# Comprehensive characterization of neurotransmitters and neuronal signaling (NT) pathway alterations in colorectal cancer (CRC)

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**Abstract ID: 3537**

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## Introduction

- Strong evidence supports the critical role of the gut-brain axis in modulating the gastrointestinal (GI) tract function and homeostasis.
- Aberrant neuronal signaling and neurotransmitters signaling have been shown to play a role in several GI cancer types, activating uncontrolled proliferation and dissemination.
- In addition, neurotransmitters can affect endothelial cells and immune cells in the tumor microenvironment to promote tumor progression.
- Original data from Lenz lab show that single nucleotide polymorphisms in the dopamine pathways are associated with outcome in mCRC patients receiving first-line treatment.
- However, a comprehensive evaluation of the incidence of alterations in these genes/signaling pathways and a molecular characterization of tumors harboring those alterations is lacking.
- Here we evaluated the distribution and molecular context of NT pathway alterations in CRC.

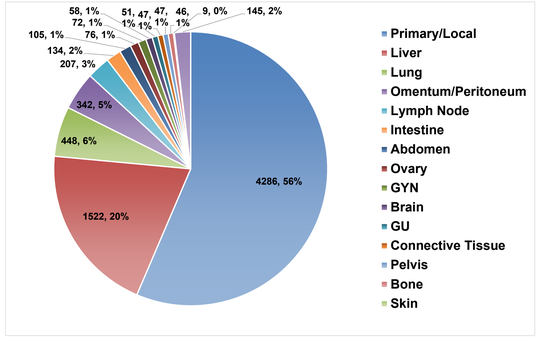
## Methods

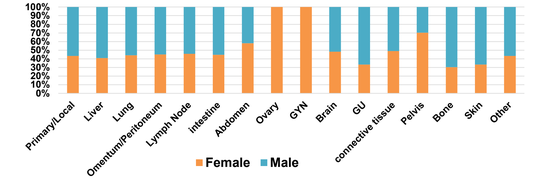
- A total of 7,595 CRC tumors tested at Caris Life Sciences (Phoenix, AZ) with NextGen Sequencing on DNA (Next Seq, 592 genes or NovaSeq, WES) and RNA (NovaSeq, WTS) were analyzed.
- ssGSEA (single-sample gene set enrichment analysis) was used to calculate pathway enrichment scores (ES) of 7 NT gene sets [GABA, nicotinic, muscarinic, dopamine (DA), reelin, glial cell line-derived neurotrophic factor and neurotrophins].
- $X^2$ and Fisher-Exact were used for comparison and significance was determined as *P*-value adjusted for multiple comparison of ($Q$) < 0.05.

| GABA Pathway | DOPAMINE Pathway | RELN Signaling | MUSCARINIC ACETYLCHOLINE Receptors | NICOTINIC ACETYLCHOLINE Receptors | Glial Cell Line–Derived Neurotrophic Factor signaling | Neurotrophins signaling |
|---|---|---|---|---|---|---|
| GABBR1 | DDC | RELN | CHRM1 | CHRNA1 | GDNF | NGF |
| GABBR2 | MAOB | APOER2 | CHRM2 | CHRNA2 | NRTN | BDNF |
| GABRA1 | COMT | DAB1 | CHRM3 | CHRNA3 | NBN | NTF3 |
| GABRA2 | DRD1 | DAB2 | CHRM4 | CHRNA4 | PSPN | NTF4 |
| GABRA4 | DRD2 | LRP8 | CHRM5 | CHRNA5 | | DCLK1 |
| GABRA5 | DRD3 | VLDLR | | CHRNA6 | | NTRK1 |
| GABRA6 | DRD4 | | | CHRNB2 | | NTRK2 |
| GABRB1 | DRD5 | | | CHRNB3 | | NTRK3 |
| GABRB3 | TAAR1 | | | CHRNB4 | | |
| GABRD2 | SLC6A3 | | | | | |
| GBARE | SLC18A2 | | | | | |
| GABRG1 | PPP1R1B | | | | | |
| GBARG2 | C14ORF28 | | | | | |
| GABRG3 | | | | | | |
| GABRQ | | | | | | |
| GABRR1 | | | | | | |
| GABRR2 | | | | | | |
| ABAT | | | | | | |
| ALDHASA1 | | | | | | |
| GAD1 | | | | | | |
| GAD2 | | | | | | |
| SLC6A1 | | | | | | |
| SLC6A13 | | | | | | |
| SLC6A11 | | | | | | |
| SLC6A12 | | | | | | |

## Results

### Figure 1. Sample Distribution and Patient Demographic.



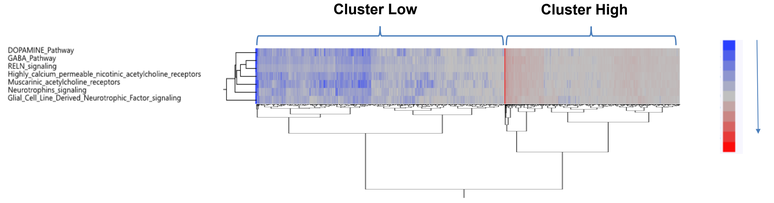




### Table 1. Enrichment Score (ES) According to Metastatic Sites.

| | Dopamine Receptor Genes | GABA receptor Genes | Glial cell Neurotrophic Factor | nACH | mACH | Neurotrophins | RELN |
|---|---|---|---|---|---|---|---|
| Primary/Local (4286) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Abdomen (105) | | * | * | * | * | | * |
| Bone (46) | | | | | | | |
| Brain (58) | * | * | * | * | * | * | * |
| Connective tissue (47) | | | | | | | |
| GU (51) | | | | | | | |
| GYN (72) | | | | | | | |
| Intestine (134) | | | | | | | |
| Liver (1522) | * | | * | * | * | * | * |
| Lung (448) | * | | | | | * | |
| Lymph Node (207) | | | | | | * | |
| Omentum/Peritoneum (342) | * | * | * | * | * | | * |
| Other (145) | | | * | * | * | | |
| Ovary (76) | * | | | | | | |
| Pelvis (47) | | | * | | | | |
| Skin (9) | | | | | | | |

