Comprehensive molecular and immune characterization of adrenergic stress-signaling receptor ADRB2 in triple negative breast cancer (TNBC)

Sachin Kumar Deshmukh[1], Sharon Wu[1], Joanne Xiu[1], Chi-Chen Hong[2], Song Yao[2], Lisa Sudmeier[3], Pawel Kalinski[2], Pavani Chalasani[4], Elizabeth A. Repasky[2], Marc S. Ernstoff[5], Jose Pablo Leone[6], Saranya Chumsri[7], Stephanie L. Graff[8], Maryam B. Lustberg[9], George W. Sledge Jr.[1], Kevin Kalinsky[3], Shipra Gandhi[2,3]

1. Caris Life Sciences, Phoenix, AZ; 2. Roswell Park Comprehensive Cancer Center; 3. Emory University Winship Cancer Institute, Atlanta, GA; 4. George Washington University, Washington, DC; 5. NCI Division of Cancer Treatment and Diagnosis, Developmental Therapy Program, Bethesda, MD; 6. Dana-Farber Cancer Institute, Boston, MA; 7. Mayo Clinic Florida, Jacksonville, FL; 8. Brown University Health Cancer Institute, The Warren Alpert Medical School of Brown University, Providence, RI; 9. Yale Cancer Center, Yale School of Medicine, New Haven, CT

BACKGROUND

- Chronic stress-mediated β2-adrenergic receptor (β2-AR) signaling promotes tumor growth via immunosuppression in the tumor microenvironment (TME) in preclinical models.
- Blockade of β2-AR has shown higher survival benefit in patients with TNBC in observational studies compared to other breast cancer (BC) subtypes.
- However, the molecular and immunological features associated with ADRB2 (gene for β2-AR) gene expression in TNBC are unknown, prompting this investigation.

METHODS

- 3,038 TNBC samples were analyzed via NGS (592-gene panel, NextSeq; WES/WTS, NovaSeq; Caris Life Sciences, Phoenix, AZ).
- TNBC *ADRB2*-high(H) and *ADRB2*-low(L) RNA expression were classified as above or below the 50 percentile, respectively.
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Pathway enrichment was determined by Gene Set Enrichment Analysis (GSEA, Broad Institute).
- Real-world overall survival (OS) was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates.
- Statistical significance was assessed using chi-square and Mann-Whitney U tests with multiple comparison adjustments (q < 0.05).

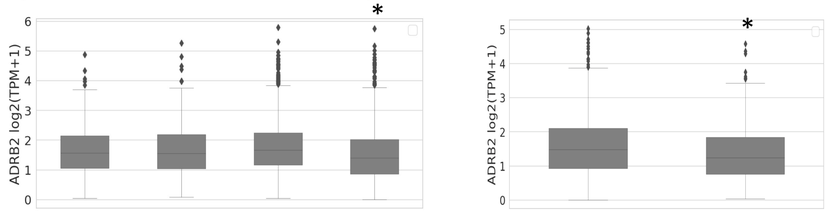
Table 1: Sample demographic information

		ADRB2 low (50th percentile)	*ADRB2* high (50th percentile)
Count (N)		1469	1469
Median age [range]		59 (22 - >89)	62 (22 - >89)
Race	White	56.32% (628/1115)	66.42% (736/1125)
	Black	33.9% (378/1115)	23.91% (269/1125)
	Asian/Pacific Islander	3.68% (41/1115)	4.44% (50/1125)
	Other	6.1% (68/1115)	6.22% (70/1125)
Ethnicity	Not Hispanic or Latino	84.6% (843/1026)	81.55% (922/1099)
	Hispanic or Latino	15.4% (183/1026)	18.45% (177/1099)
Tumor site	Primary	51.26% (753/1469)	47.45% (697/1469)
	Metastatic	48.74% (716/1469)	52.55%(772/1469)

Race/ethnicity data is self-reported

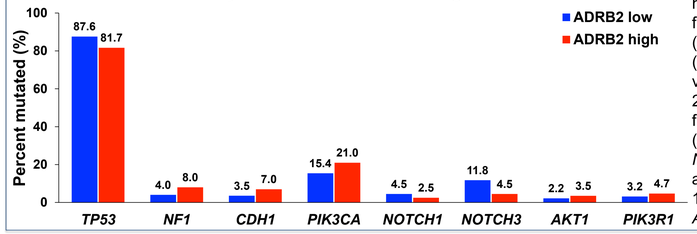
RESULTS

Figure 1. *ADRB2* expression in BC subtype and race



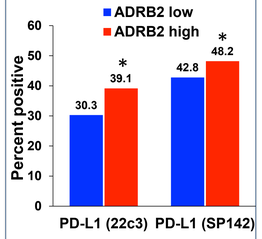
*ADRB2 gene expression was lowest in TNBC (median (TPM: 1.3) compared to N = 629 HR+HER2+ (1.5), N = 453 HR-HER2+ (1.5), and N = 4,918 HR+HER2- (1.7) BC (all q < 0.05). African American or Black patients (N = 670) had lower expression of ADRB2 compared to European American or White (N = 1,412) TNBC patients (1.2 vs 1.5), *q < 0.05.*

Figure 2. Mutation analysis of *ADRB2*-low vs high TNBC



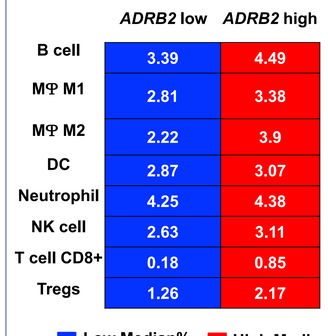
ADRB2-H TNBC had higher mutation frequency of *PIK3CA* (21% vs 15.4%), *CDH1* (7% vs 3.5%), *NF1* (8% vs 4%), *AKT1* (3.5% vs 2.1%), but lower frequency of *TP53* (81.6% vs 87.5%), *NOTCH1* (2.5% vs 4.5%) and *NOTCH3* (4.4% vs 11.7%) compared to *ADRB2*-L, all q<0.05.

