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Unveiling the Clinical Significance of RACGAP1 in a Senescence Gene and Immune Score Based Prognostic Model for Breast Cancer

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Abstract

Background: Intrinsic tumor cell factors and the tumor microenvironment (TME) are critical for breast cancer prognosis. This study established a novel prognostic model integrating senescence-associated gene expression and immune scores, and explored the clinical significance of core gene RACGAP1 and its correlation with TME.

Methods: Differentially expressed genes were screened from TCGA-BRCA cohort (n = 1222, FDR < 0.05) and combined with ESTIMATE immune scores. Eighty-one senescence-associated genes were obtained via intersection with CellAge database. Top 20 hub genes were identified by PPI network, and four prognostic genes via univariate Cox analysis; multivariate Cox and LASSO regression pinpointed RACGAP1 as the core target. Data were divided into training (n = 420) and internal validation (n = 419) sets, with GSE7390 (n = 198) for external validation.

Results: GO/KEGG analyses revealed RACGAP1 participated in immune response and cell cycle regulation. Multivariate Cox confirmed high RACGAP1 expression was an independent poor prognostic risk factor (*HR* = 1.81, 95%CI=1.10-2.98, *p* = 0.019). The RACGAP1-centered model showed strong predictive ability, with 3/5-year AUC 0.80/0.75, C-index 0.772 (internal) and 0.71 (external). The integrated nomogram had excellent calibration (Brier score < 0.2).

Conclusion: RACGAP1 is a novel prognostic biomarker for breast cancer, and the established model has reliable predictive efficacy, providing a reference for prognosis evaluation and targeted therapy.

Keywords: Breast cancer; Cellular senescence; Immune score; RACGAP1; Prognostic model

Introduction

Breast cancer remains one of the most prevalent malignancies affecting women globally. Its clinical prognosis and treatment outcomes are highly variable due to significant intratumoral heterogeneity (Sung H et al. 2021) . The tumor micro environment (TME) plays a crucial role in cancer progression (Yoshihara K et al. 2013), and the infiltration status of tumor immune cells has been established as a predictable prognostic factor. Immune scores, derived from gene expression profiles, effectively predict immune cell infiltration within tumor tissues (Asad S et al. 2022), and gene expression analysis significantly enhances both overall and individualized treatment strategies for breast cancer patients (Mostert B et al. 2015;Yang B et al. 2018).

Cellular senescence, a state of permanent cell cycle arrest, is a hallmark of biological aging, first described by Hayflick in fibroblasts (Muñoz-Espín D and Serrano M 2014). While senescent cells lose their proliferative capacity, they remain metabolically and transcriptionally active. Notably, they secrete inflammatory cytokines and other molecules through the senescence-associated secretory phenotype (SASP), thereby influencing their microenvironment (Rodier F et al. 2009). The precise role of SASP in modulating the TME in breast cancer remains contentious; some studies suggest that cellular senescence can inhibit tumor progression, while others indicate its potential to accelerate metastasis via a pro-inflammatory microenvironment (Coppé JP et al. 2010;Pare R et al. 2016;Ungvari Z et al. 2025;Wang J et al. 2019). The excessive and abnormal accumulation of senescent cells in tissues can detrimentally affect regenerative capacity and foster a pro-tumorigenic inflammatory environment (Sharpless NE et al. 2015). Therefore, the prognostic value of senescence-related genes likely depends heavily on their specific molecular context and their intricate interactions with the immune microenvironment, an area currently lacking systematic investigation.

Most existing prognostic models adopt a unidimensional approach, overlooking the combined influence of senescence genes and the immune microenvironment (Liu L et al. 2021). Although immune scores have been utilized for prognostic prediction, their mechanistic association with senescence pathways remains largely undefined (D'Angelo A et al. 2004). RACGAP1, a crucial cell cycle regulatory gene, has been implicated in promoting the development of various cancers. However, its specific function in breast cancer senescence-related processes and its relationship with immune evasion mechanisms remain elusive (Györffy B et al. 2010). These knowledge gaps impede the construction of precise prognostic models and the development of targeted therapeutic strategies.

This study aims to predict breast cancer prognosis by integrating multi-omics data, specifically focusing on the interactive network between senescence-associated genes and the immune microenvironment.

Materials and Methods

Data Sources and Preprocessing

Transcriptomic data for breast cancer samples were obtained from The Cancer Genome Atlas (TCGA-BRCA) database. The dataset comprised 1222 breast cancer samples, including 113 adjacent normal tissues and 1109 tumor tissues. Immune microenvironment scores were acquired using the ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) algorithm (Yoshihara K et al. 2013), provided by the MD Anderson Cancer Center. A total of 839 matched samples were included, with scores ranging from -2000 to 2000; higher scores indicated greater immune infiltration.

Differential Expression Analysis and Senescence-Related Gene Screening

Differential expression analysis was conducted using the DESeq2 R package (version 1.30.1).

Genes with an absolute log2FoldChange (|log2FoldChange|) > 1 and an FDR-adjusted p-value < 0.05 were considered significantly differentially expressed (Love MI et al. 2014). This analysis identified 1608 significant genes. A heatmap for hierarchical clustering was generated using the ComplexHeatmap R package (version 2.8.0), and a volcano plot, highlighting significantly differentially expressed genes in red, was created using ggplot2 (version 3.3.5). Senescence-related genes were retrieved from the CellAge database (CellAge: The Cell Senescence Gene Database), which curates 949 experimentally validated cell senescence-associated genes. We intersected these genes with the differentially expressed genes, yielding 81 breast cancer-related senescence genes. Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to validate the functional relevance of these genes, with a particular focus on cell cycle-related pathways.

