0.70-0.93, $P = 0.004$, respectively).
CCR5 expression was associated with benefit from bevacizumab-based treatment in right-sided MSS CRC (HR 0.39; 95%CI, 0.16-0.90, $P = 0.024$).

CONCLUSIONS

Our data show a strong association between *CCR5/CCL5* gene expression and distinct molecular features (including CMS, TMB, and PD-L1 expression), TME cell infiltration, patient outcome, and treatment benefit in CRC.

These findings suggest that targeting the CCR5/CCL5 axis may have relevant clinical applications in selected CRC subgroups and chemokines CCL5 and CCL2 may be important targets to modulate the immune TME.