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Identification of key genes and pathways involved in trastuzumab resistance in HER2-positive gastric cancer through integrative bioinformatics analysis

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ABSTRACT

Objective(s): Trastuzumab resistance limits therapeutic efficacy in HER2-positive gastric cancer, and its systems-level regulatory architecture remains poorly characterized. This study aimed to identify key regulatory modules, biomarkers, and candidate therapeutic targets associated with trastuzumab resistance.

Materials and Methods: Gene expression profiles from trastuzumab-sensitive and -resistant gastric cancer cell lines (GSE77346) were analyzed to identify differentially expressed genes (DEGs). Protein–protein interaction (PPI) networks were constructed, and hub genes were identified using topology-based methods and clustering. Functional enrichment analysis was performed using Gene Ontology and KEGG pathways. Prognostic relevance was evaluated using TCGA-STAD data via UALCAN. Drug–gene interactions were explored using DrugBank and PHAROS, followed by structural modeling and molecular docking.

Results: Trastuzumab resistance was linked to transcription factor-driven reprogramming, cytoskeletal and adhesion remodeling, and receptor tyrosine kinase (RTK) bypass signaling. Eleven hub genes were identified across four functional modules potentially associated with FGFR signaling, epithelial–mesenchymal transition (EMT), and lineage plasticity. Elevated PXDN and CDH2 expression was significantly associated with poor overall survival in TCGA-STAD. Molecular docking provided preliminary in silico evidence for potential interactions between selected compounds, including ADH-1, AGX51, artenimol, and phenethyl isothiocyanate, and candidate targets, including N-cadherin/CDH2, ID3, and vimentin.

Conclusion: Trastuzumab resistance may involve networks related to transcriptional plasticity, adhesion dynamics, and RTK redundancy. This study provides a hypothesis-generating framework for future biomarker-guided combination strategies, which requires validation in HER2-positive cohorts and experimental models.

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Introduction

Despite advances in therapy, gastric cancer (GC) remains one of the most frequent and fatal malignancies worldwide, ranking among the top causes of cancer-related mortality (1). The molecular heterogeneity of GC has prompted stratification of tumors by molecular type and the development of targeted agents against principal oncogenic drivers, such as the HER2 oncogene (human epidermal growth factor receptor 2)(2). The monoclonal antibody trastuzumab, targeted toward HER2, has been an invaluable agent for managing HER2-overexpressing GC, conferring a substantial survival advantage (3). Nevertheless, the development of resistance to trastuzumab severely affects patient outcomes (4). This underscores the need to overcome therapeutic resistance. Understanding the molecular processes associated with trastuzumab resistance is therefore important for generating hypotheses regarding potential combination strategies and predictive response biomarkers.

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The biological intricacy of trastuzumab resistance reflects the multilayered regulation of signaling in cancer, including transcriptional reprogramming, chromatin remodeling, and changes in receptor tyrosine kinase (RTK) networks (5-8). Before investigating downstream-specific pathways, it is important to note that network-based studies examine relationships between proteins/genes rather than scrutinizing single players. This approach provides an overall picture of cancer biology. Accumulating evidence suggests that resistance may not depend solely on single-gene alterations but may also involve dysregulation of coordinated pathways related to cell growth, adhesion, and epithelial–mesenchymal transition (EMT)(9). Network-based analyses have proven powerful in unraveling these interconnections, offering a systems-level understanding of cancer biology that transcends reductionist models (10). Previous studies have proven that the methods of systems biology are capable of unraveling mechanisms of oncogenesis in the varied portfolio of cancers, such as dysregulation of Wnt signaling in breast cancer (11), dysregulation of PI3K-Akt-mediated signaling cascade and the corresponding prognostic implication during GC (12), and gene network construction to distinguish molecular entities playing roles during the pathogenesis of GC (13). In conclusion, the current studies have shown that integrating gene expression data with interaction and pathway networks helps explain the key transcriptional regulators and signaling modules underlying tumor behavior.

Despite growing insight into the biology of trastuzumab resistance, significant knowledge gaps remain. These gaps can be framed as precise questions: How do global network architectures, rather than isolated genes and molecular cascades such as the PI3K/Akt axis, TGF-β, and EMT regulators, contribute to trastuzumab resistance? What higher-order regulatory modules coordinate resistance phenotypes? Earlier studies demonstrated the importance of gene interaction networks in identifying novel drivers of various cancers, including VAT-1, Humanin, and FOXO3/MYD88 (14-19). However, similar integrative frameworks have not been systematically applied to trastuzumab-resistant GC. Moreover, do lineage plasticity and transcription factor (TF) networks play a significant role in mediating escape from HER2 blockade, and how can these be systematically elucidated? Articulating these gaps as study questions enables us to focus our inquiry on these inadequately understood elements and methodically delineate these intricate relationships.

This study addresses existing gaps by using a systems-level bioinformatics approach that integrates differential expression profiling, protein interaction network analysis, and functional enrichment to identify candidate regulatory modules potentially associated with trastuzumab resistance in GC. This study builds on previous investigations in network-based modeling and transcriptomic profiling across various cancer types, including T-DM1 resistance in esophageal cancer (20), miRNA-mediated regulation in hepatic and glioblastoma progression (21, 22), and nanomaterial biocompatibility mechanisms (23). In this study, we used these computational methods to develop an integrated molecular platform for trastuzumab resistance. This strategy identified hub genes such as SOX2, PXDN, CDH2, VIM, and ID3, which may be associated with phenotypic variation and cell communication.

This study provides a comprehensive systems-level analysis of trastuzumab resistance in GC by integrating transcriptomic profiling, network modularity, and drug repurposing strategies. Unlike previous studies focusing on isolated mechanisms, this work explores the potential interplay between transcriptional reprogramming, RTK bypass signaling, and adhesion remodeling within a unified framework, while incorporating structural modeling to bridge computational discovery with translational therapeutic insights.

Materials and Methods

Dataset screening and selection

A thorough screening of the complete Gene Expression Omnibus (GEO) repository, encompassing 149 series of gastric tumor datasets, was conducted to identify appropriate datasets according to specific criteria: (1) The information derived from gene expression analysis using microarray technology, (2) trastuzumab-resistance projects related to GC, (3) analysis of resistant and sensitive GC samples, and (4) a minimum of two samples per condition were included. Following a rigorous screening process, the most appropriate dataset was selected for subsequent analysis (24).