Hub Gene Screening, Prognostic Model Construction and Validation

The top 20 hub genes were identified using the Maximal Clique Centrality (MCC) algorithm. Among these, four genes demonstrated prognostic relevance in univariate Cox proportional hazards regression analysis ($p < 0.05$). Age, clinical stage, lymph node status, immune score, and the identified hub genes were incorporated into a multivariate Cox analysis for adjustment. RACGAP1 emerged as an independent prognostic factor. A 10-fold cross-validation was applied to select the optimal penalty parameter λ for LASSO regression analysis, further validating RACGAP1. For internal validation, the dataset was randomly partitioned into a training set and a validation set, each containing 419 cases. An external validation cohort, the GSE7390 dataset (n = 198) from the Gene Expression Omnibus (GEO) database, was utilized to assess the model's generalizability.

Statistical Analysis

All statistical analyses were performed using the R programming language (version 4.1.0). Kaplan-Meier survival curves were generated using the survival package (version 3.2-10), and group comparisons were performed using the log-rank test. The predictive efficacy of the models was evaluated by concordance index (C-index), receiver operating characteristic (ROC) curves, and calibration curves. The C-index was used to assess predictive performance. All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant.

Results

Data Characteristics and Transcriptome Differential Analysis

Among the 1222 individuals in the TCGA-BRCA cohort, immune score differences calculated by the ESTIMATE algorithm were significant, with 49.1% of patients exhibiting a high immune infiltration status (immune score > -100.1). A total of 1608 significantly differentially expressed genes were identified (FDR < 0.05), comprising 1088 upregulated and 520 downregulated genes. Notably, mitotic genes such as RACGAP1 (log2FC = 2.3, FDR = 1.4×10^-6) were significantly upregulated in tumor tissues (**Fig. 1a**). The immune heatmap of tumor tissues revealed varying cell cycle expression patterns (**Fig. 1b**), consistent with genomic instability observed in triple-negative breast cancer (Telli ML et al. 2016) and pan-cancer immune cell infiltration patterns in TCGA (Thorsson V et al. 2019).

Senescence-Related Gene Characteristics and Molecular Network

Eighty-one senescence-related genes (**Fig. 2a**) selected from the CellAge database underwent functional enrichment analysis **(Fig. 2b-c)**. These genes were primarily enriched in pathways related to chromosome segregation (GO:0000776) and cancer central carbon metabolism (hsa05230), aligning with the senescence-associated secretory phenotype (Coppé JP et al. 2010). Protein-protein interaction network analysis identified RACGAP1 as a key node gene (degree = 12) **(Fig. 2cd)**. Its interaction

network with mitotic regulatory molecules like PLK1 suggests that mitotic abnormalities contribute to cancer cell growth (Yoshihara K et al .2013).

Prognostic Model Construction and Validation Univariate

Cox regression analysis identified (**Table 1**) four prognostic hub genes ($p < 0.05$) among the 20 analyzed: HJURP (*HR* = 2.56), ESPL1 (*HR* = 2.00), GTSE1 (*HR* = 1.92), and RACGAP1 (*HR* = 2.10). Multivariate Cox regression analysis further demonstrated that high RACGAP1 expression was an independent factor for poor prognosis (*HR* = 1.81, 95%CI: 1.10-2.98, $p = 0.019$) **(Table 2)**. This finding is consistent with recent multivariate studies in breast cancer patients (Thorsson V et al. 2019). The LASSO regression (Tibshirani R et al. 1997) **(Fig. 3a-d)** prediction model exhibited robust predictive performance in the training set (3-year AUC = 0.80, 5-year AUC = 0.753), outperforming the 21-gene test model (Sparano JA et al .2018) **(Fig. 3e)**. Internal validation showed a C-index of 0.772, and calibration curves indicated slightly better performance for the 3-year model compared to the 5-year model. The external independent cohort (GSE7390 dataset) validation yielded a C-index of 0.71, demonstrating good generalizability of the model.

Clinical Application and Risk Stratification

A nomogram integrating RACGAP1 expression, clinical stage, age, immune score, and lymph node status demonstrated excellent calibration for predicting 3-year/5-year survival, with Brier scores < 0.2 **(Fig. 4a)**. Based on the multivariate model, patients were stratified into two risk groups. The high-risk group (Nomogram total score > 250) had a median survival of 28.3 months, while the low-risk group (total score ≤ 250) had not reached median survival ($p < 0.001$) **(Fig. 4b).** This stratification remained robust across TCGA standardized data and external cohorts (Liu J et al. 2018).