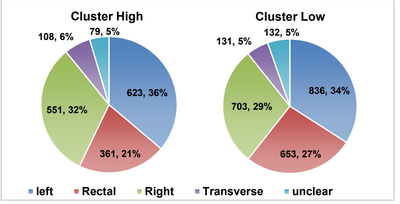
Median ES normalized to Primary/Local. RED: elevated; BLUE: decreased.
* = significantly different after correction for multiple testing

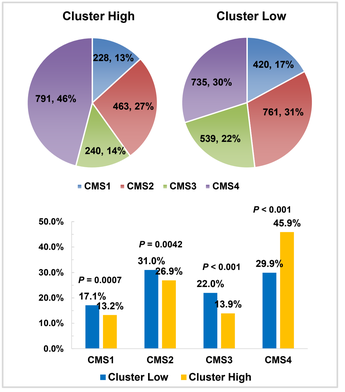
### Figure 2. Clustering of Primary Tumors According to ES.



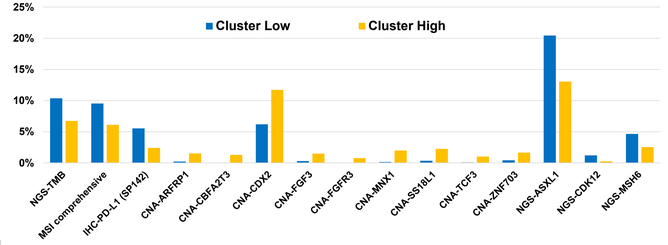

### Figure 3. Tumor Characteristics According to Enrichment Cluster.

| | | Cluster High | Cluster Low | Grand Total |
|---|---|---|---|---|
| | Average Age | 61.1 | 61.6 | 61.4 |
| Gender | Female | 738 | 1069 | 1807 |
| | Male | 984 | 1386 | 2370 |
| Sidedness | Left | 623 | 836 | 1459 |
| | Rectal | 361 | 653 | 1014 |
| | Right | 551 | 703 | 1254 |
| | Transverse | 108 | 131 | 239 |
| | unclear | 79 | 132 | 211 |
| CMS | CMS1 | 228 | 420 | 648 |
| | CMS2 | 463 | 761 | 1224 |
| | CMS3 | 240 | 539 | 779 |
| | CMS4 | 791 | 735 | 1526 |

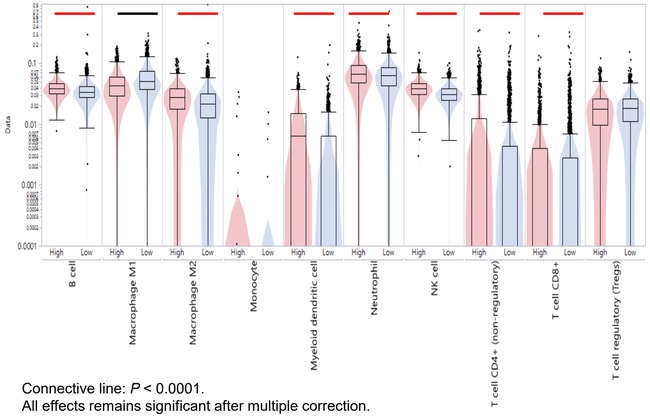



### Figure 4. Molecular Characteristics According to Enrichment Cluster.




### Figure 5. Immune Cell Infiltration According to Enrichment Cluster




Connective line: $P < 0.0001$.
All effects remains significant after multiple correction.

## Summary

- ES based on sample sites showed a substantial heterogeneity in NT enrichment.
- Notably, when compared to primary tumors, all 7 gene sets were significantly enriched in brain metastases (mets; ES ratio 1.14-1.55), while abdomen, liver, and peritoneal mets displayed significant decreases in most NT gene sets. DA was enriched in ovarian and lung mets (ES ratio: 1.18 and 1.09, respectively), the latter also showing increased neurotrophins ES (1.06) (all $Q < 0.05$).
- When investigating primary tumors grouped according to overall ES by unsupervised clustering, right-sided and CMS4 CRCs were more prevalent in the high ES cluster compared to the low ES cluster (32 vs 29%, $P = 0.02$ and 46 vs 30%, $P < 0.001$, respectively).
- Tumors in the high ES cluster showed lower prevalence of TMB-H (≥ 10mt/MB) (7 vs 10%), MSI-H (6 vs 10%) and PD-L1 (2 vs 6%), while higher copy number alterations (CAN) rates were noted in 9 genes (all $Q < 0.05$).
- High ES tumors showed significant positive associations with microenvironment infiltration of B cells, T cells (NK, CD4+ and CD8+ T cells, but not Treg), M2 Macrophages, Myeloid Dendritic Cell, Neutrophils, and an inverse association with M1 Macrophages, regardless of MSI status ($Q < 0.05$).

## Conclusions

- **This is the first and most extensive molecular profiling study to investigate NT signaling pathway alterations in CRC.**
- **Our data show a distinct distribution of pathway enrichment according to metastatic site, distinct molecular features in high vs low ES clusters in primary tumors (including CMS subtypes, TMB, MSI and PD-L1 rates), and differential immune cell infiltration.**
- **These findings support the role of NT signaling in the metastatic spread of CRC and modulation of tumor immune microenvironment.**