Comprehensive analysis identifies as a critical prognostic prediction gene in breast cancer

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Abstract

Background: Aurora kinases *(AURKs)* family plays a vital role not only in cell division but also in tumorigenesis. However, there are still rare systematic analyses of the diverse expression patterns and prognostic value of the *AURKs* family in breast cancer (BC). Systematic bioinformatics analysis was conducted to explore the biological role, prognostic value, and immunologic function of *AURKs* family in BC.

Methods: The expression, prognostic value, and clinical functions of *AURKs* family in BC were evaluated with several bioinformatics web portals: ONCOMINE Gene Expression Profiling Interactive Analysis, Kaplan–Meier plotter, cBioPortal, Metascape, GeneMANIA, and LinkedOmics; and the result was verified using human tissues.

Results: The expression of *AURKA* and *AURKB* were upregulated in BC in subgroup analyses based on tumor stage (all $P < 0.05$). BC patients with high *AURKA* and *AURKB* expression had a worse overall survival, relapse-free survival, and distant metastasis-free survival (all $P < 0.05$). Verification experiment revealed that *AURKA* and *AURKB* were upregulated in BC ($P < 0.05$). *AURKA* and *AURKB* were specifically associated with several tumor-associated kinases (polo-like kinase 1 and cyclin-dependent kinase 1), miRNAs (miR-507 and miR-381), and *E2F* transcription factor 1. Moreover, *AURKA* and *AURKB* were correlated with immune cell infiltration. Functional enrichment analysis revealed that *AURKA* and *AURKB* were involved in the cell cycle signaling pathway, platinum drug resistance signaling pathway, *ErbB* signaling pathway, *Hippo* signaling pathway, and nucleotide-binding and oligomerization domain-like receptor signaling pathway.

Conclusions: Aurora kinases *AURKA* and *AURKB* could be employed as novel prognostic biomarkers or promising therapeutic targets for BC.

Keywords: Aurora kinases; *AURKs*; Breast cancer; Prognosis; Bioinformatics analysis

Introduction

Breast cancer (BC) is the leading cause of cancer-related death in women.[1] Despite great advances in classic clinical biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2),[2] which play a critical role in helping to judge the prognosis and drug sensitivity of BC,[3,4] the prognosis in these patients remains poor.[5] Given that heterogeneity is one of the hallmarks of tumors,[6] the biomarkers we are currently using cannot provide sufficient information to predict the prognosis of BC patients, and thus, it is urgent and crucial for us to identify novel biomarkers that serve as prognostic indicators for BC or even instruct diagnosis and treatment.

Aurora kinases *(AURKs)* are members of the serine/threonine kinase family, are involved in cell division and play a vital role in regulating chromosome segregation during cell division by affecting the formation of bipolar spindles.[7] Three members of *AURKs* family have been identified in human beings, including *AURKA, AURKB,* and *AURKC*.[8] *AURKA* is mainly involved in the initiation of mitosis, separation of the centriole, accurate arrangement of the bipolar spindle apparatus, chromosome alignment in metaphase, and division of daughter cells during telophase.[9] *AURKB* chiefly participates in the bidirectional separation of chromosomes and dominates the synthesis of centromeric microtubules.[8] *AURKC* exhibits a similar function as *AURKB* during mitosis.[10]

Precisely because *AURKs* play such an important role in the mitotic process of cells, their abnormally high

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expression results in the emergence of instability in the genome and thus gives rise to carcinogenesis in different cells or tissues, including gastric/gastrointestinal cancer,[11] ovarian cancer,[12] colorectal cancer,[13] cervical cancer,[14] and BC.[15-17] During tumorigenesis, *AURKA* has been shown to affect tumor cell proliferation[18] and epithelial-mesenchymal transition,[19] and maintain the self-renewal capacity of cancer stem cells (CSCs).[20] *AURKB* has been shown to help tumor cells escape elimination by the immune system and promote the survival of malignant cells.[21,22] *AURKC* may promote tumor development based on its overlapping and complementary functions with *AURKB*, as well as gene amplification and over-expression in cancers.[17] Although certain studies about *AURKs* family in BC have been performed[15,23] the role of *AURKs* family was far from being fully clarified.

In our study, a comprehensive study about the expression, and prognosis significance of *AURKs* family in BC was constructed based on several large public databases, such as ONCOMINE (www.oncomine.org), Gene Expression Profiling Interactive Analysis (GEPIA, gepia.cancer-pku. cn), Kaplan–Meier plotter (www.kmplot.com), and cBio-Portal databases (http://www.cbioportal.org). We also conducted a functional enrichment analysis of *AURKs* in BC patients in the Metascape (http://www.metascape.org) and LinkedOmics databases (http://www.linkedomics. org). Moreover, we also evaluate the correlation between *AURKs* family and immune infiltration via the tumor immune estimation resource (TIMER, https://cistrome. shinyapps.io/timer). Our study may provide more serviceable information on the function of *AURKs* family in BC.

Methods

ONCOMINE analysis

ONCOMINE (www.oncomine.org), an online web-based cancer database for RNA and DNA sequences, was used to analyze the transcriptional expression of *AURKs* in different type of cancer. Transcriptional expression of *AURKs* in cancer samples was compared with those in normal individuals using Student's t test. Statistically significant values and fold change were demarcated as $P < 0.05$ and 2, respectively.

GEPIA dataset

GEPIA (gepia.cancer-pku.cn) is a newly developed interactive web server for analyzing the RNA sequencing (RNA-seq) expression data of 9736 tumors and 8587 normal samples from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression projects, using a standard processing pipeline. GEPIA provides customizable functions such as tumor/normal differential expression analysis, profiling according to cancer types or pathological stages, patient survival analysis, similar gene detection, correlation analysis, and dimensionality reduction analysis.[24]

Kaplan–Meier plotter

The prognostic value of *AURKs* mRNA expression was evaluated using an online database, Kaplan–Meier plotter

(www.kmplot.com),[25] which contained gene expression data and survival information of BC patients. To analyze the relapse-free survival (RFS), overall survival (OS), distant metastasis-free survival (DFMS), and post-progression survival (PPS) of patients with BC, patient samples were split into two groups by median expression (high *vs*. low expression) and assessed by a Kaplan–Meier survival plot, with the hazard ratio with 95% confidence intervals and log rank *P* value.

TCGA data and cBioPortal

TCGA had both sequencing and pathological data on 30 different cancers.[26] The breast invasive carcinoma (TCGA, provisional) dataset, including data from 1101 cases with pathology reports, was selected for further analyses of *AURKs* using cBioPortal (https://www.cbio. portal.org/results/oncoprint?session_id= 5df0b87ae4b04836b8ae2a71). The genomic profiles included mutations, putative copy number alterations from genomic identification of significant targets in cancer, and protein expression Z scores (reverse phase protein array). Co-expression and network were calculated according to the cBioPortal's online instructions.

GeneMANIA analysis

GeneMANIA (http://www.genemania.org) is a flexible, user-friendly web interface for constructing protein-protein interaction (PPI) network, generating hypotheses about gene function, analyzing gene lists, and prioritizing genes for functional assays.[27] The website can set the source of the edge of the network, and it features several bioinformatics methods: physical interaction, gene co-expression, gene co-location, gene enrichment analysis, and website prediction. We used GeneMANIA to visualize the gene networks and predict function of *AURKs*.

Functional enrichment analysis

Metascape (http://metascape.org) is a free, well-maintained, user-friendly gene-list analysis tool for gene annotation and analysis. It is an automated meta-analysis tool to understand common and unique pathways within a group of orthogonal target-discovery studies. In this study, Metascape was used to conduct pathway and process enrichment analysis of *AURK* family members and neighboring genes significantly associated with *AURK* alterations. For this, the Gene Ontology (GO) terms for biological process, cellular component, and molecular function categories, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, were enriched based on the Metascape online tool. Only terms with $P < 0.01$, minimum count of three, and enrichment factor of >1.5 were considered as significant. The most statistically significant term within a cluster was chosen as the one representing the cluster. A subset of enriched terms was selected and rendered as a network plot to further determine the relationship among terms, where terms with similarity of >0.3 were connected by edges. PPI enrichment analysis was performed using the following databases: BioGrid, InWeb_IM, and OmniPath. Furthermore, Molecular Complex Detection (MCODE)

algorithm was applied to identify densely connected network components.