Biological Significance of RACGAP1

High RACGAP1 expression was significantly associated with advanced tumor differentiation ($p <$ 0.001) and immunosuppression. In multivariate Cox analysis, the coefficients for Stage III/IV/V were significantly positive (e.g., Stage IV: coef = 2.977, p = 0.000068), indicating a higher probability of death with later tumor differentiation. The positive coefficient for RACGAP1 (coef = 0.596, p = 0.019) indicated that higher RACGAP1 expression correlated with increased mortality risk, further supporting its association with advanced tumor differentiation. Although the coefficient for the high immune score group was negative (coef = -0.596), it was not statistically significant ($p = 0.124$), possibly due to sample size or collinearity. However, the negative correlation between immune score and RACGAP1 in univariate analysis confirmed its immunosuppressive function. KEGG analysis revealed that RACGAP1-related genes were enriched in hsa04110: Cell cycle and hsa05230: Central carbon metabolism in cancer pathways. This suggests that RACGAP1 may promote genomic instability by interfering with GO:0000776: chromosome segregation (Coppé JP et al. 2010) or metabolic reprogramming, thereby inducing immune evasion through mitotic abnormalities (Telli ML et al. 2016).

Discussion

This study successfully developed a prognostic model for breast cancer leveraging RACGAP1 as a senescence gene feature and an immune scoring system, highlighting the critical importance of the tumor microenvironment. Our findings align with Zhou Y et al.'s research (2022), which reported a significant correlation between senescence features and patient survival. The prognostic typing system based on senescence-related genes established in their study is consistent with our identification of 81 senescence-related differentially expressed genes with prognostic value in breast cancer.

The training results of our model largely concur with Wang J et al.'s study (2019), which demonstrated that immune score-based nomograms improve breast cancer prognosis prediction. The

immune score's role as an independent predictor in this study confirms the involvement of the immune microenvironment in breast cancer stratification, consistent with findings by Chen L et al. (2021). Mechanistically, RACGAP1-related genes were enriched in transcription factor activity (GO:0003700) and cytoskeleton organization (GO:0016310), complementing Cai KT et al.'s meta-analysis (2019) showing that miR-204-5p targets and inhibits RACGAP1. Similarly, Chen J et al.'s study (2022) in hepatocellular carcinoma found that ANLN promotes tumor cell growth by interfering with cytokinesis fidelity. The network effect of RACGAP1 and ANLN in regulating the breast cancer cell cycle is consistent with our findings, where ANLN was identified as a hub gene. Our KEGG results showing RACGAP1-related gene enrichment in the cell cycle pathway (hsa04110) align with Liu Y et al.'s research (2024), which found a strong correlation between multiple gene risk scores (prisk score, PRS) and cell cycle-related immune gene expression. Xing T et al. (2023) also identified cell cycle-related pathways as core biological pathways of senescence characteristics through bioinformatics analysis, similar to our results. More recently, Huang Q et al. (2023) discovered that m7G modification genes play a significant role in regulating the breast cancer immune microenvironment, suggesting epigenetic modifications mediate RACGAP1's immunosuppressive features and providing avenues for future mechanistic studies of RACGAP1.

This study found that RACGAP1 overexpression is a poor prognostic factor in breast cancer, consistent with Lawson CD et al.'s research (2018) demonstrating that RACGAP1 promotes tumor progression by regulating cytokinesis during cell division in breast carcinoma in situ. From a molecular perspective, RACGAP1 is a key regulator of chromosome segregation. Dysregulation of RACGAP1 expression leads to high chromosomal instability, aligning with Mardin BR et al.'s (2013) findings that RACGAP1 affects chromosomal stability by regulating mitosis. Zhou D et al. (2021) reported that the

long non-coding RNA RACGAP1 promotes breast cancer infiltration and metastasis via the mitochondrial fission miR-345-5p/RACGAP1 axis, supporting the cell cycle pathway enrichment mechanism identified in our KEGG analysis. Furthermore, Ren K et al. (2021) showed that RACGAP1 promotes breast cancer tumor infiltration and metastasis by increasing mitochondrial mass in an ECT2-dependent manner, reinforcing RACGAP1's critical role in tumor infiltration and invasion.

Regarding immune effects, we observed an immune score paradox: RACGAP1 expression was negatively correlated with immune scores (r = -0.32). This suggests that RACGAP1 promotes immune escape by inhibiting immune infiltration, consistent with Graham R et al.'s (2023) findings that serum exosomes in triple-negative breast cancer suppress immunity by affecting T cell activity. However, Kennedy LC et al. (2023) found that dynamic changes in tumor-infiltrating lymphocytes (TILs) in HER2-positive breast cancer correlate with targeted therapy efficacy, implying that the predictive value of immune scores might be subtype-specific. Therefore, larger sample sizes are needed to validate the molecular subtype characteristics of RACGAP1's immune effect heterogeneity. In our multivariate analysis, the protective effect of high immune scores was not statistically significant (p = 0.124). This could be attributed to ESTIMATE's reliance on bulk RNA-seq for inferring immune cell infiltration, which may not accurately reflect the functional and spatial states of immune cells within the tumor microenvironment (Amjad E et al. 2020). Toda H et al.'s study (2018) found that miR-204-5p deletion in triple-negative breast cancer can increase RACGAP1 and PD-L1 expression levels, thereby inhibiting the effect of immune scores.

Our findings are consistent with Wang C et al.'s (2018) observation in ovarian cancer that RACGAP1 promotes ovarian cancer metastasis by activating Rac1. However, they contradict Nie R et al.'s (2022) finding that TRPV1 can inhibit RACGAP1 to promote anti-ovarian cancer immunity. This

discrepancy may arise because RACGAP1 expression in breast carcinoma in situ is linked to the Wnt/β-catenin pathway (Lawson CD et al. 2018), whereas in ovarian cancer, it is associated with the PI3K-AKT pathway (Wang C et al. 2018). This suggests that RACGAP1's functions may be pathway-dependent. Furthermore, the inhibitory effect of TRPV1 might be stronger in more immunogenic cancers, which could be related to the highly heterogeneous immune environment of breast cancer (Toda H et al. 2018).

