AURKA is a prognostic potential therapeutic target in skin cutaneous melanoma modulating the tumor microenvironment, apoptosis, and hypoxia

Xuan Fen Zhang (zhang_xf@lzu.edu.cn)
Lanzhou University Second Hospita

ShengYong Long
Lanzhou University Second Hospita

Research Article

Keywords: AURKA, SKCM, tumor microenvironment, apoptosis, hypoxia, molecular docking technology

Posted Date: April 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1528209/v1

AURKA is a prognostic potential therapeutic target in skin cutaneous melanoma modulating the tumor microenvironment, apoptosis, and hypoxia

ShengYong Long, Xuan Fen Zhang

Department of Plastic Surgery, Lanzhou University Second Hospita, Lanzhou, China

Abstract

Background: *AURKA*, Aurora kinase A encoding gene, is an important signaling hub gene for mitosis. In recent years, *AURKA* has been implicated in the occurrence and development of several cancers. However, its relationship with the tumor microenvironment in skin cutaneous melanoma (SKCM) and the molecular mechanisms underlying its effects are still unclear.

Method： We adopted a variety of bioinformatics methods to comprehensively analyze the potential carcinogenesis of *AURKA* in SKCM, and constructed a prognostic nomogram model. We also dentified an inhibitor targeting AURKA and verified its therapeutic effects against SKCM using the molecular docking technology.

Results: We found that abnormally high expression of *AURKA* was responsible for driving the occurrence and development of SKCM, and affected various pathological factors in SKCM. In addition, *AURKA* was established as an independent marker of poor SKCM prognosis. We also characterized the potential mechanisms by which AURKA manifests its effects in SKCM and found that AURKA inhibits the infiltration of CD8+ T cells and promotes hypoxia by activating the TGF-β signaling pathway. At the same time, the high AURKA expression group had higher tumor stemness index and promoted cell proliferation and metastasis. Finally, the small molecule compound ZNC97018978 targeting AURKA screened by molecular docking technology can inhibit the proliferation, invasion and metastasis of SKCM. The possible mechanism is that ZNC97018978 induces apoptosis by arresting the cell cycle, thereby inhibiting cell proliferation.

Conclusion: *AURKA* is the core hub gene driving the occurrence and development of SKCM, and its expression is regulated by epigenetic modifications. AURKA can regulate the infiltration level of various immune cells in the tumor microenvironment, reshape the immunosuppressive tumor microenvironment, and apoptosis, and hypoxia. Thus, it is a prognostic biomarker and potential therapeutic target in SKCM. ZNC97018978 is an effective and safe inhibitor of AURKA in vitro; its safety and effectiveness in vivo as a potential treatment for cutaneous melanoma should be further determined.

Keywords: AURKA; SKCM; tumor microenvironment; apoptosis; hypoxia; molecular docking technology.

Introduction

Skin cutaneous melanoma (SKCM) accounts for only 2% of skin cancers, yet, it is one of the deadliest skin cancers, accounting for more than 80% of skin cancer-related deaths in the world[1]. It is also one of the most malignant tumors endangering human health[2]. In recent decades, the morbidity and mortality of SKCM have gradually increased[3]. Although i immunotherapy and targeted therapy have improved the prognosis of patients with advanced melanoma, only a few patients with SKCM can benefit from it, and there is no specific treatment for patients with metastatic melanoma[4,5]. Thus, it is becoming an urgent to identify new therapeutic targets and develop more effective therapeutics against SKCM.

Tumor microenvironment (TME) refers to the complex dynamic ecosystem on which tumor cells depend for survival and development, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, various signaling molecules, and the extracellular matrix (ECM)[6,7]. Immune cells and their regulation in tumomicroenvironment play an important role in the occurrence and development [8]. Several studies have confirmed that the deterioration of malignant melanoma is closely related to alterations in the immune microenvironment, but no study has determined the immune cells associated with the metastasis of malignant melanoma[9]. As for the core hub genes and TME, it is even less reported that the influence of the transfer system constitutes the interaction system.