Identification of differentially expressed genes (DEGs)

Differential expression analysis was performed using the GEO2R online tool to compare trastuzumab-resistant and trastuzumab-sensitive GC cells in the GSE77346 dataset. GEO2R uses the GEOquery and limma packages from Bioconductor/R for expression data processing and statistical analysis. Multiple-testing correction was performed using the Benjamini–Hochberg false discovery rate (FDR) method. Genes were considered differentially expressed based on an adjusted P-value<0.05 and an absolute log2 fold-change threshold of $|\log2FC| \geq 1.5$. For downstream PPI network construction and hub gene prioritization, up-regulated genes in trastuzumab-resistant cells were selected (25).

Construction and functional analysis of the protein–protein interaction (PPI) network

The STRING webserver provides predicted and confirmed protein interactions. We used STRING v.12 to analyze the interactions of notably overexpressed genes, setting the confidence level to low (minimum interaction score: 0.15)(26). The network was then imported into Cytoscape v.3.9.1 to visualize the molecular interactions and topological relationships among the nodes. The hub proteins in the nodes were detected using CytoHubba v.0.1 by applying four topological analysis strategies: MCC, MNC, Degree, and DMNC. The four highest scored nodes in every algorithm were detected as hub nodes and the subnetwork of interactions in the hubs was visualized by applying the plugin of CytoHubba In the functional examination of subnetworks we used the Gene Ontology (GO) and KEGG databases, encompassing biological process (BP), cellular components (CC), and molecular function (MF) in the online program from STRING (12, 13).

Cluster analysis

CytoCluster v.2.1.0 was used to group the nodes in the network by IPCA to explore subnetwork clusters.

The density-based method detects dense subgraphs by analyzing edge weights with the aid of common neighbors and computes node weights by summarizing incident edge weights (27). A threshold of 0.9 was set, leading to gene analysis within each cluster using Enrichr to identify associated pathways (Reactome)(28-30).

Survival analysis and therapeutic target screening

The association between hub gene expression and overall survival was evaluated using Kaplan–Meier survival analysis based on TCGA-STAD data accessed through the UALCAN online resource (University of Alabama at Birmingham; https://ualcan.path.uab.edu/). For each hub gene, patients were stratified according to the UALCAN-defined expression groups, and survival outcomes were compared between the high-expression group and the low/medium-expression group. Statistical significance was assessed using the log-rank test, and *P*-values reported by UALCAN were used to determine the prognostic relevance of hub gene expression. To identify potential therapeutic molecules targeting the hub genes, drug-gene interaction screening was performed using the PHAROS and DrugBank databases (31).

Structure preparation of proteins and drugs

The protein structure of VIM was retrieved and identified by the PDB ID 8RVE from the RCSB Protein Data Bank. The 3D conformations of the receptors peroxidasin (PXDN), ID-3, and CDH2 are not available, so they were predicted using the Robetta online tool. The quality of the modeled structures was analyzed using MolProbity, ProSA web, ERRAT, and Ramachandran plot tools. The Ramachandran plot calculates torsion angles of residues, ProSA predicts an overall model quality Z-score, MolProbity predicts structure parameters, and ERRAT detects atomic interaction differences. The SAVES web server was used to determine an ERRAT score. The receptor structures were prepared using the Dock prep tool in Chimera 1.17.3, with hydrogen and charge added and water molecules and ligands removed. 3D structures of approved drugs were collected from the PubChem compound database and downloaded in Structure Data File (.sdf) format and prepared using AutoDock tools according to standard protocols, saving them as .pdbqt files (32).

Molecular docking (MD)

AutoDock Vina, a commercially successful and highly effective MD software package, was applied here. Compared with others like AutoDock 4, AutoDock Vina also has a doubling in processing speed, with a substantial increase in accuracy for docking mode prediction. Molecular docking analysis included VIM, PXDN, CADH2, and ID-3 receptor binding with pre-designed compounds generated by AutoDock Vina. AutoDock Vina binding affinity value prediction analysis was done to find the most effective drugs with high binding affinities. The resulting docking visualizations were generated with PyMOL v1.1.7 and Discovery Studio v4.5 (33).

Results

Dataset screening and differential expression analysis of trastuzumab sensitivity

Following a thorough screening process, the GSE77346 dataset was chosen as the preferred database, containing both trastuzumab-sensitive and -resistant GC cells. This analysis was conducted using the Illumina HumanHT-12 V4.0 Platform.

To establish molecular bases for trastuzumab sensitivity in GCs, we first performed a genome-wide differential expression analysis between sensitive and resistant cell lines. From the volcano plot (Figure 1a), a large number of significantly dysregulated genes were indicated in resistant cells (Red: upregulation, blue: down-regulation, black: non-significant). Strong distribution of genes away from the vertical axes indicates active transcriptional reprogramming with acquired trastuzumab resistance.

Further quantification of the transcriptomic changes was achieved through Venn diagram analysis (Figure 1b). Differential expression analysis revealed 4,032 genes exhibiting significant transcriptional changes (adjusted *P*-value<0.05), a testament to the broad-based nature of transcriptional alterations accompanying resistance. Such a broad-based DEG landscape suggests that trastuzumab resistance is neither controlled by a handful of gene mutations nor a single gene defect but is a highly multilayered rewiring of gene regulatory circuits.

To determine distribution and expression variation within samples, a boxplot has been generated (Figure 1c). As part of this analysis, it confirmed similar overall expression profiles within each set but highlighted significant expression-level differences between sensitive and resistant cells. Reproducibility and similarity among such patterns confirm the robustness of the DEG dataset and indicate clear transcriptional differentiation between the two phenotype states.

