Interplay between B cell and GABA metabolism (GABAm) reveals potential immune evasion in Breast Carcinoma

Frances Chow[1], Irene Kang[1], Jun Yin[2], Alex Farrell[2], Vahan Martirosian[3], Priya Jayachandran[3], Evanthia T. Roussos Torres[3], Janice M. Lu[4], Heinz-Josef Lenz[3], Cynthia X. Ma[5], Heather L. McArthur[6], Reva K Basho[7], David Spetzler[2], Josh Neman[3]

1. University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, CA; 2. Caris Life Sciences, Phoenix, AZ; CARIS Life Sciences, Irving, TX; 3. University of Southern California, Keck School of Medicine, Los Angeles, CA; 4. Stony Brook Cancer Center, S. Setauket, NY; 5. Washington University School of Medicine, St. Louis, MO; 6. University of Texas Southwestern Medical Center, Dallas, TX; 7. Cedars-Sinai Medical Center, Los Angeles, CA

Background

GABAergic signaling has been reported to play a pivotal role in breast cancer (BC) tumorigenesis and metastasis, however, its role in immune modulation remains unclear. Recent *in vitro* and *in vivo* studies [1] report the role of B cell-derived GABA metabolites in promoting anti-inflammatory macrophages (MM), thus limiting anti-tumor immunity.

In this study, we aim to characterize the interplay between B cells and the GABAm pathway, as well as their associated immune infiltrates and cytokines.

Methods

- BC tumors (n=9455) were analyzed by next generation sequencing (NextSeq, 592 Genes and WES, NovaSEQ) and whole transcriptome sequencing (WTS, NovaSeq) at Caris Life Sciences.
- Gene set variation analysis (GSVA) scores were used for GABAm pathway activity (GMPA) [2].
- IFN score to test the likelihood of a tumor's response to anti PD1 therapy and Immune cell fraction (quanTIseq) were assessed by mRNA analysis [3].
- Wilcoxon-Mann-Whitney test was applied (p without, q with multiple comparison correction). Correlation coefficients were calculated using spearman correlation.
- BC tumors with high B cell infiltration were then grouped into GMPA-high (B+G+, cutoff >median for both) or GMPA-low (B+/G-), which likely represented tumors with B cell-derived high and low GMPA group, respectively.

Results

- GMPA demonstrated a statistically significant positive correlation with B cells fraction (ρ = 0.24, p<0.0001). When stratified by classical molecular subtypes, the positive correlations were exclusive to HR+ and HER2+ BC, and absent in TNBC. GMPA was the most enriched in HR+ BC, followed by HER2+ and TNBC.

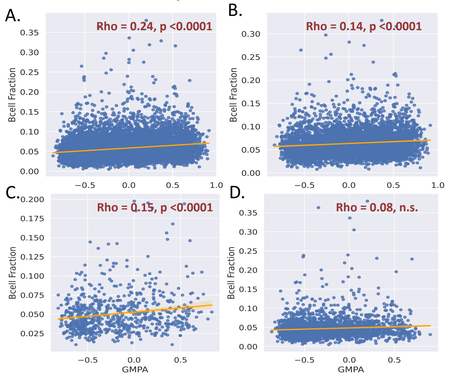

Figure 1. Correlation between GMPA and B cell in A) all breast cancer patients; B) HR + patients; C) HER2+ and D) TNBC patients (rho: spearman correlation coefficient)

- The GMPA-high group demonstrated significantly less fractions of MM1 and CD8+ T cells but greater MM2.