LinkedOmics analysis

The LinkedOmics database (http://www.linkedomics. org/login.php) is a web-based platform for analyzing 32 TCGA cancer-associated multi-dimensional data-sets.[28] The LinkFinder module of LinkedOmics was used to study genes differentially expressed in correlation with *AURKs* in the TCGA. Results were analyzed statistically using Pearson's correlation coefficient. Data from the LinkFinder results were signed and ranked, and gene set enrichment analysis (GSEA) was used to perform analyses of kinase-target enrichment, miRNA-target enrichment, and transcription factor-target enrichment. The latter two network analyses were based on the Molecular Signatures Database.[29] The rank criterion was an false discovery rate (FDR) <0.05, and 500 simulations were performed.

TIMER database analysis

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (https://cistrome.shinyapps.io/timer/).[30] The TIMER database includes 10,897 samples across 32 cancer types from TCGA to estimate the abundance of immune infiltrates. We analyzed *AURKs* expression in BC and the correlation of *AURKs* expression with the abundance of immune infiltrates, including B cells, CD4+ T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells, via gene modules. Gene expression levels against tumor purity are displayed on the left-most panel.

Patients and clinical specimens

This study was approved by the Institutional Research Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology on 01/04/2020, written informed consent was obtained from each participant. Twenty breast cancer patients (females, aged 52.71 ± 8.50 years) undergoing tumorectomy were recruited, and pairs of fresh samples of human breast cancer and corresponding paracancerous tissues were obtained for immunohistochemistry (IHC) analysis from the same hospital. The samples were stored at $-80°C$ until use.

IHC

Three millimeters tumor sections were incubated with commercial rabbit polyclonal antibodies against *AURKA, AURKB,* and *AURKC* (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1/100 dilution overnight at 4°C. Then, the sections were conjugated with horseradish peroxidase antibody (1:500 dilution; Santa Cruz Biotechnology) at room temperature for 2 h, then covered by 3, 3-diaminobenzidine (Vector Laboratories, Burlingame, CA, USA), and slides were mounted with Vectashield mounting medium (Vector Laboratories). Subsequently, all fields were observed under light microscopy (Olympus 600 auto-biochemical analyzer, Tokyo, Japan). Control experiments without primary antibody demonstrated that the signals observed were specific.

Results

The expression of AURKA and AURKB was upregulated in BC

We used the ONCOMINE database to analyze the transcription levels of the three *AURK* family members *AURKA, AURKB,* and *AURKC* in human cancer and paracancerous tissue online. The mRNA expression of *AURKA* and *AURKB* but not *AURKC* was significantly upregulated in many types of cancer, including breast carcinoma samples, in multiple datasets [Figure 1A and 1B; Table 1] (all $P < 0.05$). According to the Curtis dataset, compared with that in normal tissue, *AURKA* expression is increased in almost all types of BC, including medullary breast carcinoma with a fold change of 4.706, invasive lobular breast carcinoma with a fold change of 2.115, invasive ductal and invasive lobular breast carcinoma with a fold change of 2.339, invasive breast carcinoma with a fold change of 2.586, mucinous breast carcinoma with a fold change of 2.137, breast carcinoma with a fold change of 2.488, and ductal breast carcinoma *in situ* with a fold change of 2.433. Data from TCGA show *AURKA* levels in invasive breast carcinoma with a fold change of 3.468, invasive lobular breast carcinoma with a fold change of 2.351, intraductal cribriform breast adenocarcinoma with a fold change of 2.679, mixed lobular and ductal breast carcinoma with a fold change of 2.767, and male breast carcinoma with a fold change of 3.285. In different datasets, for *AURKA* expression, we observed ductal breast carcinoma with a fold change of 9.423 compared with that in normal breast reported by Richardson, and a similar trend was found in the Sorlie (3.194 and 3.041) and Perou (3.37) four datasets. We also found that lobular breast carcinoma had an expression fold change of 2.13 reported by Sorlie, while the fold change of 2.411 in lobular breast carcinoma was reported by Perou. In addition, we observed that compared with those in normal breast, *AURKA* levels in invasive ductal breast carcinoma showed a fold change of 3.168 in the Curtis dataset, fold change of 4.702 in TCGA, and fold change of 2.154 in the Zhao dataset [Table 1].

AURKB is another member of the *AURK* family that we focused on with respect to breast carcinoma. The mRNA expression of *AURKB* was found to be upregulated in many types of breast carcinoma compared to normal breast tissue. *AURKB* transcriptional levels in invasive ductal breast carcinoma exhibited a fold change of 2.446 in TCGA; the Curtis, Zhao, Radvanyi, and Turashvili datasets showed similar fold changes (2.655, 2.333, 2.922, and 2.733, respectively). We also observed *AURKB* overexpression in male breast carcinoma with a fold change of 2.259 and invasive breast carcinoma with a fold change of 2.691 according to the TCGA dataset; in medullary breast carcinoma the fold change was 4.696, in breast carcinoma the fold change was 2.327, in invasive breast carcinoma the fold change was 2.269 according to the Curtis dataset; in ductal breast carcinoma, the fold
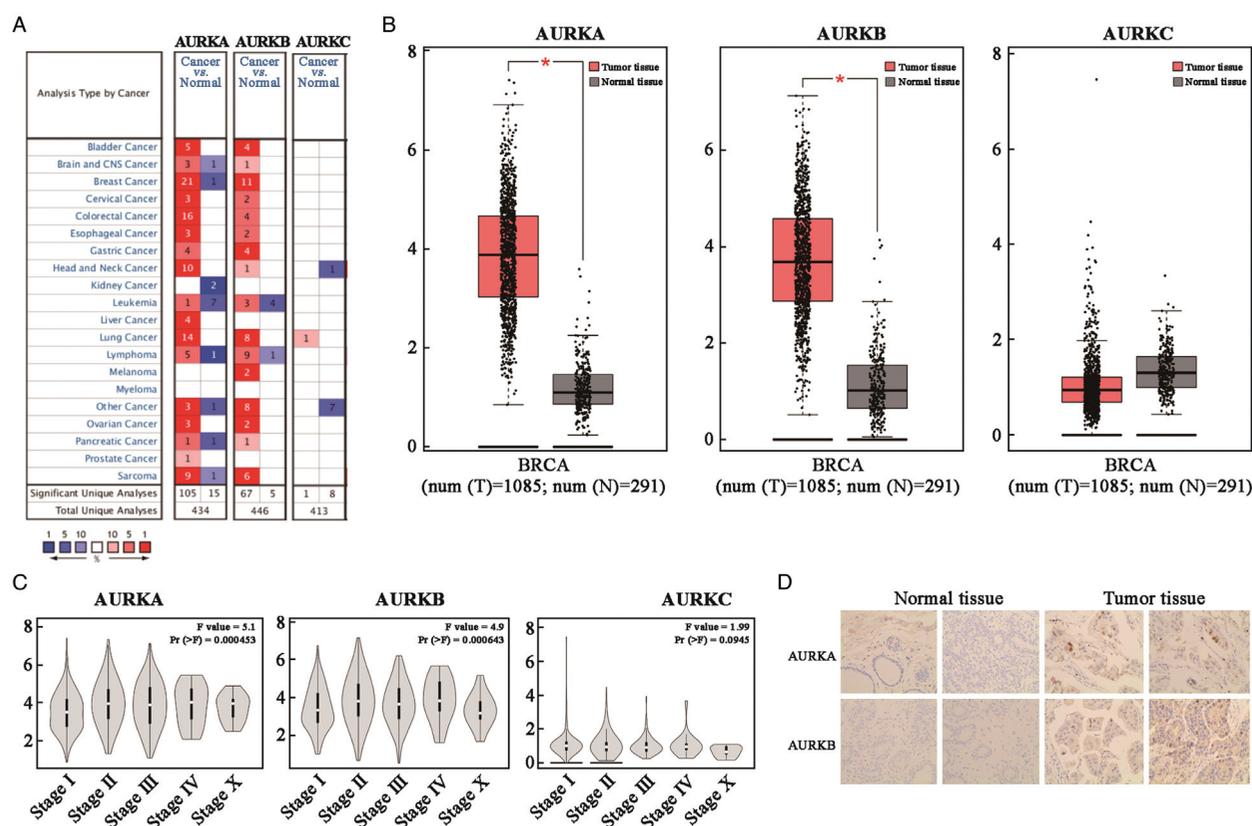
Figure 1: The expression of *AURKA* and *AURKB* were upregulated in BC. (A) The transcription levels of *AURKs* in different types of cancers (Oncomine). (B) The expression of *AURKs* in BC patients (GEPIA). (C) AURKs expression is not correlated with tumor stage in BC patients (GEPIA). (D) The expression of *AURKs* in tumor tissue and normal tissue (IHC, original magnification ×400). *AURKs*: Aurora kinases; *AURKA*: Aurora kinase A; *AURKB*: Aurora kinase B; *AURKC*: Aurora kinase C; *BRCA*: Breast cancer; CNS: Central nervous system; GEPIA: Gene expression profiling interactive analysis; IHC: Immunohistochemistry.