While the prognostic advantage of tumor stage in our nomogram was considerably stronger than that of RACGAP1, external validation confirmed RACGAP1's substantial predictive efficacy. This may be because TNM staging incorporates comprehensive information like tumor burden and lymph node metastasis, whereas RACGAP1, as a single gene indicator, might be more applicable for stratifying early-stage patients. This aligns with Huan Y et al.'s (2025) report that biomarkers exhibit better prognostic discrimination for Stage I/II patients.

Despite the systematic division of the TCGA dataset into training and validation sets, and the use of an external GEO dataset to validate the constructed model to mitigate modeling biases, our study, like others of its kind, has inherent limitations. Although multiple independent cohorts were utilized, further prospective studies are essential for validation. The direct regulatory relationship between senescence-related genes and RACGAP1 has not been experimentally validated, requiring further investigation through techniques like ChIP-seq and loss-of-function experiments (Cai KT et al. 2019;Khalid M et al. 2019). Additionally, significant differences may exist across different molecular subtypes of breast cancer, and our sample size might be insufficient for certain subgroup analyses. The retrospective design introduces potential selection bias, and the limited number of cases in the external validation dataset may have contributed to a relatively weaker model performance.

Conclusion

In summary, this study employed multi-omics analysis to construct a novel prognostic model for breast cancer based on senescence gene features and immune scores. We identified RACGAP1 as a significant biomarker for breast cancer prognosis, demonstrating its role in promoting breast cancer progression by regulating cell cycle processes and influencing the immune microenvironment. This research provides new avenues for precise diagnosis and treatment of breast cancer. Further investigation into RACGAP1's regulation of immune response and its predictive value across different molecular subtypes is warranted.

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None.

Author contributions

Conceptualization: Mengxin Liu,Yang Xiong; Methodology: Ke Wan, Ningbo Yin; Formal analysis and investigation: Dalin Shi, Ningbo Yin; Writing-original draft preparation: Ke Wan; Writing - review and editing:Mengxin Liu; Funding acquisition: Mengxin Liu; Supervision: Yang Xiong.

Data availability

The transcriptomic data of the TCGA-BRCA cohort (containing 1222 breast cancer samples) used in this study were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov). The external validation dataset GSE7390 was downloaded from the Gene Expression Omnibus (GEO) database under accession number GSE7390 (https://www.ncbi.nlm.nih.gov/geo/). The list of senescence-associated genes was retrieved from the CellAge database (https://cellage.dgdc.uned.es/). Immune scores were calculated using the ESTIMATE algorithm provided by the MD Anderson Cancer Center. All data used are publicly

available, and no new experimental data were generated in this study. Additional supporting data are available from the corresponding author upon reasonable request.

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Declarations

Conflict of interest: All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Figure Legends and Tables

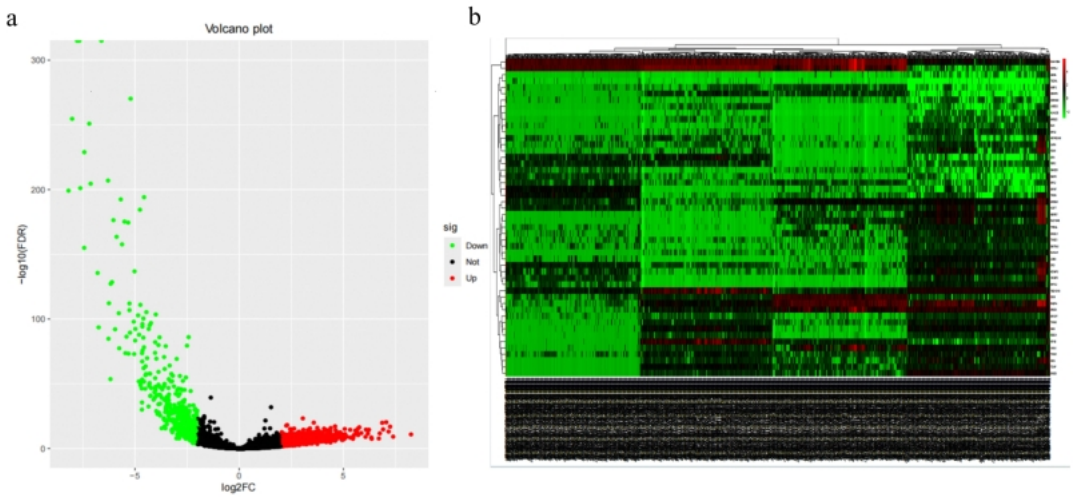


Figure 1. Differentially expressed genes (DEGs) in TCGA-BRCA cohort stratified by immune infiltration status. (a) DEGs volcano plot. Volcano plot of 1608 DEGs (FDR < 0.05) between high (n = 600, immune score > -100.1, 49.1%) and low immune infiltration groups. Red: upregulated (1088 genes, RACGAP1, $\log_2$FC = 2.3, FDR = 1.4×10^{-6}); green: downregulated (520 genes); black: non-significant. (b) Cluster heat map of DEGs in the BRCA group and the control group, showing

distinct cell cycle expression patterns consistent with triple-negative breast cancer genomic instability and pan-cancer immune infiltration features. Red: high expression; green: low expression.