In current study,, we aimed to explore potential therapeutic targets and their biological functions in SKCM. *AURKA* has been identified as the core hub gene of SKCM. studies have shown that *AURKA* as an oncogene is involved in the regulation of multiple key steps in the invasion and metastasis of malignant tumors[10,11]. It plays a key role in regulating tumor cell mobility, and is a potential therapeutic target for preventing tumor metastasis in bladder cancer[12], endometrial cancer[13] triple-negative breast cancer[14], nasopharyngeal carcinoma[15], and urothelial carcinoma[16]; it also acts as a biomarker for the diagnosis, treatment, and prognosis of the aforementioned cancers. Therefore, in this study, we investigated the role of AURKA in SKCM, and characterized the mechanism by which it manifests its effect in SKCM. In addition, recent studies have shown that *AURKA* is overexpressed in SKCM tissues and is related to the invasion and metastasis of SKCM. Downregulation of *AURKA* can inhibit the proliferation and

migration of SKCM cells and promote CM apoptosis and autophagy[17,18]. Our current study revealed that abnormally high expression of AURKA promotes SKCM invasion and metastasis in vitro and mediates the formation of an immunosuppressive microenvironment and regulates ferroptosis and hypoxia. Thus, it has several roles in SKCM and may be a prognostic biomarker and potential therapeutic target for SKCM patients. ZNC97018978 was found to be an effective inhibitor of AURKA by virtual drug screening. In vitro tests revealed that ZNC97018978 is a safe and effective drug for the treatment of cutaneous melanoma. Our findings provide an in-depth understanding of the function of AURKA in the occurrence and development of SKCM and unveil the role of AURKA in tumor immunotherapy.

Materials and methods

Data collection

Gene expression matrix from 54 cutaneous melanoma cell lines were retrieved from the CCLE database (https://portals.broadinstitute.org/ccle/about). RNA-seq data and somatic mutation data of 33 cancer types were downloaded from the GDC portal (https://portal.gdc.cancer.gov/) of TCGA (http://cancergenome.nih.gov/), together with corresponding clinical information. Normal tissue samples were obtained from the GTEx V8 database (https://gtexportal.org/home/datasets). In addition, GSE3189, GSE15605 and GSE22155 microarray datasets were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo) to further validate the expression of AURKA and its survival significance.

Collection of clinical specimens

Thirteen pairs of human melanoma tissues and paracancerous tissues were obtained from the tissue specimen bank of the Second Affiliated Hospital of Lanzhou University. The living tissue was collected in situ immediately after ex vivo, and the paraffin-embedded formalin-fixed SKCM tissue and adjacent paracancerous tissue were subjected to immunohistochemical (IHC) staining to verify the expression of AURKA protein in SKCM tissue. This study complied with the Declaration of Helsinki protocol and was approved by the Research Ethics Committee of the Second Hospital of Lanzhou University (No. 2022A-065). All participants provided written informed consent, and the patients in this study did not receive any antitumor therapy before surgery.

Immune correlation analysis

The correlation between AURKA expression and the infiltration abundance of various immune cells in SKCM patients was determined using the TIMER algorithm[19], MCP-

counter algorithm[20] xCell algorithm[21], and ssGSEA algorithm [22] of the Immuneeconv R software package [23] The xCell algorithm[21] explored immune scoring. Subsequently, based on the ESTIMATE algorithm[24] and the xCell algorithm [21] the correlations between immune score, stroma score, tumor purity score in TME, and AURKA expression in SKCM patients were analyzed. The TIMER2.0 [19] (http://timer.cistrome.org/) online database was also used to explore the correlation of AURKA expression with the infiltration levels of four types of immunosuppressive cells, namely, CAFs, MDSCs, Tregs, M2-TAMs, to determine the effect of the infiltration abundance of immune cells on the prognosis of SKCM patients. Finally, we also analyzed the correlation between AURKA and immune markers and MHC molecules in several immune cells.

Differentially expressed gene (DEG) acquisition and functional enrichment analysis

DEGs were analyzed using the Limma package in R language. The threshold for DEG screening was set as "adj $p<0.05$ and $|Log2(FC)| <1$". To gain an in-depth understanding of the potential biological functions of AURKA in SKCM, we used the R language ClusterProfiler package to perform gene set enrichment analysis (GSEA). Gene set enrichment analysis (GSVA) was performed using the R software GSVA package.

Tumor stemness analysis

We used the OCLR algorithm constructed by Malta et al. to calculate the mRNA expression-based stemness (mRNAsi) index[25]and compared the mRNAsi of different groups.

Identification of the SKCM HUB gene.

Based on the SKCM dataset of the cancer genome atlas (TCGA) and the GSE3189 and GSE15605 datasets of the gene expression omnibus (GEO) database, the DEGs in the tissues of SKCM patients and normal patients were analyzed, and 10 HUB genes were identified using the cytoHubba plugin in Cytoscape (version 3.7.2) [26] The association of these10 genes with mutation, survival, and diagnosis was further analyzed.

