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 EZF 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),[2,3] which play a critical role in helping to judge the prognosis and drug sensitivity of BC,[1,2,3] the prognosis in these patients remains poor.[3]

Given that heterogeneity is one of the hallmarks of tumors,[4] 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
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 AURK family in BC have been performed[15,23] the role of AURK family was far from being fully clarified.

In our study, a comprehensive study about the expression, and prognosis significance of AURK 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 cBioPortal 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 AURK 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 AURK 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.cbioportal.org/results/oncoprint?session_id=5f40b87a4b04836b8ae2a71). 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, InWeb1M, 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 TCGA cancer-associated multi-dimensional datasets. The LinkFinder module of LinkedOmics was used to study genes differentially expressed in correlation sets. 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.

**TIMER database analysis**

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (https://cistrome.shinyapps.io/timer/). The TIMER database includes 10,897 samples across 32 cancer types from TCGA to estimate the abundance of immune infiltrates. We analyzed AURK expression in BC and the correlation of AURK 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 change was 2.351. In TCGA, the Curtis, Zhao, and Radvanyi datasets showed similar fold changes (2.655, 2.333, and 2.922, respectively).
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=5d0fb87ae4b04836b8ae2a71) 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...
AURKB closely associated with CSNK1D, PAK1, RPS6KB2, SDCCAG8, NEK7, ATK3, TACC1, H3F3B, H3F3A, STK3, NEK2, NSL1, SKA2, NUF2, CENPL, RPS27, CENPF, PMF1, RAD21, KIF2B, AHCCTFI, NUP85, NUP153, 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

| 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 | Radvarani Breast |
| | Invasive ductal breast carcinoma vs. normal | 2.733 | 3.30E-02 | 2.169 | Turashvili Breast |

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

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.
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.
AURK family members and neighboring 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 oligomerization 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 network...
network was also related mainly to the \(2\)F family, including \(E2F_0, E2F_1, E2F_2\), and \(E2F_3\). For \(AURK\), we found that the kinase-target networks were associated with ataxia-telangiectasia mutated serine/threonine kinase (\(ATM\)), mesenchymal-epithelial transition proto-oncogene, receptor tyrosine kinase (\(MET\)), and \(CDK1\). The miRNA-target network was associated with (CAGTGTG) miR-128A and MIR 128B, (ACATTCC) miR-1 and miR-206, (AGCAGTG) miR-93, miR-302A, miR-302B, miR-302C, miR-372, miR-373, miR-520A, miR-520B, miR-520C, miR-520D, and miR-520E [Table 5 and Supplementary Tables 1-3, http://links.lww.com/CM9/A960].

**AURK**s 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 \(AURK\)A 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 \((r = 0.127, P = 2.60e-08)\) 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 \(AURK\), 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 \(AURK\)s may play an important role in \(BC\) infiltration by immune cells, especially for B cells, macrophages, and DCs.

### 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:0002226 | 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:0045598 | 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         |

**AURK**: 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).

| KEGG | 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        |

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

Figure 5: ERBB-signaling pathway regulated by the AURK alteration in BC. AURKs: Aurora kinases; BC: Breast cancer.
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   |
|       |                      | GTGCGAAA, MIR-507| 39             | 0.61700   |
|       |                      | GGATCCG, MIR-127| 2              | 0.60979   |
|       | Transcription factor | VSE2F1_Q6       | 75             | 0         |
|       |                      | VSE2F_Q6        | 71             | 0         |
|       |                      | VSE2F_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  |
|       |                      | CTGGTAT, MIR-381 | 64             | 0.004324  |
|       |                      | ATGTAGC, MIR-221, MIR-222 | 50 | 0.004340 |
|       | Transcription factor | VSE2F_Q6        | 69             | 0         |
|       |                      | VSE2F1_Q6       | 79             | 0         |
|       |                      | VSE2F_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         |
|       |                      | ACATCC, MIR-1, MIR-206 | 95 | 0         |
|       |                      | AGCAGTT, MIR-93, MIR-302A, MIR-302B, MIR-302C, MIR-302D, MIR-372, MIR-373, MIR-520E, MIR-520A, MIR-520B, MIR-520C, MIR-520D | 113 | 0         |
|       | Transcription factor | VSETF_Q6        | 38             | 0         |
|       |                      | TAANNYSGCCG_UNKNOWN | 25 | 0         |
|       |                      | VSE2F1DP1_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.