change was 5.424 according to the Richardson dataset [Table 1]. Combined with above results, the expressions of *AURKA* and *AURKB* were downregulated in BC.

Using the GEPIA dataset, the mRNA expression levels of *AURKs* in BC and breast tissues were examined. The results suggested that the expression levels of *AURKA* and *AURKB* were higher in BC tissues than normal tissues ($P < 0.05$), whereas the expression levels of *AURKC* were not significantly different between BC tissues and normal tissues [Figure 1B]. We also explored the expression of *AURKs* relative to tumor stage in BC. The *AURKA* and *AURKB* groups varied significantly ($P < 0.05$), whereas the *AURKC* group did not differ significantly [Figure 1C]. In addition, we also applied IHC to detect *AURK* protein expression in BC tissues and their counterparts and to examine the expression of *AURKs* in BC patients. The results suggested that *AURKA* and *AURKB* protein levels were higher in BC tissues than normal tissues ($P < 0.05$) [Figure 1D]. Thus, *AURKA* and *AURKB* may help to detect invasive BC patients.

AURKA and AURKB served as prognostic biomarkers in BC

We further explored the correlation between *AURK* expression and survival in BC patients. The Kaplan–Meier plotter tools were used to analyze the mRNA level of *AURKs* and the survival of BC patients. The

Kaplan–Meier curves are shown in Figure 2 and Table 2. The increased mRNA levels of *AURKA* and *AURKB* were significantly associated with poor RFS, OS, and distant metastasis-free survival (DMFS; $P < 0.05$), whereas the *AURKC* expression was not related to OS, DMFS, or PPS [Figure 2] of all of the patients with BC. However, the increased expression of *AURKB* was not significantly associated with PPS, whereas the increased level of *AURKA* was associated with poor PPS. We also observed that the decreased level of *AURKC* was significantly associated with poor RFS ($P < 0.05$). Therefore, *AURKA* and *AURKB* served as prognostic biomarkers in BC.

Changes in the functions and pathways of AURKs and their related altered neighboring genes in BC patients

We used cBioPortal (https://www.cbioportal.org/results/oncoprint?session_id=5df0b87ae4b04836b8ae2a71) to analyze the variation frequency of *AURK* gene mutations in BC patients. *AURKs* were altered in 96 samples from 1101 patients with invasive breast carcinoma (9%). The *AURKA, AURKB,*and*AURKC* genetic alteration percentage was 6%, 0.9%, and 3%, respectively, for individual genes based on the TCGA provisional dataset [Figure 3A].

We also investigated the correlations of *AURKs* with each other by analyzing their mRNA expression (RNA-seq

Table 1: The significant changes of *AURKs* expression in transcription level between different types of BC and normal breast tissues (ONCOMINE database).

Gene	Type of breast cancer *vs.* normal breast tissue	Fold change	*P* value	*t* test	Source and/or reference
AURKA	Ductal breast carcinoma *vs.* normal	9.423	1.18E–14	15.440	Richardson Breast 2
	Medullary breast carcinoma *vs.* normal	4.706	7.88E–19	16.701	Curtis Breast
	Invasive ductal breast carcinoma *vs.* normal	3.168	8.44E–120	40.777	Curtis Breast
	Invasive lobular breast carcinoma *vs.* normal	2.115	5.80E–44	19.905	Curtis Breast
	Invasive ductal and invasive lobular breast carcinoma *vs.* normal	2.339	1.02E–31	15.363	Curtis Breast
	Invasive breast carcinoma *vs.* normal	2.586	9.96E–07	6.466	Curtis Breast
	Mucinous breast carcinoma *vs.* normal	2.137	8.28E–16	10.774	Curtis Breast
	Breast carcinoma *vs.* normal	2.488	1.46E–05	6.120	Curtis Breast
	Ductal breast carcinoma *in situ vs.* normal	2.433	1.00E–03	3.996	Curtis Breast
	Invasive ductal breast carcinoma *vs.* normal	4.702	5.39E–53	25.633	TCGA
	Invasive breast carcinoma *vs.* normal	3.468	5.85E–26	13.374	TCGA
	Invasive lobular breast carcinoma *vs.* normal	2.351	7.20E–14	9.223	TCGA
	Intra-ductal cribriform breast adenocarcinoma *vs.* normal	2, 679	2.03E–05	11.823	TCGA
	Mixed lobular and ductal breast carcinoma *vs.* normal	2.767	9.38E–04	4.894	TCGA
	Male breast carcinoma *vs.* normal	3.285	6.00E–03	7.141	TCGA
	Lobular breast carcinoma *vs.* normal	2.130	1.20E–02	5.006	Sorlie Breast
	Ductal breast carcinoma *vs.* normal	3.194	9.98E–04	6.672	Sorlie Breast
	Lobular breast carcinoma *vs.* normal	2.411	1.00E–02	5.109	Perou Breast
	Ductal breast carcinoma *vs.* normal	3.370	1.00E–03	3.370	Perou Breast
	Invasive ductal breast carcinoma *vs.* normal	2.154	2.34E–07	6.348	Zhao Breast
	Ductal breast carcinoma *vs.* normal	3.041	3.00E–03	6.787	Sorlie Breast 2
AURKB	Male breast carcinoma *vs.* normal	2.259	6.15E–24	18.820	TCGA
	Invasive ductal breast carcinoma *vs.* normal	2.446	1.39E–36	19.045	TCGA
	Invasive breast carcinoma *vs.* normal	2.691	4.37E–21	11.200	TCGA
	Invasive ductal breast carcinoma *vs.* normal	2.655	2.04E–123	39.109	Curtis Breast
	Medullary breast carcinoma *vs.* normal	4.696	1.11E–17	16.006	Curtis Breast
	Breast carcinoma *vs.* normal	2.327	9.54E–06	6.388	Curtis Breast
	Invasive breast carcinoma *vs.* normal	2.269	2.37E–05	5.120	Curtis Breast
	Ductal breast carcinoma *vs.* normal	5.424	7.32E–11	9.458	Richardson Breast 2
	Invasive ductal breast carcinoma *vs.* normal	2.333	6.59E–10	14.979	Zhao Breast
	Invasive ductal breast carcinoma *vs.* normal	2.922	2.70E–02	2.503	Radvanyi Breast
	Invasive ductal breast carcinoma *vs.* normal	2.733	3.30E–02	2.169	Turashvili Breast

