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Tumor-derived ZAG expression is associated with immunologically cold tumors and poor response to neoadjuvant chemotherapy in TNBC

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Purpose

An immunosuppressive tumor microenvironment (TME) contributes to therapeutic resistance in breast cancer[1]. Immune checkpoint inhibitors (ICIs) combined with chemotherapy have emerged as a promising strategy to overcome this barrier. Understanding immune regulation within the TME is essential for developing effective biomarkers and therapies. Our previous work suggested an immunomodulatory role for ZAG (*AZGP1*), with both clinical and in vitro findings consistently indicating its immunosuppressive activity[2], potentially contributing to therapeutic resistance. This study evaluates the relationship between ZAG expression and response to neoadjuvant chemotherapy (NAC) in triple-negative breast cancer (TNBC).

Methods

To determine the cellular source of *AZGP1*, we reanalyzed a public TNBC single-cell RNA-seq dataset[3] using Seurat v5.2.1. UMAP clustering was used to annotate cell types. Feature plots and violin plots were employed to visualize cell type-specific *AZGP1* expression. Gene set enrichment analysis (GSEA) was performed using bulk transcriptomic data from four NAC-treated TNBC cohorts to explore pathways associated with pathological complete response (pCR) and *AZGP1* expression. ROC analysis was used to assess the predictive value of *AZGP1* for pCR and to determine optimal cut-off values. For validation, we analyzed NAC-treated TNBC cases at our institution. Pre-treatment core needle biopsy (CNB) samples were evaluated for tumor-infiltrating lymphocytes (TILs) based on International TILs Working Group criteria. ZAG expression was assessed by immunohistochemistry. The ZAG score was defined as the percentage of tumor cells showing moderate-to-strong staining. ROC analysis was also performed to evaluate the ZAG score's predictive value for pCR (ypT0/isN0, ypT0N0), overall ypN0, and ypN0 in cN1 cases.

Results

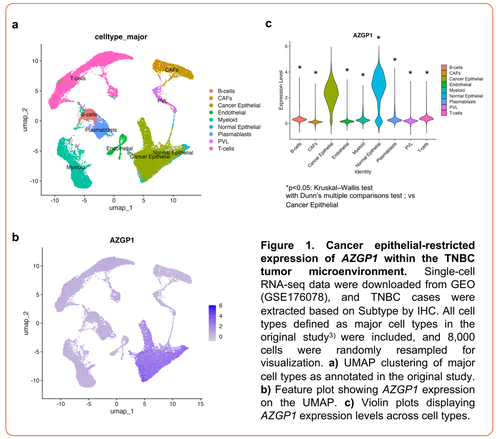
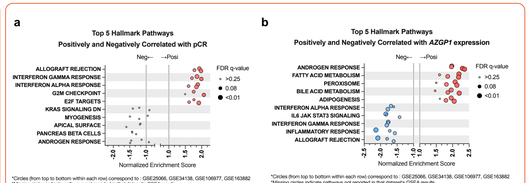


Figure 1. Cancer epithelial-restricted expression of *AZGP1* within the TNBC tumor microenvironment. Single-cell RNA-seq data were downloaded from GEO (GSE176078), and TNBC cases were extracted based on Subtype by IHC. All cell types defined as major cell types in the original study[3] were included, and 8,000 cells were randomly resampled for visualization. **a)** UMAP clustering of major cell types as annotated in the original study. **b)** Feature plot showing *AZGP1* expression on the UMAP. **c)** Violin plots displaying *AZGP1* expression levels across cell types.



*Circles (from top to bottom within each row) correspond to : GSE25066, GSE34138, GSE106977, GSE163882
*Missing circles indicate pathways not reported in that dataset's GSEA results.

Table 1. Datasets* used in the analysis

Data Set (n)	Platform	Chemotherapy Regimen	Definition of pCR
GSE25066 (178)	Array	Sequential anthracycline- and taxane-based regimens	ypT0N0isN0
GSE163882 (90)	RNA-Seq	Taxane-based regimens	N/A
GSE34138 (58)	Array	Dose-dense anthracycline and cyclophosphamide	ypT0N0isN0
GSE106977 (119)	Array	Anthracycline and/or taxane +/- carboplatin	ypT0N0isN0

*: Datasets containing at least 50 TNBC cases were selected for analysis.