To depict the comprehensive separation among expression patterns, Uniform Manifold Approximation and Projection (UMAP) analysis was performed (Figure 1d). The UMAP plot delineated complete segregation among trastuzumab-sensitive and -resistant samples to the extent that they did not intersect. Such clear segregation signifies

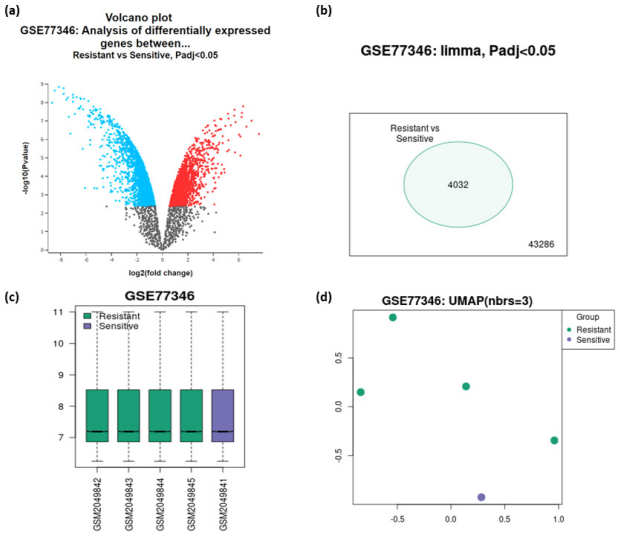


Figure 1. Graphical representations of DEGs between trastuzumab-sensitive and -resistant GC cells
(a) Volcano plot: red=up-regulated, blue=down-regulated, black=no change. (b) Venn diagram showing 4,032 DEGs (adjusted *P*<0.05). (c) Boxplot comparing expression profiles of sensitive vs. resistant cells. (d) UMAP plot showing complete separation between the two groups

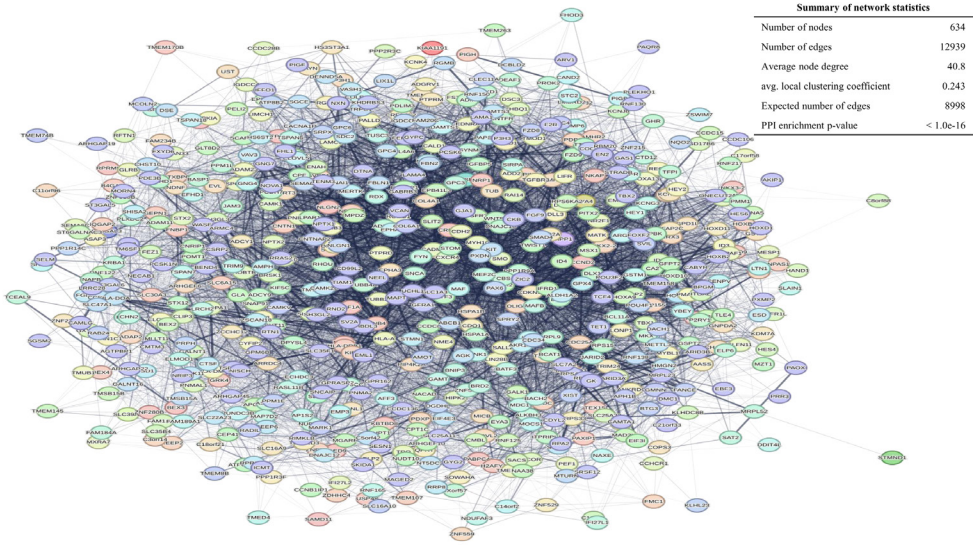


Figure 2. Network representation of up-regulated genes in trastuzumab-resistant gastric cancer (GC) cells along with their established interactors, identified using the CytoHubba application

the fundamental biological dissimilarity between these phenotypes and supports the validity of DEG identification. In general, such analyses collectively offer strong evidence that trastuzumab resistance among GCs is accompanied by extensive transcriptomic rebuilding that opens avenues to subsequent pathway enrichment as well as network-based investigations.

Reconstruction and analysis of PPI networks

Construction of the protein–protein interaction (PPI) network revealed 634 nodes and 12,939 edges, which were markedly enriched relative to random expectation (P<1.0e−16)(Figure 2). Strong functional interrelationships among the DEGs arise from high-density connectivity (average node degree 40.8). CytoHubba analysis identified eleven hub genes with high centrality values (Table 1). These included transcription factors (SOX2, MESP1, SIX4, NKX3-2, ID3), signal mediators (WNT5A, FGF9, BMP2), and structural/adhesive proteins (CDH2, VIM, PXDN). Of these proteins, SOX2 ranked first, underscoring its central regulatory role. As a set, they represent a concentrated molecular signature that can be harnessed as markers to predict resistance and identify new therapeutic targets.

Functional enrichment analysis of DEGs

Using DEGs contrasting trastuzumab-resistant (R) versus -sensitive (S) GC cells in GSE77346, enrichment analysis revealed a developmental/plasticity program with exceptionally strong multiple-hypothesis–corrected support (Figure 3). In BP, broad developmental terms dominated, including development of multicellular organisms, system development, morphogenesis of anatomical structures, development of the nervous system, generation of neurons, tube development, and mesenchyme development. Many leading-edge genes in these terms (e.g., TWIST1, SMO, HOX paralogs, ID2/3/4, SOX2, POU3F2, PITX2, FGFs/FGFR3) were up-regulated in resistant cells, suggesting enrichment of lineage plasticity and morphogenetic programs under HER2-targeted pressure.

CC results localize this program to structures that coordinate genome regulation and cell–cell/ECM communication. The most significant terms included cell junction, chromatin, cell surface, anchoring junction, cell projection, chromosome, and synapse. Representative genes

Table 1. CytoHubba analysis identified 11 hub genes as the most central nodes within the constructed protein–protein interaction (PPI) network

Gene	ID	Ensembl	Description	Rank	Method	Position
SOX2	6657	ENSG00000181449	SRY-box transcription factor 2	1, 1, 1	MCC, MNC, Degree	3q26.33
WNT5A	7474	ENSG00000114251	Wnt family member 5A	2	MCC	3p14.3
FGF9	2254	ENSG00000102678	Fibroblast growth factor 9	3	MCC	13q12.11
BMP2	650	ENSG00000125845	Bbone morphogenetic protein 2	4	MCC	20p12.3
MESP1	55897	ENSG00000166823	Mesoderm posterior bHLH transcription factor 1	1	DMNC	15q26.1
SIX4	51804	ENSG00000100625	SIX homeobox 4	2	DMNC	14q23.1
NKX3-2	579	ENSG00000109705	NK3 homeobox 2	3	DMNC	4p15.33
ID3	3399	ENSG00000117318	Inhibitor of DNA binding 3	4	DMNC	1p36.12
PXDN	7837	ENSG00000130508	Peroxidasin	2, 2	MNC, Degree	2p25.3
CDH2	1000	ENSG00000170558	Cadherin 2	3, 3	MNC, Degree	18q12.1
VIM	7431	ENSG00000026025	Vimentin	4, 4	MNC, Degree	10p13