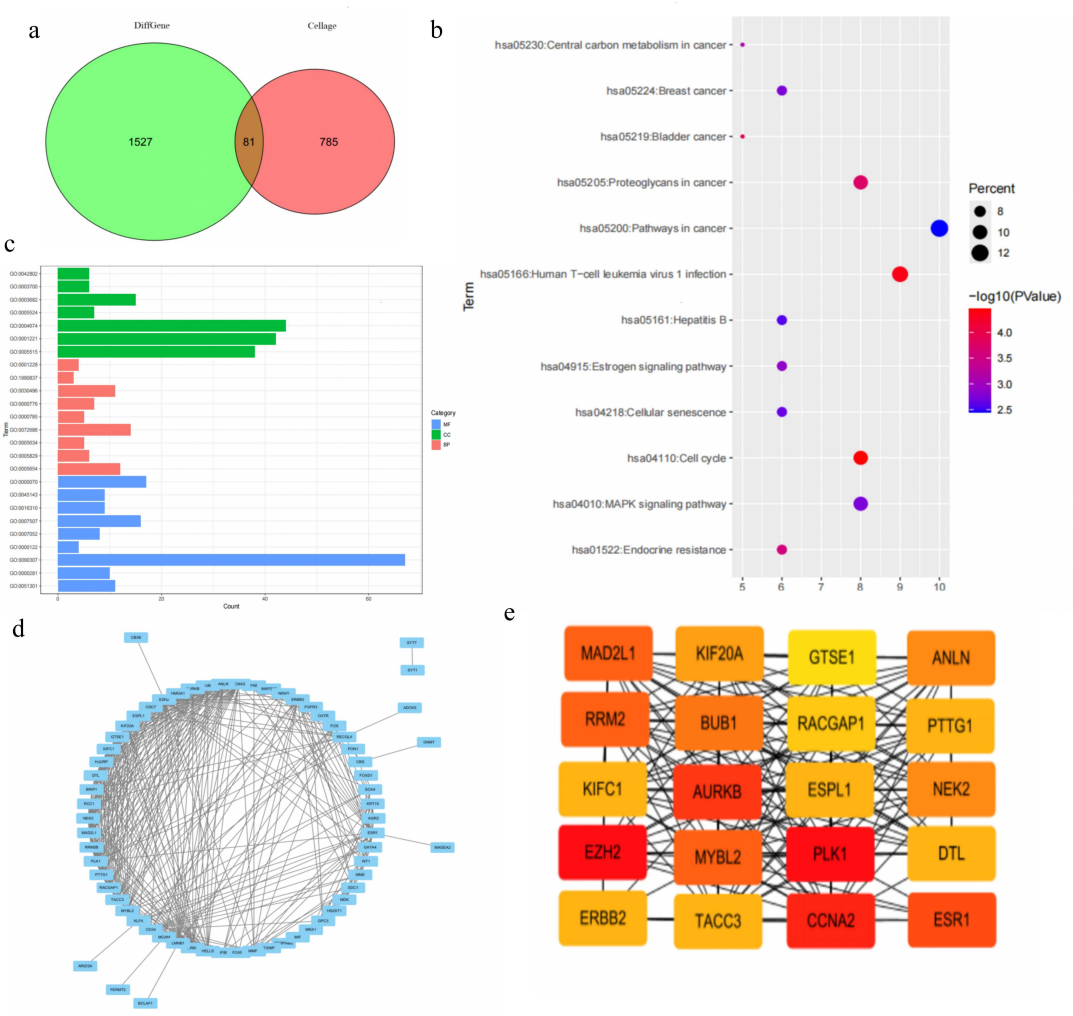


Figure 2. Functional enrichment and network analysis of genes related to aging. (a) The Venn diagram of genes among DiffGene and cellage.Venn diagram showing 81 overlapping senescence-related genes between differentially expressed genes (DiffGene) and CellAge database. (b) KEGG enrichment analysis. KEGG pathway enrichment bubble plot of the 81 genes, highlighting enrichment in cancer-related and cell cycle pathways. (c) GO enrichment analysis. GO enrichment bar plot, categorized by BP, CC, and MF, with top enrichment in chromosome segregation. (d) Transcription factor protein interaction network related to characteristic genes.PPI network of senescence-related characteristic genes. (e) Hub gene screening TOP20. Top 20 hub gene PPI network,

with RACGAP1 (degree = 12) as a key node interacting with mitotic regulators. Bubble color: -$\log_{10}$ (*p*-value); bubble size: gene percentage; node color: connectivity degree.