AURKs: Aurora kinases; *AURKA:* Aurora kinase A; *AURKB:* Aurora kinase B; *AURKC:* Aurora kinase C; BC: Breast cancer; TCGA: The Cancer Genome Atlas.

version (v.)2 RNA-Seq by Expectation Maximization (RSEM) via the cBioPortal online tool for breast carcinoma (TCGA, provisional), and Pearson's correction is shown in Figure 3B and 3C. The results indicated a significant and positive correlation between *AURKA* and *AURKB*. We then explored the *AURK* network and the 50 most frequently altered neighboring genes by using cBioPortal. The data showed that *NSL1, SKA2, NUF2, CENPL, RPS27, CENPF, PMF1, RAD21, KIF2B, AHCTF1, NUP85, NUP133, BIRC5, PPP2R5A, CHMP4C, PLEC, TP53, RPL8, PRKDC, MAPKAPK2, NEK7, ATK3, TACC1, H3F3B, H3F3A, STK3, NEK2,*

CSNK1D, PAK1, RPS6KB2, SDCCAG8, and *FADD* are closely associated with *AURK* alterations [Figure 3D]. GeneMANIA was used to explore the correlation among *AURK* family members at the gene level [Figure 3E]. The results showed co-expression, co-localization, and physical interaction relationships between *AURKA* and *AURKB*. Shared protein domains were noted among

AURKA, AURKB, and *AURKC.* In addition, the results of the Kaplan–Meier plotter and log-rank test indicated no significant difference but a tendency in OS and disease-free survival between the cases with changes in one of the queried genes (*P* value, 0.232 and 0.610, respectively) [Figure 3F].

Functional enrichment analysis of AURKs in BC patients

We performed GO and KEGG enrichment analyses of *AURK* family members and their adjacent genes using the Metascape online tool. The top 20 GO enrichment items were classified into three functional groups: biological process group (13 items), cellular component group (four items), and molecular function group [three items; Figure 4A and 4B; Table 3]. The *AURK* family members and their neighboring genes were mainly enriched in the cell cycle, embryogenesis, protein kinase activity regulation, and transcriptional regulation biological processes such as microtubule cytoskeleton organization, protein
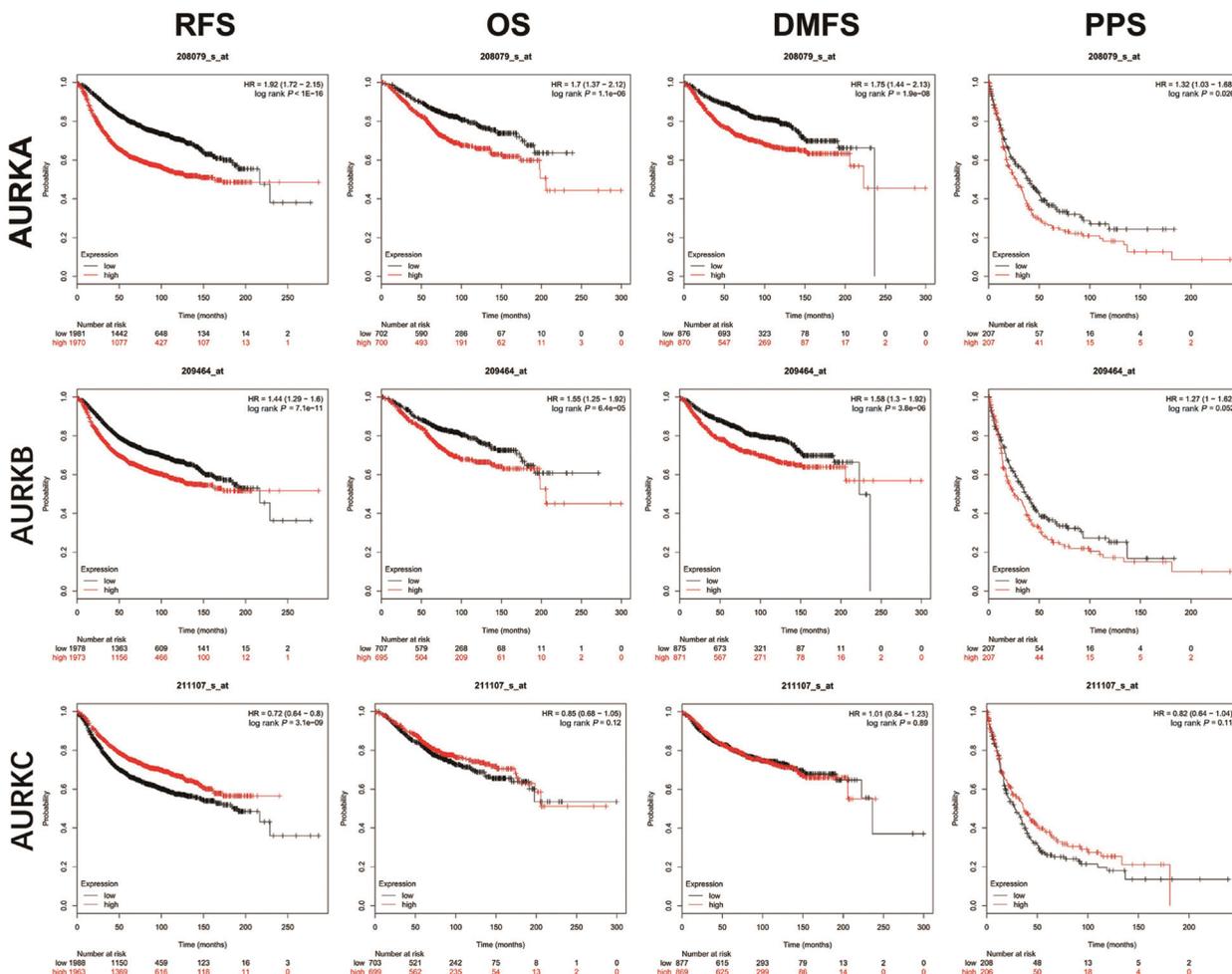
Figure 2: The prognostic value of mRNA level of *AURKs* In BC patients (Kaplan–Meier plotter). *AURKs.* Aurora kinases; *AURKA:* Aurora kinase A; *AURKB.* Aurora kinase B; *AURKC.* Aurora kinase C; BC: Breast cancer; DMFS: Distant metastasis-free survival; OS: Overall survival; PPS: Post-progression survival; RFS: Relapse-free survival; HR: Hazard ratio.

Table 2: Prognostic association of *AURKs* expression in BC based on Kaplan–Meier plotter.

Factors	Variable	Cutoff value expression	Expression	*P* value	HR	No. of patients
RFS	*AURKA*	440	8–9823	<1e–16	1.92 (1.72–2.15)	3951
	AURKB	181	4–2537	7.10E–11	1.44 (1.29–1.60)	3951
	AURKC	116	2–2027	3.10E–09	0.72 (0.64–0.80)	3951
OS	*AURKA*	519	44–4215	3.60E–08	1.83 (1.47–2.28)	1402
	AURKB	187	6–1135	6.40E–05	1.55 (1.25–1.92)	1402
	AURKC	107	3–2027	0.1231	0.85 (0.68–1.05)	1402
DMFS	*AURKA*	414	8–4306	1.90E–08	1.75 (1.44–2.13)	1746
	AURKB	180	7–1135	3.80E–06	1.58 (1.30–1.92)	1746
	AURKC	118	3–2027	0.8864	1.01 (0.84–1.23)	1746
PPS	*AURKA*	513	70–4306	0.0257	1.32 (1.03–1.68)	414
	AURKB	193	10–1063	0.0523	1.27 (1.00–1.62)	414
	AURKC	106	3–755	0.1056	0.82 (0.64–1.04)	414