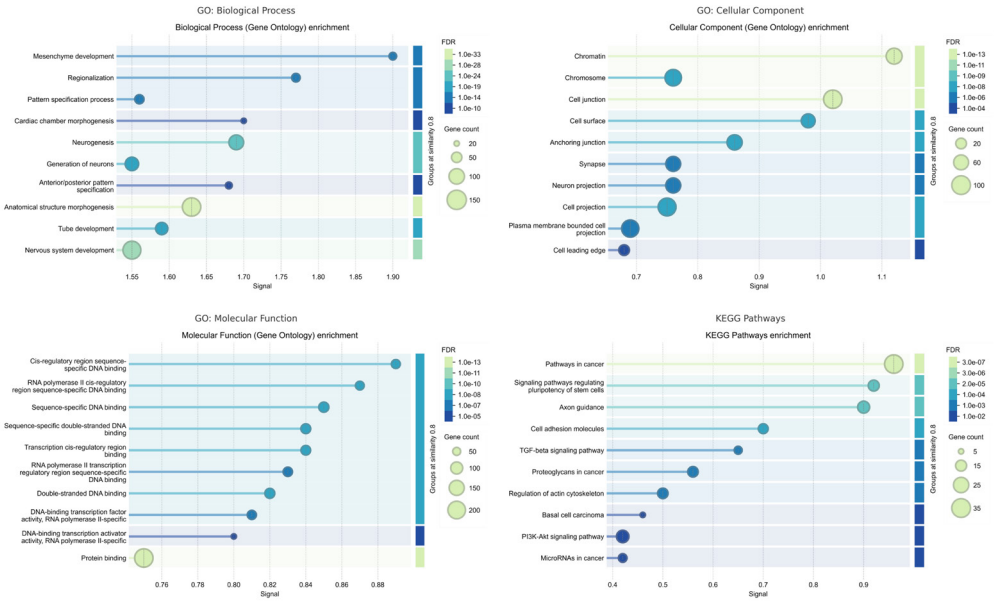


Figure 3. GO and KEGG enrichment analysis of DEGs in trastuzumab-resistant GC cells across biological process, cellular component, and molecular function categories
GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; DEG: Differentially expressed gene; GC: Gastric cancer

span CDH2 (N-cadherin), VCAN, NRP1, EPHA4, CXCR4, ROBO1/SLIT2, ENAH, ITGAE, and chromatin regulators (TFAP2C, SMAD4, JARID2). The pattern in R cells supports chromatin reprogramming together with remodeling of adhesive interfaces and protrusive structures, features consistent with EMT-like and neural-like dedifferentiation-associated programs that may be related to motility and niche adaptation.

MF converges on transcriptional control via sequence-specific DNA interactions: specific binding to DNA sequences, RNA polymerase II cis-regulatory region binding, cis-regulatory region sequence-specific DNA binding, and DNA-binding transcription factor activity, RNA Pol II-specific. The leading gene set is enriched for candidate regulators (TFAP2C, SIX4, HOXA5/HOXA9, HOXD9/10/11, TBX2, TWIST1, SMAD4, MEF2C, POU3F2, OLIG2), suggesting the presence of a compact TF network potentially associated with the resistant transcriptome.

KEGG pathways embed these ontologies in cancer-relevant circuits. The significant terms were Pathways in cancer, Axon guidance, Pluripotency of stem cells, Cell adhesion molecules, TGF-β signaling, Proteoglycans in cancer, Regulation of actin cytoskeleton, and PI3K-Akt signaling. Gene-level markers suggest potential links between these pathways and resistance-associated biological processes: PI3K-Akt (CCND2, FGFR3, KIT, CDKN1A, GHR, laminins/collagens,) may reflect survival/growth bypass-associated signaling under HER2 blockade; TGF-β components (SMAD4, BMP2/6, ID2/3/4, RGMB/PITX2) are consistent with EMT-associated and plasticity-related processes; CAM/actin modules (CDH2, VCAN, SDC2, PTPRM, ARHGEF6, TIAM1, ENAH, VAV3, RDX) support adhesion/FAK–Rho signaling and motility; and axon-guidance factors (WNT5A, EPHA3/4, ROBO1/SLIT2, RGMA, NRP1, CXCR4) are consistent with neuronal-mimicry associated programs that may contribute to invasive behavior. The enrichment of MicroRNAs in cancer further shows post-transcriptional regulation of these states.

Integrating across ontologies, trastuzumab-resistant GC cells showed enrichment of transcription-factor-related and chromatin-associated programs, together with developmental patterning and stem-like signatures. Concurrent enrichment of adhesion, cytoskeletal, CAM/proteoglycan, and axon-guidance pathways suggests that resistant cells may acquire biological features related to altered cell–cell communication, motility, and microenvironmental interaction. These pathway-level findings are consistent with potential involvement of PI3K-Akt, TGF-β-associated processes, adhesion remodeling, and transcriptional plasticity in the resistant phenotype. However, these associations remain computationally inferred and require functional validation. Therefore, the identified pathways and candidate master regulators, including TWIST1, TFAP2C, HOX-family genes, MEF2C, and POU3F2, should be considered priorities for future experimental testing rather than confirmed mechanistic drivers.

Cluster analysis of the network

Network clustering of resistance-associated genes resolved four dense, single-component modules with high clustering and network density (Figure 4). A shared transcriptional core, RUNX2/RUNX3 control of CDKN1A and BCL2L11 (BIM), AP-2/TFAP2 regulation of cell-cycle genes, and MECP2-mediated TF control converge on cell-cycle and apoptotic regulation (34-36). Another recurrent pattern involved FGFR3-related signaling, including ligand/mutant activation and PLC cascades, which may reflect alternative RTK-associated signaling under HER2 blockade (37). The augmentation of endocrine-lineage programs (NEUROG3^{+}/beta-cell) indicates lineage plasticity and the reorganization of differentiation pathways (38, 39).