Figure 2. Gene set enrichment analysis of pCR and *AZGP1* expression in TNBC cohorts. Bulk transcriptomic data summarized in Table 1 were analyzed using Hallmark gene sets. For datasets including all breast cancer subtypes, only TNBC cases were extracted for analysis. GSEA was performed separately for each dataset, and pathways were ranked by the average normalized enrichment score (NES). The top five positively and negatively enriched pathways (based on average NES) are shown. **a)** Pathways correlated with pCR. **b)** Pathways correlated with *AZGP1* expression. Circles within each row represent the individual datasets. Gray circles indicate results with FDR q > 0.25.

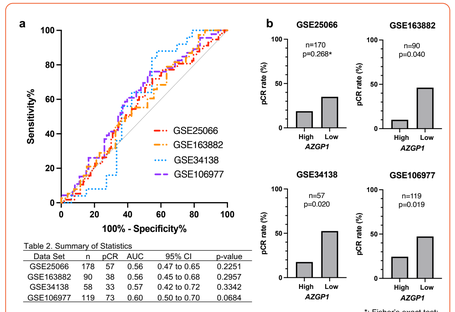


Table 2. Summary of Statistics

Data Set	n	pCR	AUC	95% CI	p-value
GSE25066	178	57	0.56	0.47 to 0.65	0.2251
GSE163882	90	38	0.56	0.45 to 0.68	0.2957
GSE34138	58	33	0.57	0.42 to 0.72	0.3342
GSE106977	119	73	0.60	0.50 to 0.70	0.0684

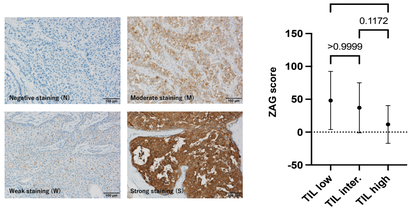
*: Fisher's exact test:

Figure 3. ROC analysis of AZGP1 expression for predicting pCR in NAC-treated TNBC cohorts. Bulk transcriptomic data from the same datasets as in Figure 2 were analyzed. The optimal cut-off was determined using Youden's index, and patients were stratified into *AZGP1*-high and *AZGP1*-low groups. **a)** ROC curves evaluating the ability of *AZGP1* expression to predict pCR in each dataset, with summary statistics provided in Table 2. **b)** Comparison of pCR rates between *AZGP1*-high and *AZGP1*-low groups in each dataset.

Table 3. Patient Characteristics

	n=51		n=51
Age		**NAC Regimen**	
Mean ± SD	57.2 ± 10.5	Sequential A →T*	44 (86.3%)
Location		ICI combination	3 (5.9%)
L	26 (51.0%)	Other regimens	4 (7.8%)
R	25 (49.0%)	**Clinical Response to NAC**	
Menopausal state		PD	5 (9.8%)
Unknown	2	SD	7 (13.7%)
Post	34 (69.4%)	PR	29 (56.9%)
Pre	15 (30.6%)	CR	10 (19.6%)
Clinical Stage (UICC TNM)		**ypT0**	
1	3 (5.9%)	No	40 (78.4%)
2	30 (58.8%)	Yes	11 (21.6%)
3	18 (35.3%)	**ypT0/is**	
Histological Type		No	32 (62.7%)
Unknown	1	Yes	19 (37.3%)
IDC	48 (96.0%)	**ypN0**	
Special type	2 (4.0%)	No	11 (21.6%)
Nuclear Grade		Yes	40 (78.4%)
Unknown	2	**pCR ypT0N0**	
1	21 (42.9%)	No	40 (78.4%)
2	11 (22.4%)	Yes	11 (21.6%)
3	17 (34.7%)	**pCR ypT0/isN0**	
Ki67 (%)		No	35 (68.6%)
Mean ± SD	54.1 ± 25.6	Yes	16 (31.4%)
TIL Score			
Unknown	2		
0	19 (38.8%)		
1	17 (34.7%)		
2	13 (26.5%)		

*: Anthracycline and Taxane



*: Kruskal–Wallis test with Dunn's multiple comparisons test

Figure 4. Institutional cohort analysis of ZAG expression by immunohistochemistry in NAC-treated TNBC cases. Table 3 summarizes the patient characteristics. Pre-treatment core needle biopsy specimens were evaluated for tumor-infiltrating lymphocytes (TILs) according to International TILs Working Group criteria. ZAG expression was assessed by immunohistochemistry, and the ZAG score was defined as the percentage of tumor cells showing moderate-to-strong staining. **a)** Representative images of ZAG immunostaining (negative, weak, moderate, strong). **b)** Association between ZAG score and TIL score categories.