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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 transcriptional regulation biological processes, which is consistent with their normal physiological functions.\textsuperscript{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.\textsuperscript{17} 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.\textsuperscript{36-38} We found that in BC, 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.\textsuperscript{36-38} Thus, AURKA and AURKB may regulate DNA damage repair, cell cycle progression, and embryo-gensis 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 tumorigenesis.\textsuperscript{19,40} 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.\textsuperscript{141,42} The expression of miR-507 and miR-127 has been reported to be inversely correlated with the invasion potential and proliferation of BC.\textsuperscript{143,44} With respect to AURKB, miR-142 has been found to be correlated with the immune and inflammatory response,\textsuperscript{45} and miR-381 has been reported to be dysregulated in BC and may play a tumor-suppressor role in cancer.\textsuperscript{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 undefined.\textsuperscript{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.\textsuperscript{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.\textsuperscript{31,52} 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, it is specifically expressed in the testis tissue of mammals.\textsuperscript{153} 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,\textsuperscript{24} 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.\textsuperscript{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 (CAGTTC) miR-128A and MIR 128B, (ACATTCC) miR-1 and miR-206, (AGACTT) 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.
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 tumorogenesis.

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Conflicts of interest
None.

References
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30. doi: 10.3322/caac.21442.
2. Dai X, Xiang L, Li T, Bai Z. Cancer hallmarks, biomarkers and breast cancer molecular subtypes. J Cancer 2016;7:1281–1294. doi: 10.7150/jca.13141.
3. Harbeck N, Grant M. Breast cancer. Lancet 2017;389:1134–1150. doi: 10.1016/S0140-6736(16)31891-8.
4. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 2015;5:2929–2943.
5. Cardoso F, Harbeck N, Fallowfield L, Lienert N, Mortimer D, Samejima K, Ogawa H, et al. AURKB overexpression to low survival rate in BCRA and HDAC synergistically regulate survival and proliferation of lymphoma cell via AKT, mTOR and Notch pathways. Eur J Pharmacol 2016;779:1–7. doi: 10.1016/j.ejphar.2015.11.045.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–674. doi: 10.1016/j.cell.2011.02.013.
7. Vagner G, Lens SM. The Aurora kinase family in cell division and cancer. Biochim Biophys Acta 2008;1786:60–72. doi: 10.1016/j.bbabio.2008.07.003.
8. Teng A, Gao K, Chu L, Zang R, Yang J, Zheng J. Aurora kinases: novel therapy targets in cancers. Oncotarget 2017;8:23397–23394. doi: 10.18632/oncotarget.14893.
9. Nikonova AV, Asatsuroun I, Serebriiski IG, Dunbrack RL Jr, Golemis EA. Aurora A/B kinase(AURKA) in normal and pathological cell division. Cell Mol Life Sci 2013;70:661–687. doi: 10.1007/s00018-012-1073-7.
10. Quartuccio SM, Schindler K. Functions of Aurora kinase C in meiosis and cancer. Front Cell Dev Biol 2015;3:50. doi: 10.3389/fcel.2015.00050.
11. Honma K, Nakashima T, Nakano T, Ando K, Saeki H, Oki E, et al. Contribution of Aurora-A and -B expression to DNA aneuploidy in gastric cancers. Surg Today 2014;44:454–461. doi: 10.1007/s00595-013-0581-x.
12. Davidson B, Nymoen DA, Elgaaen BV, Staff AC, Trope CG, Kaern M, Alvero AB, et al. AURKA and AURKB mRNA in advanced-stage ovarian serous carcinoma. Cancer Res 2015;75:707–717. doi: 10.1158/0008-5472.CAN-14-2812.
13. Casorzo L, Dell’Aglio C, Sarotto I, Risio M. Aurora kinase A gene copy number is associated with the malignant transformation of colorectal adenomas but not with the serrated neoplasia progression. Hum Pathol 2015;46:411–418. doi: 10.1016/j.humpat.2014.11.016.
14. Twu NF, Yuan CC, Yen MS, Lai CR, Chao KC, Wang PH, et al. Expression of Aurora kinase A and B in normal and malignant cervical tissue: high Aurora A kinase expression in squamous cervical cancer. Eur J Obstet Gynecol Reprod Biol 2009;142:57–63. doi: 10.1016/j.ejogrb.2008.09.012.
36. Gheghiani L, Loew D, Lombard B, Mansfeld J, Gavet O. PLK1 activation in late G2 sets up commitment to mitosis. Cell Rep 2017;19:2060–2073. doi: 10.1016/j.celrep.2017.05.031.