Module-level features correspond with specific escape routes; Module 1: FGFR3 signaling + RUNX3→CDKN1A/BIM and TFAP2 cell-cycle control, may be associated with RTK bypass signaling, Module 2: NEUROG3/beta-cell

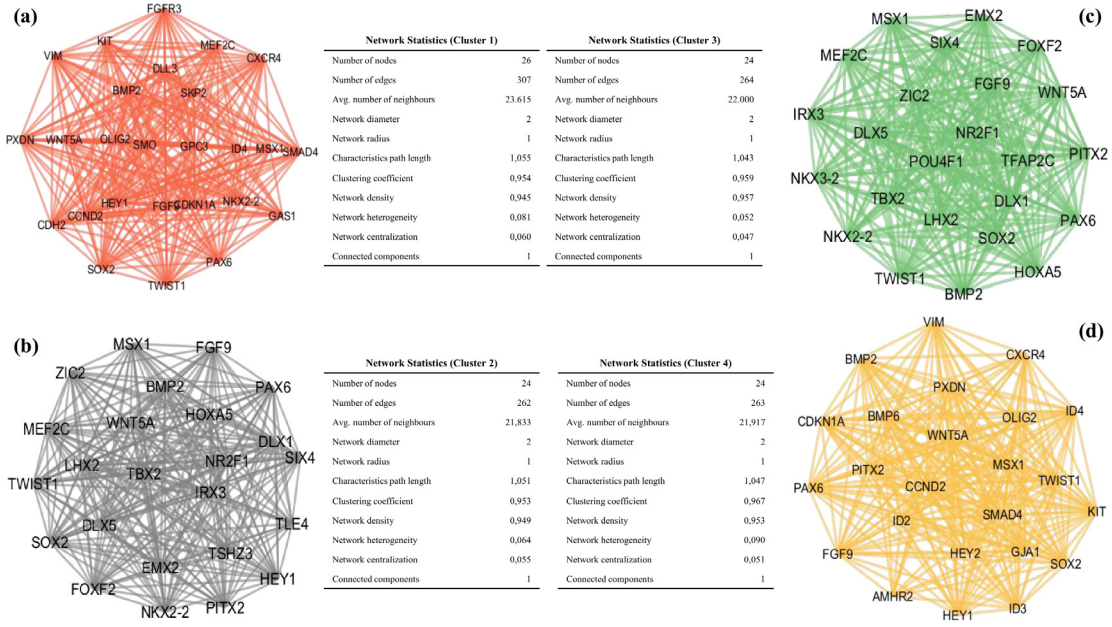


Figure 4. Visualization of four protein–protein interaction (PPI) network clusters: (a) Cluster 1, (b) Cluster 2, (c) Cluster 3, and (d) Cluster 4, constructed using CytoCluster (v2.1.0)

programs, OCT4/SOX2/NANOG, RUNX2, and WNT5A internalization, indicates a progenitor-like condition, Module 3: strengthened AP-2/TFAP2 (including negative regulation), MECP2 TF control, and RUNX2 with FGFR3b input, suggests epigenetic/TF-driven reprogramming overlaid atop RTK signaling; Module 4: RUNX3 cell cycle/apoptosis with BMP/RUNX2 development, FOXO targets, and RB1-linked G1/S defects, suggests potential relaxation of checkpoint-associated programs and survival-related signaling.

The presence/absence heatmap (Figure 5) shows which pathways are shared and which are cluster-specific. Endocrine-lineage control, MECP2-regulated transcription, TFAP2-mediated cell-cycle control, RUNX2 programs, and RUNX3 regulation of CDKN1A/BIM recur across multiple modules. By contrast, FGFR3/PLC signaling appears only in selected modules (RTK bypass), while FOXO, BMP–RUNX2, and RB1 signatures define a cell-cycle/differentiation subnetwork. Overall, the cluster-level findings suggest that trastuzumab resistance may be associated with a shared transcriptional–epigenetic framework: (i) alternative RTK input (notably FGFR3), (ii) transcription-factor/epigenetic

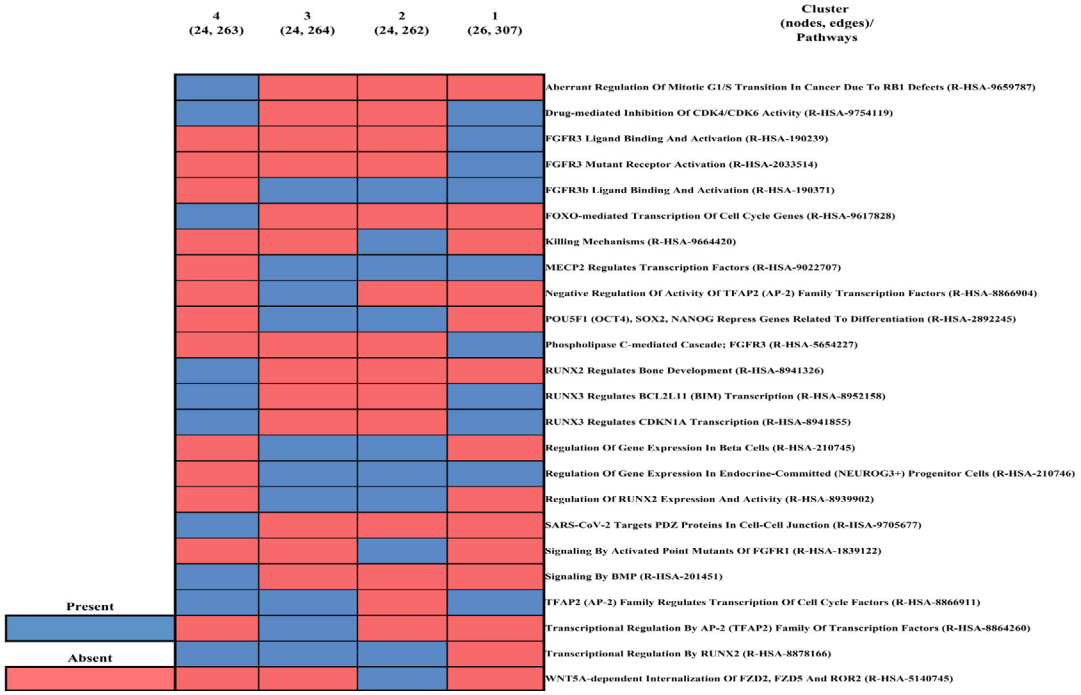


Figure 5. Pathway membership across clusters
Presence/absence heatmap showing which Reactome pathways occur in each network module. Rows: pathways; columns: clusters 1-4. Red=present (1), blue=absent (0)

rewiring that resets CDKN1A/BCL2L11, and (iii) lineage plasticity (NEUROG3, RUNX2/BMP). RB1/FOXO features suggest G1/S checkpoint relaxation, allowing proliferation despite HER2 blockade.