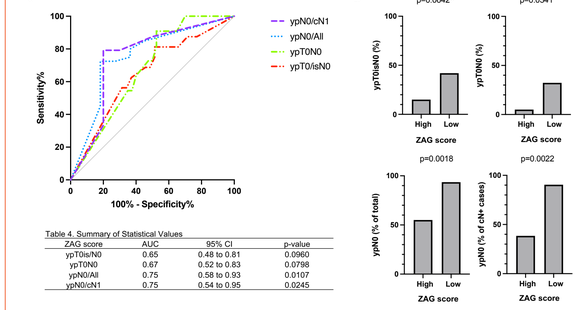


Table 4. Summary of Statistical Values

ZAG score	AUC	95% CI	p-value
ypT0/isN0	0.65	0.48 to 0.81	0.0960
ypT0N0	0.67	0.52 to 0.83	0.0798
ypN0/All	0.75	0.58 to 0.93	0.0107
ypN0/cN1	0.75	0.54 to 0.95	0.0245

*: Fisher's exact test:

Figure 5. ROC analysis of ZAG score for predicting treatment response in NAC-treated TNBC cases. ROC analysis was performed to evaluate the predictive value of ZAG score for pCR (ypT0/isN0, ypT0N0), overall ypN0, and ypN0 in cN1 cases. The optimal cut-off was determined using Youden's index, and patients were stratified into ZAG-high and ZAG-low groups. **a)** ROC curves evaluating the ability of ZAG score to predict each outcome, with summary statistics provided in Table 4. **b)** Comparison of outcome proportions between ZAG-high and ZAG-low groups in each category.

Summary

- **Figure 1.** Single-cell RNA-seq analysis confirmed that *AZGP1* expression was restricted to tumor epithelial cells.
- **Figure 2.** GSEA revealed that pCR was associated with enrichment of immune-related gene set, while *AZGP1* expression negatively correlated with these pathways.
- **Figure 3.** In four public TNBC cohorts, *AZGP1* showed modest predictive value for pCR, but high-*AZGP1* groups had significantly lower pCR rates in three cohorts.
- **Figure 4.** In our institutional cohort of 51 cases (94.1% stage II–III, mean age 57.2), 86.3% received anthracycline–taxane regimens, and pathological CR (ypT0/isN0) was achieved in 31.4% of cases. ZAG scores were significantly higher in tumors with low TILs ($p < 0.05$).
- **Figure 5.** High ZAG expression was associated with poorer response to NAC, particularly in terms of nodal clearance in cN1 cases.

Conclusions

ZAG (*AZGP1*), shown to be tumor-derived via single-cell RNA-seq, acts as a paracrine immune modulator[4] in TNBC, supporting its validity as a tumor-intrinsic marker in bulk transcriptomic analyses. High *AZGP1* expression correlated with suppressed immune gene programs and reduced chemotherapy sensitivity. Our data confirmed that elevated ZAG expression predicts poor NAC response and reduced nodal clearance, particularly in cN1 cases. ZAG represents a promising tumor-intrinsic biomarker and potential immunotherapeutic target in TNBC.

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Conflict of interest

The authors declare no conflicts of interest.

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References

1. Tilsed, C. M., Fisher, S. A., Nowak, A. K., Lake, R. A. & Lesterhuis, W. J. Cancer chemotherapy: insights into cellular and tumor microenvironmental mechanisms of action. Frontiers in oncology 12, 960317 (2022).
2. Hanamura, T. et al. Investigating the immunological function of alpha-2-glycoprotein 1, zinc-binding in regulating tumor response in the breast cancer microenvironment. Cancer Immunol Immunother 73, 42 (2024).
3. Wu, S. Z. et al. A single-cell and spatially resolved atlas of human breast cancers. Nature genetics 53, 1334-1347 (2021).
4. Hanamura, T. et al. Secreted indicators of androgen receptor activity in breast cancer pre-clinical models. Breast Cancer Research 23, 102 (2021).

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