AURKs: Aurora kinases; *AURKA:* Aurora kinase A; *AURKB:* Aurora kinase B; *AURKC:* Aurora kinase C; BC: Breast cancer; DMFS: Distant metastasis free survival; HR: Hazard ratio; OS: Overall survival; PPS: Post-progression survival; RFS: Relapse-free survival.

autophosphorylation, regulation of cell cycle G2/M phase transition, positive regulation of transferase activity, organelle localization, regulation of microtubule-based processes, meiotic cell cycle, T-cell differentiation in the thymus, positive regulation of translation initiation, brain development, cellular response to peptides, nuclear organization, and regulation of lymphocyte apoptotic processes. The genes are involved in chromosome,
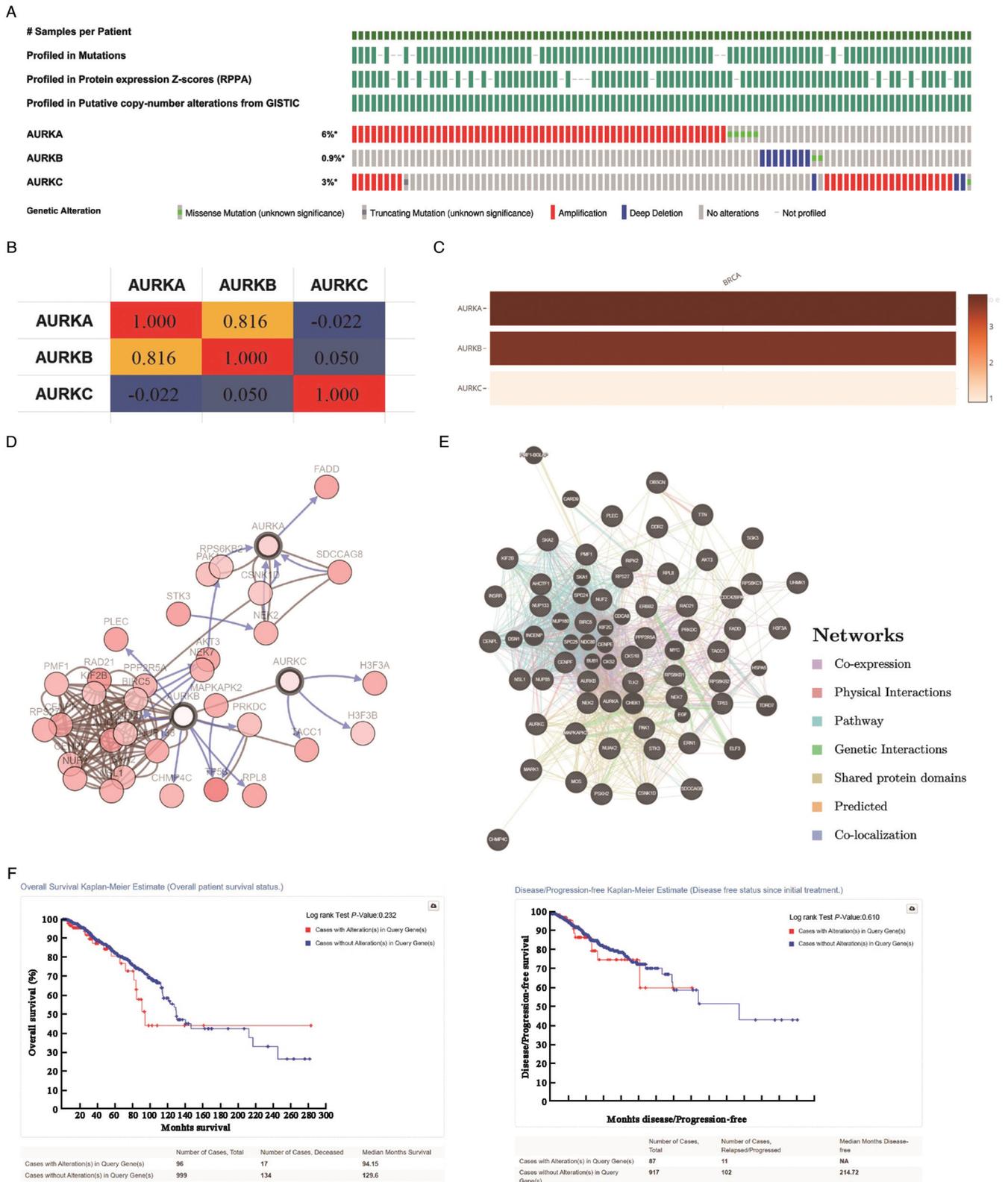
Figure 3: *AURKs* gene expression and mutation analysis in BC (cBioPortal). (A) *AURKs* gene expression and mutation analysis in BC (cBioPortal). (B) Pearson correlation of *AURK* gene family members. (C) Correction between different *AURK* in BC (cBioPortal). (D) The network for *AURK* and the 50 most frequently altered neighbor genes (cBioPortal). (E) PPI network among *AURK* family members in the GeneMANIA dataset. (F) Kaplan–Meier plots comparing OS and DFS in cases with/without *AURKs* family member alterations. *AURKs* Aurora kinases; *AURKA:* Aurora kinase A; *AURKB:* Aurora kinase B; *AURKC* Aurora kinase C; BC: Breast cancer; DFS: Disease-free survival; OS: Overall survival; PPI: Protein-protein interaction.
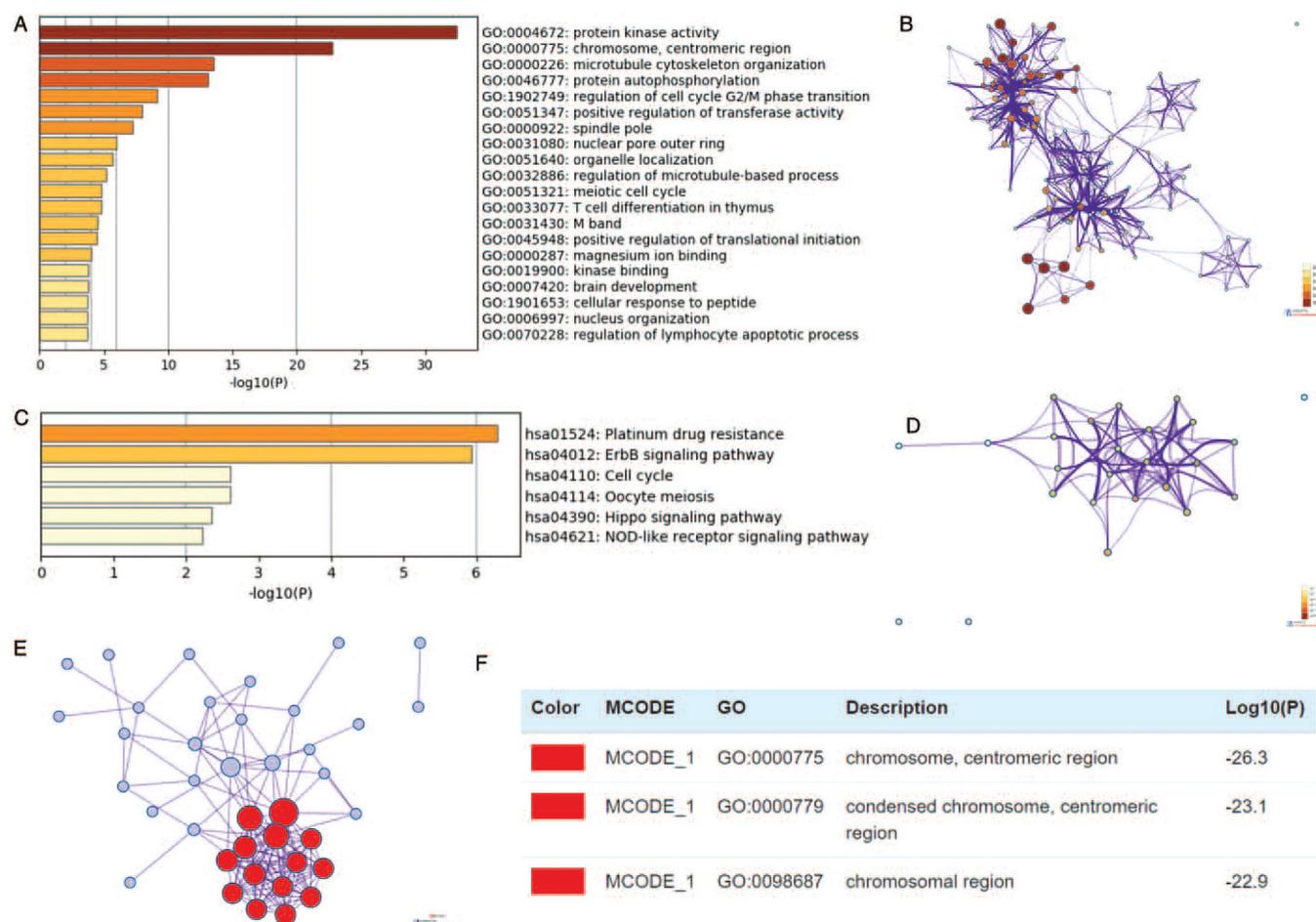
Figure 4: The enrichment analysis of *AURK* family members and neighboring genes in BC (Metascape). (A) Heatmap of GO enriched terms colored by *P* values. (B) Network of GO enriched terms colored by *P* value, where terms containing more genes tend to have a more significant *P* value. (C) Heatmap of KEGG enriched terms colored by *P* values. (D) Network of KEGG enriched terms colored by *P* value, where terms containing more genes tend to have a more significant *P* value. (E) PPI network and the most significant MCODE component form the PPI network. (F) Independent functional enrichment analysis of three MCODE components. *AURKs* Aurora kinases; BC: Breast cancer; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genome; MCODE: Molecular Complex Detection; PPI: Protein-protein interaction.