From a hypothesis-generating perspective, these modules may help prioritize route-specific combination strategies for future experimental testing. For RTK-bypass-associated clusters, combined evaluation of trastuzumab with FGFR inhibitors or PLC/PKC-axis modulators may be considered in preclinical models. For cell-cycle/differentiation-associated clusters, combinations involving CDK4/6 inhibitors or BMP/SMAD- or FOXO-related modulators may warrant future investigation. Across these contexts, the shared transcription-factor backbone, including RUNX3, TFAP2, and MECP2, should be functionally tested using approaches such as CRISPRi/a to determine whether these factors represent true druggable co-dependencies.

Survival analysis

In the unselected TCGA-STAD cohort queried via UALCAN, Kaplan–Meier analysis showed that high PXDN expression (n=100) and high CDH2 expression (n=95) were each associated with poorer overall survival compared with the corresponding low/medium-expression groups (PXDN: n=292, log-rank P=0.017; CDH2: n=297, log-rank P=0.037; Figure 6). These findings suggest that PXDN and CDH2 may serve as adverse prognostic markers in GC. Their known roles, ECM remodeling/TGF-β activation (PXDN) and EMT/mesenchymal adhesion (CDH2), are consistent with the invasive, plastic programs highlighted by our network analysis. However, because the TCGA cohort lacks information on trastuzumab exposure or resistance, these results do not establish predictive value for anti-HER2 response. The increased expression of the remaining nine hub genes showed no significant correlation with reduced overall patient survival with GC (*P*-value>0.05).

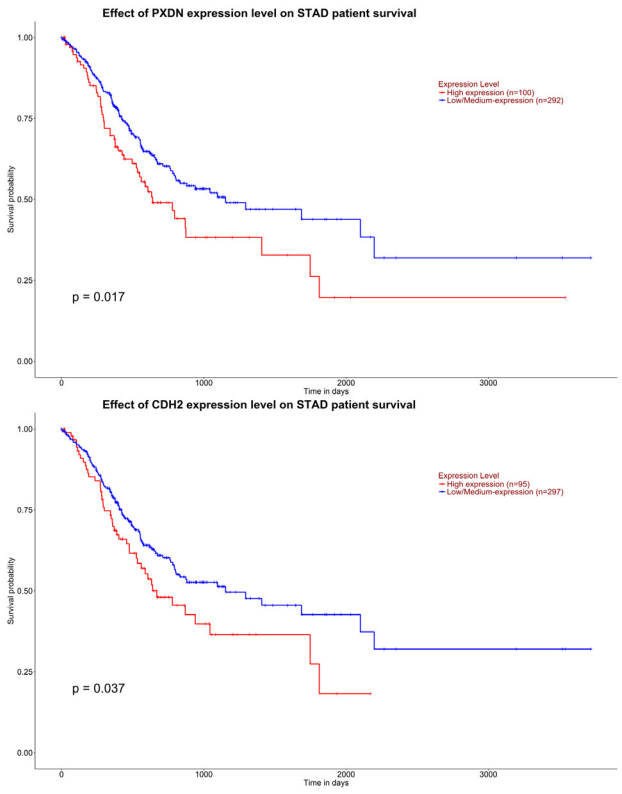


Figure 6. Kaplan-Meier survival analysis was performed to evaluate the prognostic impact of the 11 identified hub genes in gastric cancer (GC), using data retrieved from the UALCAN portal

Although this TCGA-STAD/UALCAN analysis provides independent prognostic support for PXDN and CDH2 in GC, it should not be interpreted as trastuzumab-specific validation. The TCGA-STAD cohort does not include detailed information regarding trastuzumab exposure, HER2-targeted treatment response, or acquired trastuzumab resistance. Therefore, the survival findings support the potential clinical relevance of PXDN and CDH2 as adverse prognostic markers, but further validation in independent HER2-positive GC cohorts with documented trastuzumab response is required to determine their predictive value for anti-HER2 resistance.

Therapeutic target screening

We analyzed drug interactions with genes by using DrugBank and PHAROS. This screening facilitated the identification of candidate molecules with reported interactions or relevance to selected hub genes implicated in GC biology and resistance-associated processes. Combining protein action data from PHAROS with drug information from DrugBank enabled us to identify gene-drug pairs that may warrant future experimental evaluation.

We found that PXDN was linked to phloroglucinol. Because PXDN has been implicated in extracellular matrix organization and tumor-associated remodeling, this interaction may warrant further exploratory investigation (40). CDH2, or N-cadherin, is associated with ADH-1, a compound that blocks tumor cell adhesion. ADH-1 has been investigated in the context of solid tumors, suggesting that the CDH2–ADH-1 interaction may be relevant for future experimental testing in GC models (41).

ID3 is linked to AGX51, an experimental compound that blocks ID proteins and may be relevant to transcriptional plasticity-related programs and should be experimentally evaluated in trastuzumab-resistant models (42). VIM was linked to phenethyl isothiocyanate and artenimol, compounds previously associated with redox stress, cytoskeletal regulation, or anticancer-related effects in other contexts (43, 44).

In summary, DrugBank and PHAROS screening identified several candidate compounds with reported interactions or biological relevance to genes involved in cell adhesion, differentiation, and cytoskeletal organization. These results should be interpreted as preliminary computational prioritization rather than evidence of therapeutic efficacy. The identified drug-target pairs, including phloroglucinol–PXDN, ADH-1–CDH2, AGX51–ID3, and artenimol or phenethyl isothiocyanate–VIM, may provide starting points for future experimental screening. Further biochemical, cellular, and preclinical studies are required to determine whether these candidates can modulate trastuzumab-resistant phenotypes or enhance response to HER2-targeted therapy.

Receptor preparation and structural validation

The web servers ProSA-web, Ramachandran plot, ERRAT, and MolProbity were used to validate the structural quality of the predicted 3D models of the PXDN, CADH2, and ID-3 receptors. The evaluation results are shown in

Table 2. Data analysis showed that the modeled structures for PXDN, CADH2, and ID-3 receptors had acceptable scores and were comparable to the quality of previously determined crystallographic structures of natural proteins. Based on these analyses, we can proceed with confidence into molecular docking. These validation results support the use of the modeled receptor structures for subsequent molecular docking analysis. These well-established receptor models facilitate inhibitory selectivity at PXDN's active site, evaluation of binding interactions, and planning subsequent experiments.