37. Fang H, Niu K, Mo D, Zhu Y, Tan Q, Wei D, et al. RecQL4-Aurora B kinase axis is essential for cellular proliferation, cell cycle progression, and mitotic integrity. Oncogenesis 2018;7:68. doi: 10.1038/s41389-018-0080-4.

38. Herrera MC, Chymkowitch P, Robertson JM, Eriksson J, Boe SO, Alseth I, et al. Cdk1 gates cell cycle-dependent tRNA synthesis by regulating RNA polymerase III activity. Nucleic Acids Res 2018;46:11698–11711. doi: 10.1093/nar/gky846.

39. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014;20:460–469. doi: 10.1016/j.molmed.2014.06.005.

40. Bayoumi AS, Sayed A, Broskova Z, Teoh JP, Wilson J, Su H, et al. Crosstalk between long noncoding RNAs and microRNAs in health and disease. Int J Mol Sci 2016;17:356. doi: 10.3390/ijms17030356.

41. Yang J, Sheng YY, Wei JW, Gao XM, Zhu Y, Jia HL, et al. MicroRNA-219-5p promotes tumor growth and metastasis of hepatocellular carcinoma by regulating cadherin 1. Biomed Res Int 2018;2018:4793971. doi: 10.1155/2018/4793971.

42. Si A, Wang L, Miao K, Zhang R, Ji H, Lei Z, et al. miR-219 regulates liver cancer stem cell expansion via E-cadherin pathway. Cell Cycle 2019;18:3530–3561. doi: 10.1080/15384101.2019.1691762.

43. Lu G, Li Y, Ma Y, Lu J, Chen Y, Jiang Q, et al. MicroRNA-127 is downregulated by Tudor-SN protein and contributes to metastasis and proliferation in breast cancer cell line MDA-MB-231. Anat Rec (Hoboken) 2013;296:1842–1849. doi: 10.1002/ar.22823.

44. Sharma S. Immunomodulation: a definitive role of microRNA-142. Dev Comp Immunol 2017;77:150–156. doi: 10.1016/j.dci.2017.08.001.

45. Ming J, Zhou Y, Du J, Fan S, Pan B, Wang Y, et al. miR-381 suppresses CEBPalpha-dependent Cx43 expression in breast cancer cells. Biosci Rep 2015;35:e00266. doi: 10.1042/BSR20150167.

46. Qiao G, Li J, Wang J, Wang Z, Bian W. miR381 functions as a tumor suppressor by targeting ETS1 in pancreatic cancer. Int J Mol Med 2019;44:593–607. doi: 10.3892/ijmm.2019.4206.

47. Iida M, Hazama S, Tsunedomi R, Tanaka H, Takenouchi H, Kaneko Y, et al. Overexpression of miR221 and miR222 in the cancer stroma is associated with malignant potential in colorectal cancer. Oncol Rep 2018;40:1621–1631. doi: 10.3892/or.2018.6575.

48. Ravagnani G, Cangrini S, Sammarini G, Zanotti F, Bermejo JL, Hrelia P, et al. Prognostic role of miR-221 and miR-222 expression in cancer patients: a systematic review and meta-analysis. Cancers (Basel) 2019;11:970. doi: 10.3390/cancers11070970.

49. Polager S, Ginsberg D. E2F - at the crossroads of life and death. Trends Cell Biol 2008;18:528–535. doi: 10.1016/j.tcb.2008.08.003.

50. Sun CC, Li SJ, Hu W, Zhang J, Zhou Q, Liu C, et al. Comprehensive analysis of the expression and prognosis for E2Fs in human breast cancer. Mol Ther 2019;27:1153–1165. doi: 10.1016/j.ymthe.2019.03.019.

51. Lu G, Li Y, Ma Y, Lu J, Chen Y, Jiang Q, et al. Long noncoding RNA LINCC00511 contributes to breast cancer tumourigenesis and stemness by inducing the miR-185-3p/E2F1/Nanog axis. J Exp Clin Cancer Res 2018;37:289. doi: 10.1186/s13046-018-0945-6.

52. Tseng TC, Chen SH, Hsu YP, Tang TK. Protein kinase profile of sperm and eggs: cloning and characterization of two novel testis-specific protein kinases (AIE1, AIE2) related to yeast and fly chromosome segregation regulators. DNA Cell Biol 1998;17:823–833. doi: 10.1089/dna.1998.17.823.

53. Li X, Sakashita G, Matsuzaki H, Sugimoto K, Kimura K, Hanaoa F, et al. Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora-C. J Biol Chem 2004;279:47201–47211. doi: 10.1074/jbc.M403029200.