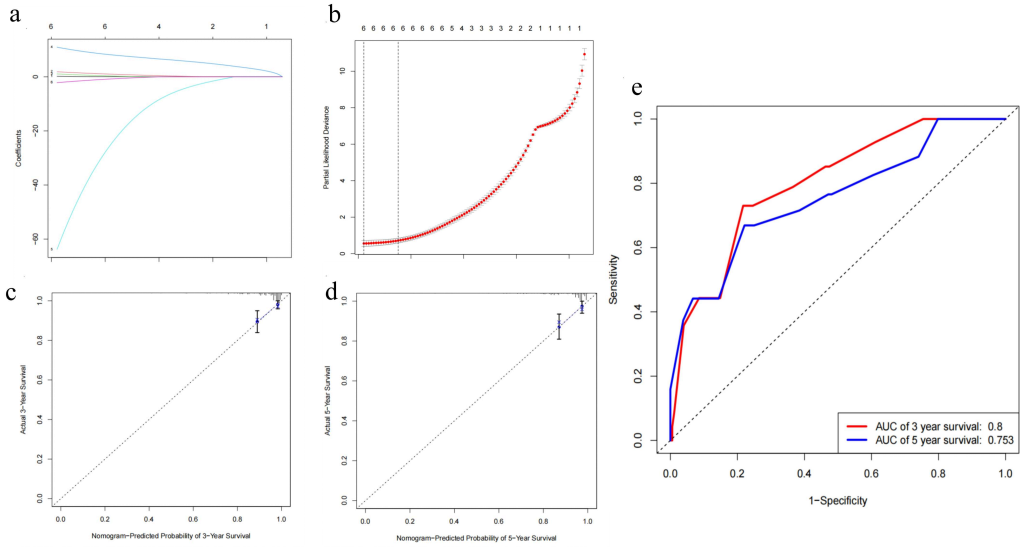


Figure 3. LASSO regression-based prognostic model construction and validation in breast cancer.

(a) Choose the optimal model complexity. LASSO coefficient shrinkage path for the 4 prognostic hub genes. (b) The coefficient shrinkage path of lasso regression.Partial likelihood deviance plot for optimal λ selection. (c) Calibration curve for three-year survival analysis. (d) Calibration curve for five-year survival analysis,showing good consistency between predicted and actual survival. (e) BRCA 3-5 year survival analysis ROC curve.Time-dependent ROC curves, with 3-year AUC = 0.80 and 5-year AUC = 0.753, demonstrating robust predictive performance of the model.

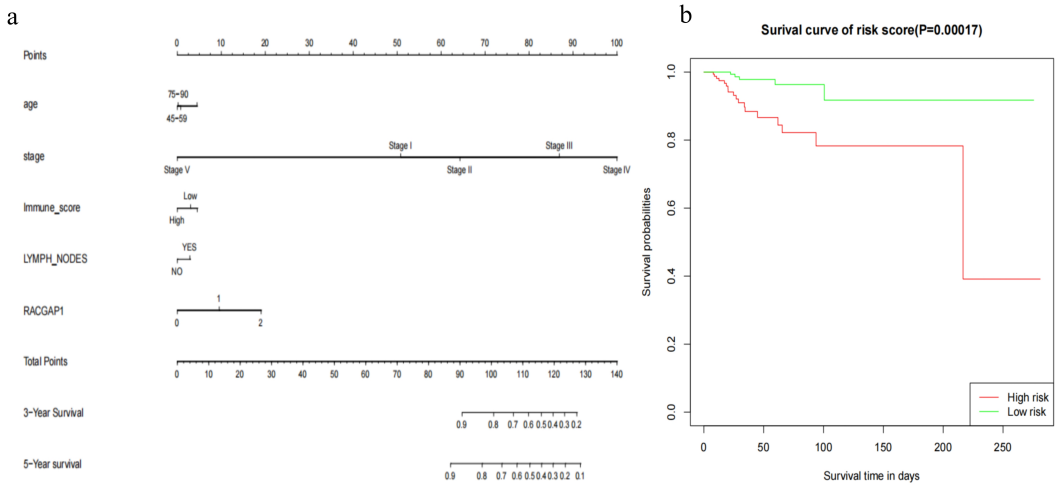


Figure 4. Prognostic nomogram and risk stratification for BRCA patients. (a) Fuse multi-factor to predict 3/5-year BRCA survival rate nomogram.A nomogram integrating RACGAP1 expression, clinical stage, age, immune score, and lymph node status to predict 3-year and 5-year overall survival, with excellent calibration (Brier scores < 0.2). (b) Kaplan-Meier survival curves based on the nomogram risk score, the high-risk group had significantly worse prognosis than the low-risk group (p = 0.00017).

Table 1 20 hub genes in univariate Cox regression analysis

Factor	HR	*P*
stage	4.213610	0.000845
TXNIP	3.206042	0.003320
GATA4	0.319324	0.004185
HJURP	2.564230	0.007483
NFKB2	3.706572	0.013502
SDC1	2.909433	0.014032
survival_time	0.000000	0.017410
OXTR	2.358787	0.017532
TWIST2	2.198870	0.027546
ESPL1	1.996195	0.030977
MDK	2.207187	0.031675
FOS	1.993836	0.037039
ADCK5	2.148794	0.038435
CBX8	2.643298	0.038585
MMP9	1.904983	0.041631
RRM2	2.294707	0.042846
GTSE1	1.920511	0.044688
RACGAP1	2.094731	0.048467