Molecular docking

Protein-ligand docking simulations were conducted to determine interaction patterns between ligands and target proteins, and the lowest-energy conformations were evaluated. Interactions observed between the selected ligands and receptors are shown in Table 3. The strongest

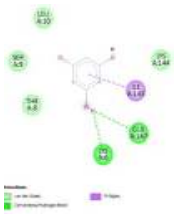
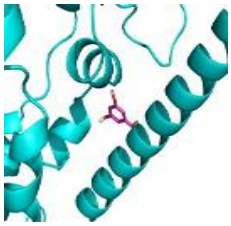
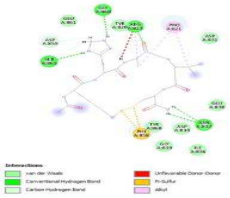
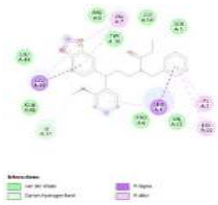
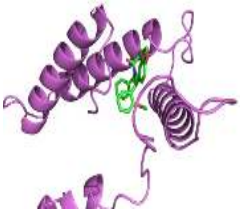
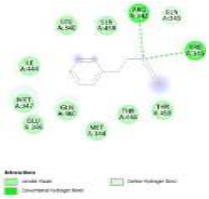
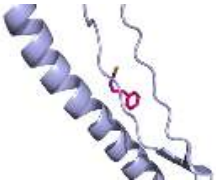
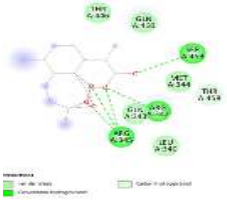
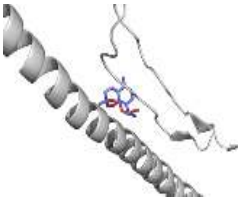
Table 2. Evaluation of the tertiary structure of PXDN, CADH2, and ID-3 receptors using ProSA-web, Ramachandran plot, ERRAT, and MolProbity servers

Predicted structure	ERRAT	MolProbity score	Clashscore	Residues in allowed region (%)	Residues in disallowed region (%)	Z-Score
PXDN	98.75	1.53 (94%*)	4.73 (94%*)	99.5%	0.5%	-5.45
CADH2	90.5374	1.59 (93%*)	6.75 (88%*)	99.2%	0.8%	-8.03
ID-3	95.1456	1.12 (100%*)	3.27 (97%*)	100%	0%	-4.23

*of the most ideal structural resolution

PXDN: Peroxidasin; CADH2: Cadherin-2 (N-cadherin); ID-3: Inhibitor of DNA binding 3

Table 3. Interaction of potential drugs with VIM, PXDN, CADH2 and ID-3 receptors

Receptor	Drug name	Affinity (kcal/mol)	2D Illustration	3D Illustration
PXDN	Phloroglucinol	-4.2		
CADH2	ADH-1	-7.7		
ID-3	AGX51	-7.6		
VIM	Phenethyl Isothiocyanate	-5.2		
VIM	Artenimol	-8.2		

VIM: Vimentin; PXDN: Peroxidasin; CADH2: Cadherin-2 (N-cadherin); ID-3: Inhibitor of DNA binding 3

interaction in the ligand-receptor study was related to the Artenimol drug with the VIM receptor (binding energy: -8.2 kcal/mol). ADH-1 with CADH2 (binding energy: -7.7 kcal/mol) and AGX51 with ID-3 (binding energy: -7.6 kcal/mol) also showed a strong interaction. These docking results should be interpreted strictly as *in silico* predictions. Binding affinity scores and predicted ligand-receptor interactions do not confirm biological activity, target engagement under physiological conditions, or therapeutic efficacy. Therefore, the compounds identified in this analysis should be considered candidate molecules for future experimental validation rather than confirmed therapeutic agents.

Discussion

This study aimed to explore molecular features potentially associated with trastuzumab resistance in HER2-positive GC using an integrative bioinformatics approach. The results suggest that coordinated alterations in transcriptional regulation, cell adhesion, cytoskeletal organization, developmental plasticity, and receptor tyrosine kinase signaling may characterize trastuzumab-resistant cells. In particular, the identified hub genes and network modules point to several candidate biological themes, including FGFR-related bypass signaling, EMT-associated adhesion remodeling involving CDH2, VIM, and PXDN, and transcriptional plasticity involving regulators such as RUNX, TFAP2, MECP2, SOX2, and ID3. However, because the present study is computational and correlative, these findings should be interpreted as potential associations and hypothesis-generating mechanisms rather than as experimentally confirmed causal drivers of trastuzumab resistance.

These findings are consistent with previous studies suggesting that EMT-like programs, integrin/FAK signaling, TGF-β-associated activity, and RTK redundancy may contribute to reduced sensitivity to HER2-targeted therapy. For example, previous reports have described the involvement of EMT-associated processes in therapeutic resistance and the potential role of FGFR-related signaling as an alternative pathway during HER2 blockade. The recurrent endocrine/neuronal patterning signals observed in the present analysis, including NEUROG3-related and axon-guidance-associated signatures, are also consistent with reports of neuronal mimicry and lineage plasticity in aggressive solid tumors. Our network-centered analysis extends these observations by organizing the resistance-associated transcriptomic changes into discrete modules and identifying a shared transcription-factor backbone, including RUNX3, TFAP2, and MECP2, that may coordinate multiple downstream adaptation programs. Importantly, survival analysis in an unselected TCGA-STAD cohort showed that higher PXDN and CDH2 expression was associated with poorer overall survival. Although this analysis does not evaluate trastuzumab response directly, it provides prognostic support for the clinical relevance of matrix remodeling, adhesion remodeling, and EMT-associated features in GC (45-48).

The translational implications of these findings should be considered cautiously. The modular architecture identified in this study may help generate hypotheses for future biomarker-guided combination strategies. For example, tumors with RTK-bypass-associated signatures may be investigated in future studies for potential sensitivity to combined HER2 and FGFR/PLC-axis targeting. In contrast, tumors with cell-cycle or differentiation-associated signatures may warrant evaluation of combinations involving cell-cycle, BMP/SMAD, or FOXO-related modulators. In addition, drug-gene screening and molecular docking prioritized candidate drug-target pairs that may be relevant to the identified hubs. ADH-1, a reported N-cadherin antagonist, was linked to the CDH2/adhesion-related axis; AGX51, an ID-family inhibitor, was linked to the ID3-associated transcriptional plasticity axis; and compounds associated with VIM, including phenethyl isothiocyanate and artenimol/dihydroartemisinin, were linked to cytoskeletal and redox-associated programs. Nevertheless, these compounds should be regarded as preliminary candidates for future experimental screening rather than validated therapeutic agents. The docking results provide *in silico* prioritization only and require biochemical validation, target-engagement assays, resistant cell-line models, and preclinical studies before any therapeutic conclusion can be made.