centromeric region, spindle pole, nuclear pore outer ring, and M band; the molecular functions for these genes are mainly the regulation of protein kinase activity, magnesium ion binding, and kinase binding.

The top six KEGG pathways for the *AURK* family members and their neighboring genes are shown in Figure 4C and 4D; Table 4. Among these pathways, the cell cycle signaling pathway, platinum drug resistance signaling pathway, *ErbB* signaling pathway, *Hippo* signaling pathway, and nucleotide-binding and oligo-meri-zation domain-like receptor signaling pathway were found to be associated with multiple tumor development and play important roles in the tumorigenesis and pathogenesis of BC [Figure 5]. In addition, to better comprehend the relationship between *AURK* family members and BC, we performed a Metascape PPI enrichment analysis. The PPI network and MCODE components identified in the gene lists are shown in Figure 4E and 4F. Through enrichment analysis of pathways and biological processes for each MCODE component, we found that the biological processes were

mainly related to chromosome, centromeric region, condensed chromosome, and chromosomal region.

Kinase, miRNA, or transcription factor network targets of AURKs in BC patients

To further explore the targets of *AURKs* in BC, we used the GSEA online tool to analyze kinases, miRNAs, and transcription factors. For *AURKA,* the top three most significant target networks were the kinase-target networks related primarily to cyclin-dependent kinase 1 (*CDK1*), polo-like kinase 1 *(PLK1),* and *AURKB.* The miRNA-target network was associated with (GACAATC) miR-219, (GTGCAAA) miR-507, and (GGATCCG) miR-127. The transcription factor-target network was related mainly to the *E2F* transcription factor *(E2F)* family, including *E2F1_Q6, E2F_Q6,and* E2F_Q4. Regarding *AURKB*, the kinase-target networks were associated with *PLK1* , *CDK1* , and cyclin-dependent kinase 2 *(CDK2).* The miRNA-target network was associated with (ACTT-TAT) miR-142-5P, (CTTGTAT) miR-381, (ATGTAGC) miR-221 and miR-222. The transcription factor-target

Table 3: The GO function enrichment analysis of *AURK* family members and neighbor genes in BC (GeneMANIA).

GO	Category	Description	Count	%	Log10 (*P*)	Log10 (*q*)
GO:0000226	GO biological processes	Microtubule cytoskeleton organization	16	30.77	−13.52	-10.32
GO:0046777	GO biological processes	Protein autophosphorylation	12	23.08	−13.10	-9.95
GO:1902749	GO biological processes	Regulation of cell cycle G2/M phase transition	9	17.31	−9.14	-6.28
GO:0051347	GO biological processes	Positive regulation of transferase activity	12	23.08	−7.99	-5.26
GO:0051640	GO biological processes	Organelle localization	10	19.23	−5.65	-3.15
GO:0032886	GO biological processes	Regulation of microtubule-based process	6	11.54	−5.14	-2.72
GO:0051321	GO biological processes	Meiotic cell cycle	6	11.54	−4.81	-2.42
GO:0033077	GO biological processes	T cell differentiation in thymus	4	7.69	−4.80	-2.42
GO:0045948	GO biological processes	Positive regulation of translational initiation	3	5.77	−4.44	-2.11
GO:0007420	GO biological processes	Brain development	8	15.38	−3.79	-1.60
GO:1901653	GO biological processes	Cellular response to peptide	6	11.54	−3.74	-1.57
GO:0006997	GO biological processes	Nucleus organization	4	7.69	−3.71	-1.55
GO:0070228	GO biological processes	Regulation of lymphocyte apoptotic process	3	5.77	−3.69	-1.54
GO:0000775	GO cellular components	Chromosome, centromeric region	17	32.69	−22.73	-19.08
GO:0000922	GO cellular components	Spindle pole	7	13.46	−7.22	-4.56
GO:0031080	GO cellular components	Nuclear pore outer ring	3	5.77	−5.95	-3.39
GO:0031430	GO cellular components	M band	3	5.77	−4.53	-2.19
GO:0004672	GO molecular functions	Protein kinase activity	29	55.77	−32.45	-28.10
GO:0000287	GO molecular functions	Magnesium ion binding	5	9.62	−4.03	-1.77
GO:0019900	GO molecular functions	Kinase binding	8	15.38	−3.80	-1.61

AURKs: Aurora kinases; BC: Breast cancer; GO: Gene ontology.

Table 4: The KEGG function enrichment analysis of AURK family members and neighbor genes in BC (GeneMANIA).

GO	Category	Description	Count	%	Log10 (*P*)	Log10 (*q*)
hsa01524	KEGG pathway	Platinum drug resistance	5	9.62	−6.29	−3.6
hsa04012	KEGG pathway	ErbB signaling pathway	5	9.62	−5.94	−3.54
hsa04110	KEGG pathway	Cell cycle	3	5.77	−2.61	−1.41
hsa04114	KEGG pathway	Oocyte meiosis	3	5.77	−2.61	−1.41
hsa04390	KEGG pathway	*Hippo* signaling pathway	3	5.77	−2.35	−1.2
hsa04621	KEGG pathway	NOD-like receptor signaling pathway	3	5.77	−2.23	−1.09

AURKs: Aurora kinases; BC: Breast cancer; KEGG: Kyoto Encyclopedia of Genes and Genome; NOD: Nucleotide-binding and oligomerization domain.

network was also related mainly to the *E2F* family, including *E2F_Q6, E2F1_Q6,* and *E2F_Q4.* For *AURKC,* we found that the kinase-target networks were associated with ataxia-telangi-ectasia mutated serine/threonine kinase *(ATM),* mesenchy-mal-epithelial transition proto-oncogene, receptor tyrosine kinase *(MET),* and *CDK1.* The miRNA-target network was associated with (CACTGTG) miR-128A and MIR 128B, (ACATTCC) miR-1 and miR-206, (AGCACTT) miR-93, miR-302A, miR-302B, miR-302C, miR-302D, miR-372, miR-373, miR-520A, miR-520B, miR-520C, miR-520D, miR-520E, and miR-526B [Table 5 and Supplementary Tables 1-3, http://links.lww.com/CM9/A960].