Table 2 Multivariate Cox regression analysis

Variable	coef	Exp (coef)	Exp (-coef)	Se (coef)	lower.95	upper.95	z	Pr (> |Z|)
age45-59	0.7403	2.097	0.477	0.3833	0.9892	4.444	1.932	0.053399
age60-74	0.05705	1.059	0.945	0.4257	0.4596	2.439	0.134	0.893391
age75-90	-15.67	0.000000156	6420000	2572	0	Inf	-0.006	0.995138
stageStage II	0.5857	1.796	0.557	0.3737	0.8635	3.737	1.567	0.117057
stageStage III	1.459	4.303	0.232	0.3926	1.9933	9.29	3.717	0.000202
stageStage IV	2.977	19.62	0.051	0.7473	4.5356	84.885	3.983	0.000068
stageStage V	3.826	45.88	0.0218	1.155	4.7707	441.196	3.313	0.000923
Immune_scoreMedium	-0.03816	0.9626	1.04	0.4054	0.4349	2.131	-0.094	0.925007
Immune_scoreHigh	-0.5961	0.551	1.82	0.3879	0.2576	1.178	-1.537	0.124332
LYMPH_NODESNO	1.071	2.92	0.343	0.3962	1.343	6.348	2.704	0.006845
RACGAP1	0.5958	1.814	0.551	0.2537	1.1035	2.983	2.348	0.018861
HJURP	-0.1905	0.8265	1.21	0.2624	0.4942	1.382	-0.726	0.467799
ESPL1	-0.2132	0.808	1.24	0.2392	0.5056	1.291	-0.891	0.37272
GTSE1	0.1541	1.167	0.857	0.2104	0.7724	1.762	0.732	0.463877

References

[1] Amjad E, Asnaashari S, Sokouti B, Dastmalchi S (2020) Systems biology comprehensive analysis on breast cancer for identification of key gene modules and genes associated with TNM-based clinical stages. *Sci Rep*. Jul 2 2020;10(1):10816.https://doi.org/10.1038/s41598-020-67643-w

[2] Asad S, Damicis A, Heng YJ, et al (2022) Association of body mass index and inflammatory dietary pattern with breast cancer pathologic and genomic immunophenotype in the nurses' health study. *Breast Cancer Res* 24(1):78. https://doi.org/10.1186/s13058-022-01573-5

[3] Cai KT, Liu AG, Wang ZF, et al (2019) Expression and potential molecular mechanisms of miR-204-5p in breast cancer, based on bioinformatics and a meta-analysis of 2,306 cases. *Mol Med Rep* 19(2):1168–1184. https://doi.org/10.3892/mmr.2018.9764

[4] Chen J, Li Z, Jia X, et al (2022) Targeting anillin inhibits tumorigenesis and tumor growth in hepatocellular carcinoma via impairing cytokinesis fidelity. *Oncogene* 41(22):3118–3130. https://doi.org/10.1038/s41388-022-02274-1

[5] Chen L, Dong Y, Pan Y, et al (2021) Identification and development of an independent immune-related genes prognostic model for breast cancer. *BMC Cancer* 21(1):329. https://doi.org/10.1186/s12885-021-08041-x

[6] Coppé JP, Desprez PY, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 5:99–118. https://doi.org/10.1146/annurev-pathol-121808-102144

[7] D'Angelo A, Garzia L, André A, et al (2004) Prune cAMP phosphodiesterase binds nm23-H1 and promotes cancer metastasis. *Cancer Cell* 5(2):137–49. https://doi.org/10.1016/s1535-6108(04)00021-2

[8] Graham R, Gazinska P, Zhang B, et al (2023)Serum-derived extracellular vesicles from breast cancer patients contribute to differential regulation of T-cell-mediated immune-escape mechanisms in breast cancer subtypes. *Front Immunol* 14:1204224. https://doi.org/10.3389/fimmu.2023.1204224

[9] Győrffy B, Lanczky A, Eklund AC, et al (2010) An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat* 123(3):725–31. https://doi.org/10.1007/s10549-009-0674-9

[10] Huan Y, She P, Cai X, et al (2025) Identification of key biomarkers in breast cancer based on bioinformatics analysis and experimental verification. *J Egypt Natl Canc Inst* 37(1):5. https://doi.org/10.1186/s43046-025-00260-8

[11] Huang Q, Mo J, Yang H, et al (2023) Analysis of m7G-Related signatures in the tumour immune microenvironment and identification of clinical prognostic regulators in breast cancer. *BMC Cancer* 23(1):583.https://doi.org/10.1186/s12885-023-11012-z

[12] Kennedy LC, Kazerouni AS, Chau B, et al (2023) Associations of Multiparametric Breast MRI Features, Tumor-Infiltrating Lymphocytes, and Immune Gene Signature Scores Following a Single Dose of Trastuzumab in HER2-Positive Early-Stage Breast Cancer. *Cancers (Basel)* 15(17)https://doi.org/10.3390/cancers15174337

[13] Khalid M, Idichi T, Seki N, et al (2019) Gene Regulation by Antitumor miR-204-5p in Pancreatic Ductal Adenocarcinoma: The Clinical Significance of Direct RACGAP1 Regulation. *Cancers (Basel)* 11(3)https://doi.org/10.3390/cancers11030327

[14] Lawson CD, Der CJ (2018) Filling GAPs in our knowledge: ARHGAP11A and RACGAP1 act as oncogenes in basal-like breast cancers. *Small GTPases* 9(4):290–296. https://doi.org/10.1080/21541248.2016.1220350

[15] Liu J, Lichtenberg T, Hoadley KA, et al (2018) An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell* 173(2):400–416.e11. https://doi.org/10.1016/j.cell.2018.02.052

[16] Liu L, Lv J, Lin Z, et al (2021) Co-Overexpression of GRK5/ACTC1 Correlates With the Clinical Parameters and Poor Prognosis of Epithelial Ovarian Cancer. *Front Mol Biosci* 8:785922. https://doi.org/10.3389/fmolb.2021.785922