Figure 3. PD-L1 positivity



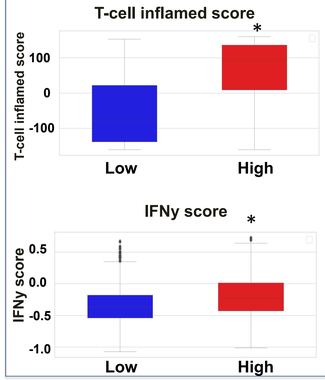
*ADRB2-H had greater PD-L1 positivity for clone 22C3 (39.1% vs 30.2%) and SP142 (42.8% vs 48.2%) , *q < 0.05*

Figure 4. Immune cell infiltration



ADRB2-H had higher infiltration of B cells (4.5% vs 3.4%), M1 Mφ (3.4% vs 2.8%), M2 Mφ (3.9% vs 2.2%), Tregs (2.2% vs 1.3%), NK cells (3.1% vs 2.6%), DC (3.1% vs 2.9%), CD8 T cells (0.9% vs 0.2%), all q< 0.05.

Figure 6. T-cell inflamed and IFNy score



ADRB2-high TNBC had higher T-cell inflamed score (95 vs -80) and *IFNγ* score (-0.23 vs -0.37) compared to *ADRB2*-low. *q < 0.05;*

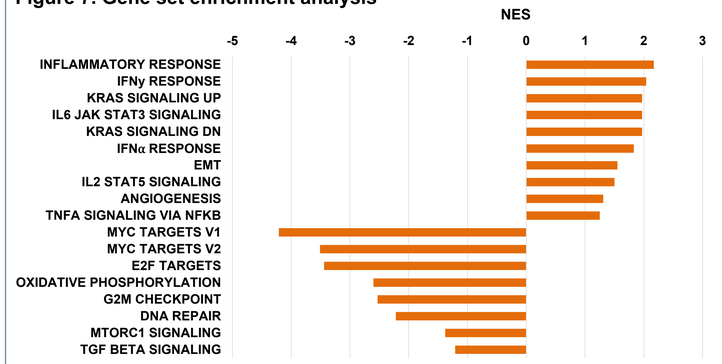
Figure 5. Immune-checkpoint gene expression (TPM)

	ADRB2 low	*ADRB2* high
CD274	2.79	6.66
PDCD1	0.29	0.79
PDCD1LG2	0.98	2.12
CTLA4	0.91	2.91
LAG3	2.58	5.22
HAVCR2	12.83	26.99
FOXP3	1.71	3.74
IDO1	1.42	3.94
TNFSF14	0.30	0.62
TIGIT	0.67	2.08
BTLA	0.77	2.86
CEACAM1	19.43	31.23
CD47	42.66	67.83
CD80	3.06	6.90
CD86	6.09	13.12
CD160	2.62	4.23

Low TPM (Median) High TPM (Median)

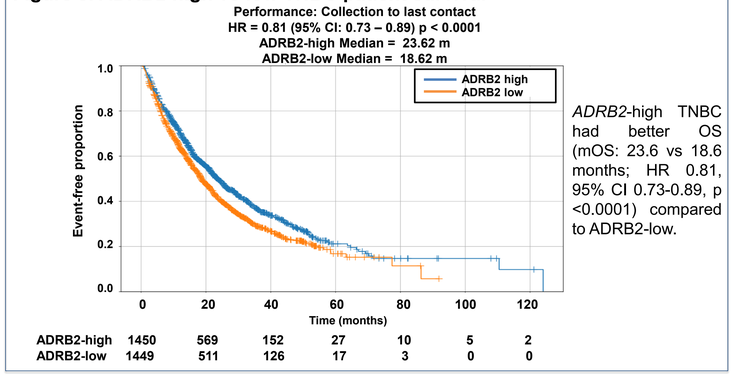
ADRB2-H TNBC had higher expression of immune checkpoint genes (*CD274, PDCD1, PDCD1LG2, CTLA4, LAG3, HAVCR2, FOXP3, IDO1, TNFSF14, TIGIT, BTLA, CEACAM1, CD47;* fold change: 1.6-3.7, all q < 0.05).

Figure 7. Gene set enrichment analysis



ADRB2-H tumors had higher expression of genes related to inflammatory response, *IFNγ* response, *IL6-JAK-STAT3* signaling (normalized enrichment score (NES): 1.9 – 2.1), while *ADRB2-L* had enrichment of *MYC* targets V1, *MYC* targets V2, E2F targets and G2M checkpoint (NES: 2.5– 4.2), all FDR < 0.01.

Figure 8. *ADRB2* high vs low TNBC patient survival



ADRB2-high TNBC had better OS (mOS: 23.6 vs 18.6 months; HR 0.81, 95% CI 0.73-0.89, p <0.0001) compared to ADRB2-low.

CONCLUSIONS

High *ADRB2* expression in TNBC is associated with better survival and an immune enriched TME, elevated immune checkpoints and other targetable vulnerabilities. Future studies are needed to investigate ADRB2 as a potential stress biomarker and therapeutic target.