AURKs correlated with immune infiltrates in BC

Using TIMER databases, we analyzed the relationship between *AURK* gene family members and various infiltrating immune cells in BC, including B cells, CD8 + T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells [Figure 6]. The results indicated that the *AURKA* expression level was slightly positively correlated with tumor purity and the infiltration level of B cells

($r = 0.169, P = 1.02e–07$) and DCs ($r = 0.121, P = 1.91e-04$), whereas it was weakly negatively correlated with the level of CD4+ T cell ($r = −0.039, P = 2.03e–01$) and macrophage ($r = −0.124, P = 9.52e–05$) infiltration but not significantly correlated with infiltration by CD8+ T cells and neutrophils. Regarding *AURKB,* we found that its expression level was positively correlated with tumor purity and the proportion of B cell ($r = 0.177, P = 2.60e–08$) and DC ($r = 0.102, P = 1.66e–03$) infiltration, whereas it was mildly negatively correlated with infiltration by CD8+ T cells ($r = −0.107, P = 8.27e–04$) and macrophages ($r = −0.228, P = 4.45e–13$) but not significantly correlated with CD4+ T cell and neutrophil infiltration. For *AURKC,* we found that its expression level was negatively correlated with tumor purity and the infiltration by CD8+ T cells ($r = −0.029, P = 3.72e–01$), whereas it was positively correlated with infiltration by DCs ($r = 0.107, P = 9.13e–04$) but not significantly correlated with infiltration by B cells, CD4+ T cells, macrophages, and neutrophils. These findings suggest that *AURKs* may play an important role in BC infiltration by immune cells, especially for B cells, macrophages, and DCs.

Figure 5: ERBB-signaling pathway regulated by the *AURKs* alteration in BC. *AURKs.* Aurora kinases; BC: Breast cancer.

Discussion

Numerous studies have suggested that the *AURK* family plays a vital role not only in cell division but also in tumorigenesis.[7,8] The role of *AURKs* in tumorigenesis and the prognosis of several cancers has been partially uncovered. Casorzo *et al*[13] demonstrated that *AURKA* is involved in the adenoma-carcinoma sequence of the large bowel and acts solely in adenomas in which malignant transformation actually occurs. Increasing experimental evidence indicates that *AURKA* overexpression could promote the metastasis of BC and give rise to chemoresistance.[15,31] Moreover, many *AURKA* inhibitors have been applied and demonstrated enhanced therapeutic efficiency.[32] Honma *et al*[11] investigated whether the overexpression of Aurora-B might contribute to DNA aneuploidy in gastric cancer by promoting chromosomal instability. Zhang *et al*[16] found that elevated Aurora-B expression contributes to chemoresistance and poor prognosis in BC. It has been reported that the over-expression of *AURKB* in BC is significantly associated with poor prognosis, and the inhibitor reversine may be an effective drug based on its anti-tumor effects in BC, especially for triple-negative breast cancer.[33] Zekri *et al*[17] showed that *AURKC* mRNA is overexpressed in invasive models of breast and prostate cancer; further biogenic analysis of the *AURKs* in BC remains to be performed. Our study is the first to explore the *AURKs*

expression patterns, prognostic value, genetic alterations, correlations, potential functions, and relationships with immune cells in BC patients. We hope that the results of this study will help to improve the poor prognosis of BC patients and provide new targets for BC treatment.

In our study, the ONCOMINE and GEPIA datasets showed that the expression of *AURKA* and *AURKB* was higher in human BC than normal tissue. The level was associated with the clinical parameters of BC patients. Using Kaplan–Meier plotter, we determined the prognostic value of *AURKA* and *AURKB* in BC patients and found that the increased expression level of *AURKA* was significantly associated with poor OS, RFS, DMFS, and PPS, whereas the increased expression level of *AURKB* was significantly associated with poor OS, RFS, DMFS but not PPS in all BC patients. Copy number variations (CNVs) can have major genomic implications, disrupt genes, and alter genetic content, leading to phenotypic differences.[34] The analysis of transcriptional sequencing data from clinical samples in the TCGA database showed that mRNA levels and CNVs of *AURKA* and *AURKB* are significantly higher in tumor tissue than normal breast tissue. These findings suggest that *AURKA* and *AURKB* overexpression occurs in many BC patients, but their efficacy as potential diagnostic and prognostic markers still needs further clinical validation. Our results reveal that the copy number of *AURKA* and *AURKB* is increased

Table 5: The kinase, miRNA, and transcription factor-target networks of *AURKs* in BC (LinkedOmics).

Gene	Enriched category	Geneset	LeadingEdgeNum	FDR
AURKA	Kinase target	Kinase_CDK1	73	0
		Kinase_PLK1	26	0
		Kinase_AURKB	31	0
	miRNA target	GACAATC, MIR-219	40	0.42216
		GTGCAAA, MIR-507	39	0.61700
		GGATCCG, MIR-127	2	0.60979
	Transcription factor target	V$E2F1_Q6	75	0
		V$E2F_Q6	71	0
		V$E2F_Q4	71	0
AURKB	Kinase target	Kinase_PLK1	27	0
		Kinase_CDK1	69	0
		Kinase_CDK2	87	0
	miRNA target	ACTTTAT, MIR-142-5P	120	0.004151
		CTTGTAT, MIR-381	64	0.004324
		ATGTAGC, MIR-221, MIR-222	50	0.004540
	Transcription factor target	V$E2F_Q6	69	0
		V$E2F1_Q6	79	0
		V$E2F_Q4	68	0
AURKC	Kinase target	Kinase_ATM	41	0.052484
		Kinase_MET	5	0.057733
		Kinase_CDK1	73	0.093815
	miRNA target	CACTGTG, MIR-128A, MIR-128B	99	0
		ACATTCC, MIR-1, MIR-206	95	0
		AGCACTT, MIR-93, MIR-302A, MIR-302B, MIR-302C, MIR-302D, MIR-372, MIR-373, MIR-520E, MIR-520A, MIR-526B, MIR-520B, MIR-520C, MIR-520D	113	0
	Transcription factor target	V$ETF_Q6	38	0
		TAANNYSGCG_UNKNOWN	25	0
		V$E2F1DP1_01	66	0.005299

ATM: Ataxia-telangiectasia mutated; *AURKs:* Aurora kinases; *AURKA:* Aurora kinase A; *AURKB:* Aurora kinase B; *AURKC:* Aurora kinase C; BC: Breast cancer; *CDK1:* Cyclin-dependent kinase 1; *MET:* Mesenchymal-epithelial transition; *PLK1:* Polo-like kinase 1; FDR: False discovery rate.
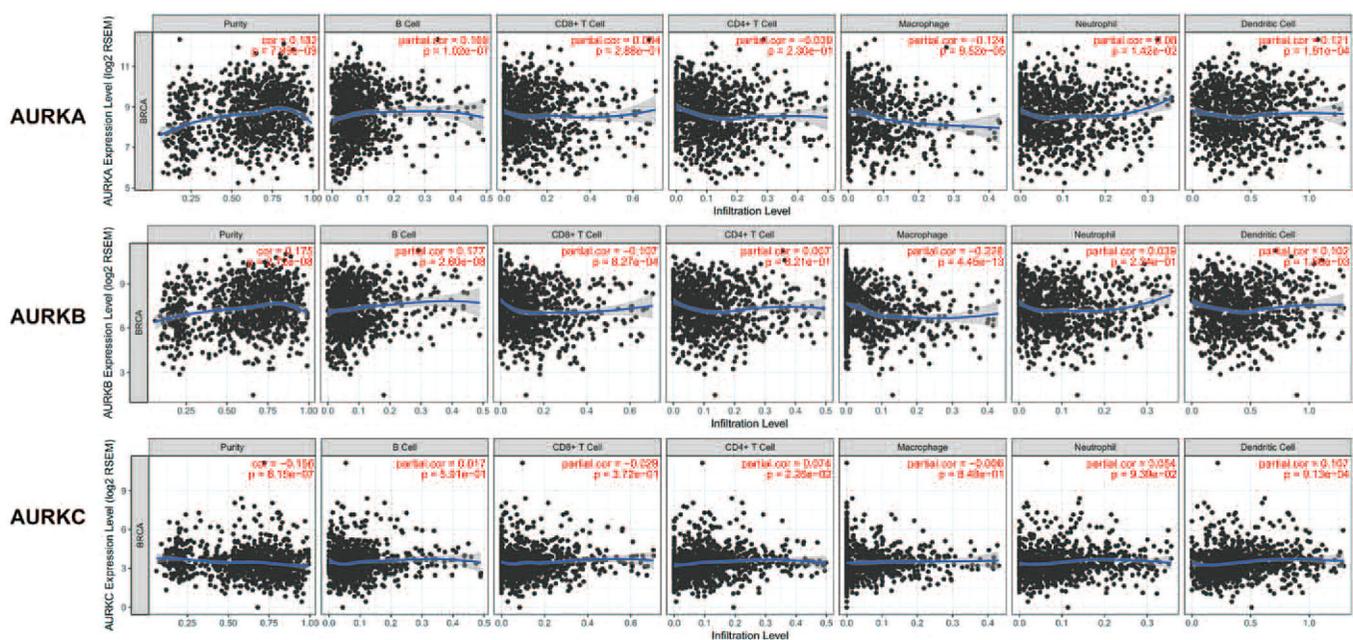
Figure 6: Correlation of *AURKs* expression with immune infiltration level in BC. *AURKs:* Aurora kinases; *AURKA:* Aurora kinase A; *AURKB:* Aurora kinase B; *AURKC* Aurora kinase C; BC: Breast cancer.