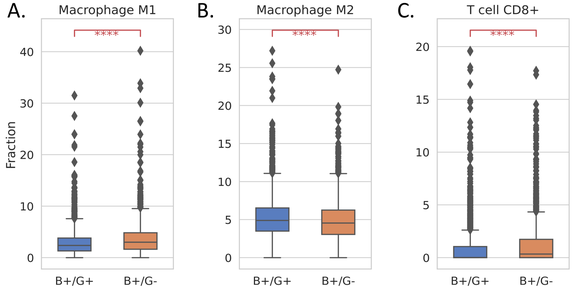

Figure 2. Differential fraction of immune cell populations (A, Macrophage M1; B, Macrophage M2; C, T cell CD8+) between B+/G+ and B+/G- groups

- mRNA levels of the MM2 marker IL10, a proposed marker of immune evasion, was significantly overexpressed in the B+/G+ group compared to the B+/G- group. mRNA levels of GAD1, a GABA-generating enzyme, were higher in B+/G+ than B+/G-. B+/G+ group had notably less IFN score than B+/G- group.

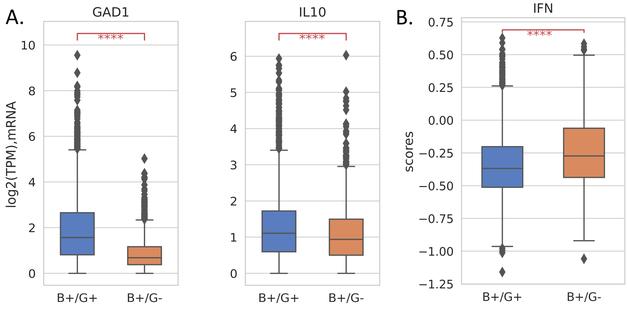

Figure 3. Differential expression of A) GAD1, IL10 and B) IFN scores between B+/G+ and B+/G-.

- When further stratified into molecular subtypes, concurrent more MM2 and less CD8+ T cell fractions were found in B+/G+ compared to B+/G- in HR+ tumors, but not in HER2+ or TNBC tumors.

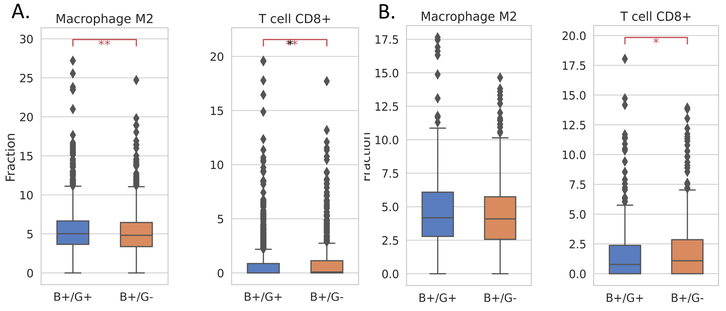

Figure 4. Differential fraction of immune cell populations between B+/G+ and B+/G- when stratified by molecular subtypes (A, HR positive tumors; B, HR negative tumors)

- B+/G+ group also demonstrated a lower IFN score in HR+ tumors. Additionally, IL10 and GAD1 were consistently overexpressed in B+/G+ regardless of subtype.

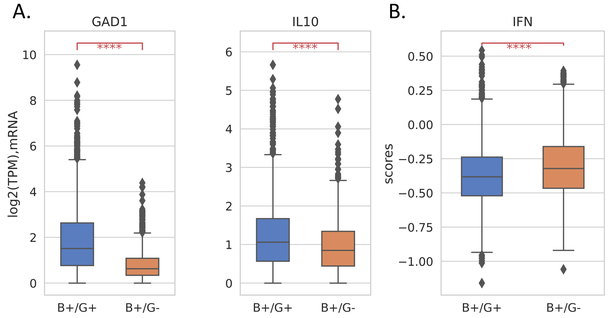

Figure 5. Differential expression of A) GAD1, IL10 and B) IFN scores between B+/G+ and B+/G- in HR positive tumors

Conclusions

- Our study is the largest clinical dataset to demonstrate the association of interplay between B cell and GABAm with immunogenicity.
- Our results support the potential role of B cell-derived GABAm metabolites in immune modulation in BC in a subtype-specific manner.
- Targeting small metabolites to modulate immune evasion in BC warrants further investigation.

References

1. Zhang et al., *Nature*, 2021
2. Hanzelmann et al., *BMC Bioinformatics,* 2013
3. Cristescu et al., *Science*, 2018