Overall, the present findings suggest a hypothetical model in which trastuzumab resistance in GC may be associated with transcription-factor-related plasticity, adhesion and cytoskeletal remodeling, and RTK redundancy. This framework integrates transcriptomic, network-based, enrichment, survival, drug-screening, and docking analyses to prioritize candidate genes, pathways, and drug-target pairs for future investigation. However, the proposed model remains computationally inferred and requires validation in independent HER2-positive GC cohorts with documented trastuzumab response, as well as functional confirmation in trastuzumab-sensitive and trastuzumab-resistant experimental models.

Limitations

Several limitations should be considered when interpreting the findings of this study. First, the analysis was based mainly on a publicly available cell-line transcriptomic dataset; therefore, the results may be influenced by dataset-specific characteristics, cell-line-specific biology, limited sample representation, and microarray-related technical variability. In addition, because the analysis relied on public GEO microarray data, potential platform effects, batch effects, and preprocessing-related biases cannot be fully excluded. Second, although TCGA-STAD survival analysis was used as an independent prognostic cross-check and showed that high PXDN and CDH2 expression was associated with poorer overall survival, this cohort does not provide trastuzumab exposure, HER2-targeted treatment-response information, or acquired trastuzumab-resistance annotation. Therefore, these findings support the potential prognostic relevance of PXDN and CDH2 but cannot establish their predictive value for anti-HER2 response or trastuzumab resistance. Third, the study is computational and correlative in nature; therefore, the identified genes, pathways, and modules should be interpreted as candidate resistance-associated features rather than experimentally confirmed drivers of trastuzumab resistance. Finally, the drug screening and molecular docking analyses provide preliminary *in silico* prioritization only and require validation through biochemical assays, cell-line experiments, resistant models, patient-derived models, and preclinical studies before any therapeutic conclusion can be drawn. Future studies should validate the identified hub

genes and network modules in independent HER2-positive GC cohorts with documented trastuzumab sensitivity or resistance.

Future perspectives

The findings of this study provide a hypothesis-generating framework for future investigations into trastuzumab resistance in HER2-positive GC. First, the predictive relevance of the identified genes and network modules should be evaluated in independent HER2-annotated GC cohorts with known trastuzumab exposure and treatment-response information. In particular, future studies should determine whether FGFR-related, EMT-associated, adhesion-remodeling, or transcription-factor-driven module scores can stratify patients according to trastuzumab sensitivity or resistance. Second, the shared transcription-factor backbone identified in this study, including RUNX3, TFAP2, and MECP2, as well as key hub genes such as PXDN, CDH2, and ID3, should be functionally investigated using loss-of-function and gain-of-function approaches in trastuzumab-sensitive and trastuzumab-resistant GC models. Such experiments may help determine whether these genes are required for maintaining the resistant phenotype or whether they represent druggable co-dependencies. Third, the candidate therapeutic combinations suggested by drug screening and molecular docking should be experimentally evaluated. For example, combinations of trastuzumab with ADH-1 in adhesion-dominant profiles or with ID-axis inhibition using AGX51 in plasticity-driven profiles may be tested through biochemical binding assays, target-engagement studies, cell viability assays, invasion assays, and re-sensitization experiments. In addition, protein-level validation using immunohistochemistry and microenvironment-aware models may help clarify the contribution of extracellular matrix remodeling, stromal interaction, and cell-adhesion pathways to trastuzumab resistance. These future directions may support the development of more precise biomarker-guided combination strategies for HER2-positive GC.

Conclusion

This study integrated differential gene expression analysis, PPI network reconstruction, clustering, pathway enrichment, survival analysis, drug screening, structural modeling, and molecular docking to explore molecular features potentially associated with trastuzumab resistance in HER2-positive GC. The results suggest that resistance may be associated with coordinated changes in transcriptional regulation, cell adhesion, cytoskeletal organization, and alternative receptor tyrosine kinase signaling, particularly FGFR-related pathways. Eleven key network hubs, including SOX2, ID3, CDH2, VIM, and PXDN, were identified as candidate resistance-associated genes. Among them, higher expression levels of PXDN and CDH2 were associated with poorer overall survival in the TCGA-STAD cohort, although this analysis does not establish predictive value for trastuzumab response. Drug screening and molecular docking prioritized several candidate drug-target pairs, including ADH-1–CDH2, AGX51–ID3, and artenimol or phenethyl isothiocyanate–VIM. However, these findings should be interpreted as preliminary *in silico* predictions rather than evidence of therapeutic efficacy or immediate drug repurposing potential. These candidate compounds may be considered for future experimental screening in combination with trastuzumab, but biochemical validation, target-engagement assays, resistant cell-line models, and preclinical studies are required before any therapeutic conclusion can be drawn. Overall, this study provides a hypothesis-generating framework for future investigation of biomarker-guided combination strategies in HER2-positive GC. Further validation in independent HER2-positive cohorts with known trastuzumab exposure and experimental confirmation in trastuzumab-sensitive and trastuzumab-resistant models are required.

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Ethics Statement

This study used publicly available datasets (GEO, TCGA); therefore, ethical approval and informed consent were not required.

Data and Materials Availability

The data underlying this study are fully available in the manuscript. The transcriptomic dataset GSE77346 was retrieved from the GEO repository (https://www.ncbi.nlm.nih.gov/geo/).

Authors' Contributions

NM D, B S, and MSR R designed the experiments; NM D, F W, F A, B S, N A, HMN T, MSR R, F Y, MJ N, HMR D, and N L performed the experiments and collected data; NM D, B S, F A, F Y, and N A discussed the results and strategy; NM D supervised, directed, and managed the study; NM D approved the final version for publication.

Conflicts of Interest

The authors of this manuscript declare that they have no significant competing interests to disclose.

Declaration

In the preparation of this study, we utilized OpenAI's ChatGPT, Paper Pal, QuillBot, and Grammarly for language enhancement. After using these tools, the authors evaluated the text and made any necessary revisions, taking full responsibility for the publication's content.

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