in BC and that the major alteration type of *AURKA* is amplification, whereas *AURKB* mainly had deep deletions, which also correlated with reduced survival times. Since *AURKA* and *AURKB* are essential for several important physiological pathways, their alteration and dysfunction may have a negative influence on different downstream signaling pathways, such as the functional networks related to kinase binding, the cell cycle signaling pathway, the platinum drug resistance signaling pathway, the *ErbB* signaling pathway, and the *Hippo* signaling pathway. We hypothesize that the altered *AURKA* and *AURKB* expression patterns may be caused by alterations in chromosomal structure. Thus, the network of *AURKA* and *AURKB* alterations is involved in embryogenesis, cell cycle regulation, protein kinase activity regulation, and transcrip-tional regulation biological processes, which is consistent with their normal physiological functions.[7,9] We performed an enrichment analysis of *AURKs,* which helped us to uncover the important related *AURK* target networks of kinases, miRNAs, and transcription factors. The results show that the functional network of *AURKA* and *AURKB* participates primarily in the chromosome, centromeric region, kinase regulation, and cell cycle. These findings are consistent with the finding that *AURKA* and *AURKB* are critical for efficient and faithful partitioning of chromosomes into daughter cells.[7] Furthermore, it is important to clarify how the alteration in a crucial protein that ensures normal transcription could result in major dysfunction or even carcinoma such as BC. The fundamental hallmarks of cancer cells are also genomic instability and mutagenesis, but kinases and their associated signaling pathways could contribute to stabilizing or restoring genomic DNA.[35] We foundtha-tinBC,*AURKA* is associated with a network of kinases, including *CDK1, PLK1,* and *AURKB.* For *AURKB,* the main associated kinases were *PLK1, CDK1,* and *CDK2.* These kinases regulate the cell cycle and mitosis.[36-38] Thus, *AURKA* and *AURKB* may regulate DNA damage repair, cell cycle progression, and embryo-genesis via *PLK1, CDK1* and *CDK2* kinases. We also identified several miRNAs that are correlated with *AURKA* and *AURKB.* These short non-coding RNAs normally participate in the posttranscriptional regulation of gene expression and can contribute to human tumorigene-sis.[39,40] The particular miRNAs in our study have been linked to tumor proliferation, invasion, metastasis, cell cycle, and drug resistance. In fact, miR-219 promotes tumor growth and metastasis of liver cancer and also promotes the self-renewal capacity, tumorigenicity, and chemoresistance of liver CSCs.[41,42] The expression of miR-507 and miR-127 has been reported to be inversely correlated with the invasion potential and proliferation of BC.[43,44] With respectto *AURKB,* miR-142 has been found to be correlated with the immune and inflammatory response,[45] and miR-381 has been reported to be dysregulated in BC and may play a tumor-suppressor role in cancer.[46,47] In addition, several studies have revealed that the overexpression of miR-221 and miR-222 is associated with metastatic activity and malignancy potential and that the overexpression of miR-222 is associated with poor prognosis in cancer patients; furthermore, the significance of miR-221 remains unde-

fined.[48,49] Our findings show that the different influences of *AURKA* and *AURKB* on different miRNAs remain to be explored, and the dysregulation of these miRNAs needs further study for confirmation. In addition, we found that the transcription factor-target network of *AURKA* and *AURKB* was mainly correlated with the *E2F* family. *E2F1* plays an important role in regulating the cell cycle.[50] Aberrant *E2F1* expression is significantly associated with the occurrence and development of BC, and several studies have demonstrated that the increased expression of *E2F1* is related to poor prognosis in BC patients.[51,52] Our findings are consistent with the aforementioned findings that *AURKA* and *AURKB* are vital targets and regulate the cell cycle and propagation of BC. We also investigated the relationship between *AURKA* and *AURKB* with the infiltrating immune cells in patients with BC and found that the expression levels of *AURKA* and *AURKB* were moderately associated with B cells and DCs, which suggests that the dysregulation of *AURKA* and *AURKB* expression may influence the immune cells infiltrating the tumor microenvironment. Therefore, the expression level changes of *AURKA* and *AURKB* in patients with BC may serve as a potential marker in the clinic.

In terms of *AURKC,*unlike*AURKA* and *AURKB,* itis specifically expressed in the testis tissue of mammals.[53] Since some studies have shown that the forced expression of mutant *AURKC* in mouse oocytes causes oocyte cell cycle arrest at meiosis I and the formation of aneuploid eggs,[54] it has been speculated that *AURKC* plays a critical role in meiotic chromosome segregation. There is also a study showing that *AURKC* is involved in the development and promotion of cancer based on its overlapping and complementary function with *AURKB* and gene alterations in tumors.[17] However, in contrast to *AURKA* and *AURKB, AURKC* did not show any expression variation between human BC and normal tissue or association with clinical parameters. The prognostic value of *AURKC* in BC was not similar to that of the two other *AURK* family members, and *AURKC* expression did not effectively predict the outcomes of BC patients. Through the analysis of *AURKC* protein alterations, we found that *AURKC* in BC is associated with a network of kinases, including ATM, *MET,* and *CDK1.* The miRNA-target network was associated with (CACTGTG) miR-128A and MIR 128B, (ACATTCC) miR-1 and miR-206, (AGCACTT) miR-93, miR-302A, miR-302B, miR-302C, miR-302D, miR-372, miR-373, miR-20A, miR-20B, miR-20C, miR-20D, miR-20E, and miR-526B. Regarding the relationship with infiltrating immune cells, we found that *AURKC* was slightly associated with DCs.

In summary, this study provided evidence for the upregulation of *AURKA* and *AURKB* in BC. In addition, we found that *AURKA* and *AURKB* had prognostic and diagnostic value for BC. *AURKA* and *AURKB* act as upstream molecules regulating their target molecules including kinase, miRNA, and transcription factor. However, whether or not the target molecules will regulate or influence the expression of *AURKs* in turn or interact with each other still needs further verification.

Conclusions

In conclusion, our results suggested *AURKA* and *AURKB* could be employed as prognosis biomarkers and were associated with immune infiltration in BC, and provided more serviceable information on the role of *AURKA* and *AURKB* in tumorigenesis.

Funding

Conflicts of interest

None.

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