[17] Liu Y, Peng C, Brorson IS, et al (2024) Germline polygenic risk scores are associated with immune gene expression signature and immune cell infiltration in breast cancer. *Am J Hum Genet* 111(10):2150–2163. https://doi.org/10.1016/j.ajhg.2024.08.009

[18] Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550. https://doi.org/10.1186/s13059-014-0550-8

[19] Mardin BR, Isokane M, Cosenza MR, et al (2013) EGF-induced centrosome separation promotes mitotic progression and cell survival. *Dev Cell* 25(3):229–40. https://doi.org/10.1016/j.devcel.2013.03.012

[20] Mostert B, Sieuwerts AM, Kraan J, et al (2015) Gene expression profiles in circulating tumor cells to predict prognosis in metastatic breast cancer patients. *Ann Oncol* 26(3):510–6. https://doi.org/10.1093/annonc/mdu557

[21] Muñoz-Espín D, Serrano M (2014) Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Biol* 15(7):482–96. https://doi.org/10.1038/nrm3823

[22] Nie R, Liu Q, Wang X (2022)TRPV1 Is a Potential Tumor Suppressor for Its Negative Association with Tumor Proliferation and Positive Association with Antitumor Immune Responses in Pan-Cancer. *J Oncol* 2022:6964550. https://doi.org/10.1155/2022/6964550

[23] Pare R, Shin JS, Lee CS (2016) Increased expression of senescence markers p14(ARF) and p16(INK4a) in breast cancer is associated with an increased risk of disease recurrence and poor survival outcome. *Histopathology* 69(3):479–91. https://doi.org/10.1111/his.12948

[24] Ren K, Zhou D, Wang M, et al (2021)RACGAP1 modulates ECT2-Dependent mitochondrial quality control to drive breast cancer metastasis. *Exp Cell Res* 400(1):112493. https://doi.org/10.1016/j.yexcr.2021.112493

[25] Rodier F, Coppé JP, Patil CK, et al (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol* 11(8):973–9. https://doi.org/10.1038/ncb1909

[26] Sharpless NE, Sherr CJ (2015) Forging a signature of in vivo senescence. *Nat Rev Cancer* 15(7):397–408. https://doi.org/10.1038/nrc3960

[27] Sparano JA, Gray RJ, Makower DF, et al (2018) Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med* 379(2):111–121. https://doi.org/10.1056/NEJMoa1804710

[28] Sung H, Ferlay J, Siegel RL, et al (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 71(3):209–249. https://doi.org/10.3322/caac.21660

[29] Telli ML, Timms KM, Reid J, et al (2016) Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. *Clin Cancer Re* 22(15):3764–73. https://doi.org/10.1158/1078-0432.Ccr-15-2477

[30] Thorsson V, Gibbs DL, Brown SD, et al (2019) The Immune Landscape of Cancer. *Immunity* 51(2):411–412. https://doi.org/10.1016/j.immuni.2019.08.004

[31] Tibshirani R (1997) The lasso method for variable selection in the Cox model. *Stat Med* 16(4):385–95. https://doi.org/10.1002/(sici)1097-0258(19970228)16:4<385::aid-sim380>3.0.co;2-3

[32] Toda H, Kurozumi S, Kijima Y, et al (2018) Molecular pathogenesis of triple-negative breast cancer based on microRNA expression signatures: antitumor miR-204-5p targets AP1S3. *J Hum Genet* 63(12):1197–1210. https://doi.org/10.1038/s10038-018-0510-3

[33] Ungvari Z, Ungvari A, Fekete M, et al (2025) Senescence-related genes as prognostic indicators in breast cancer survival. *Geroscience* 47(3):2995–3006. https://doi.org/10.1007/s11357-024-01384-w

[34] Wang C, Wang W, Liu Y, et al (2018) Rac GTPase activating protein 1 promotes oncogenic progression of epithelial ovarian cancer. *Cancer Sci* 109(1):84–93. https://doi.org/10.1111/cas.13434

[35] Wang J, Li Y, Fu W, et al (2019) Prognostic nomogram based on immune scores for breast cancer patients. *Cancer Med* 8(11):5214–5222. https://doi.org/10.1002/cam4.2428

[36] Xing T, Hu Y, Wang H, Zou Q (2023) A senescence-related signature for predicting the prognosis of breast cancer: A bioinformatics analysis. *Medicine (Baltimore)* 102(19):e33739. https://doi.org/10.1097/md.0000000000033739

[37] Yang B, Chou J, Tao Y, et al (2018) An assessment of prognostic immunity markers in breast cancer. *NPJ Breast Cancer* 4:35. https://doi.org/10.1038/s41523-018-0088-0

[38] Yoshihara K, Shahmoradgoli M, Martínez E, et al (2013) Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun* 4:2612. https://doi.org/10.1038/ncomms3612

[39] Zhou D, Ren K, Wang M, et al (2021) Long non-coding RNA RACGAP1P promotes breast cancer invasion and metastasis via miR-345-5p/RACGAP1-mediated mitochondrial fission. *Mol Oncol* 15(2):543–559. https://doi.org/10.1002/1878-0261.12866

[40] Zhou Y, Xiao L, Long G, et al (2022) Identification of senescence-related subtypes, establishment of a prognosis model, and characterization of a tumor microenvironment infiltration in breast cancer. *Front Immunol* 13:921182. https://doi.org/10.3389/fimmu.2022.921182