Comprehensive analysis of AURKA in cutaneous melanoma

The data from CCLE, TCGA, GTEx, GEO and HPA (https://www.proteinatlas.org) databases were used to analyze expression of AURKA at mRNA and protein levels in cutaneous melanoma. TCGA-SKCM data were applied to study the relationship between AURKA mRNA level and clinical characteristics of cutaneous melanoma. R package "maftools" and online databases muTarget (http://www.mutarget.com/), cbiportal (https://www.cBioPortal.org/) and MethSurv 2017 (https://biit.cs.ut.ee/MethSurv/) were adopted and visited to analyze the mutation and methylation profiles of AURKA in cutaneous melanoma. Kaplan-Meier method was used to analyze the survival significance of AURKA in overall survival (OS) and disease-specific survival (DSS) of cutaneous

melanoma patients based on TCGA-SKCM and GSE22155 data. Multi-variate Cox regression analysis was performed to explore clinical factors that potentially affected the survival of cutaneous melanoma patients based on TCGA-SKCM data.[27][28] In addition, we also performed immune correlation, functional enrichment, tumor stemness score, and hypoxia correlation analysis.

AURKA expression analysis

To further study the expression characteristics of AURKA in SKCM, we used the R software ConsensusClusterPlus package to perform consistent clustering analysis on the DEGs between the AURKA-high and -low expression groups. We also conducted survival analysis, computed immune scores, and immune examinations based on the clustering results. Furthermore, we determined point-related gene expression levels, tumor stemness index scores, and performed functional enrichment analysis.

Screening of small molecule inhibitors for AURKA

Crystal structure of AURKA protein was retrieved from the PDB database (https://www.rcsb.org/) and optimized using the Prime module of the Schrodinger20152 software to obtain the three-dimensional structure of biological activity. The SiteMap module was used to predict small molecule ligand binding sites on AURKA, and the Virtual Screening Workflow tool of the ZINC database (www.zinc15.docking.org) was applied to screen out small molecule ligands.

Cell culture and RT-PCR

Human cutaneous melanoma cell line A875 and normal cell line HaCaT were purchased from BnBio (Shanghai, China). The two cell lines were cultured in DMEM (Solarbio, 31600) supplemented with 10% FBS in an incubator with 5% CO_2 at 37 ℃. Until cells reached a confluency of 70%, total RNA was extracted from the cells using the TRIeasy^{TM} Total RNA Extraction Reagent (YESEN, HB170809), following the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the PrimeScript RT reagent Kit with gDNA Eraser (YESEN, 11123ES60). With the cDNA as the template, RT-PCR was run with the SYBR Premix Ex TaqⅡ Kit (YESEN, 11203ES08). The relative mRNA expression of target genes was calculated by $2^{-\triangle\triangle Ct}$. Primers used in amplification were sequenced as: AURKA-Forward: 5'- ACCCACTCCTCCACCTTTGA-3', AURKA-Reverse: 5'-ACCGAGCCATTTCATTTCTG-3'; β-actin-Forward: 5'-GGCACCCAGCACAATGAAG-3', β-actin-Reverse: 5'-CCGATCCAC ACGGGAGTACTTG-3'.

MTT assay

A875 cells were seeded in a 96-well plate at 1.0×10^5 cells/well. Until cells reached a confluency of 75%, ZNC97018978 of different concentrations (20, 40, 80, 160, 320 μM) were added. After 48 h, the cells were further incubated with 5 mg/mL MTT (Solabio, M1020) for 4 h. Then, the medium was removed and DMSO (Solarbio, D8371) was subsequently added. The optical density (OD) values were determined with A microplate reader at 490 nm.

Flow cytometry

A875 cells (1.0×10^5 cells/mL) were cultured in a flask. The culture medium was aspirated once the cells were attached. ZNC97018978 of different concentrations was then added. After 48 h, the cells were collected, washed once with pre-cooled PBS, and resuspended. The suspension (1 mL) was

centrifuged at 1,000 rpm for 5 min. The supernatant was discarded. The remaining fraction was fixed with pre-cooled 70% ethanol, washed with PBS and centrifuged. Then, the cells were suspended by 100 μL RNase A solution, followed by water bath at 37 ℃ for 30 min. Propidium iodide (PI) staining buffer (400 μL) was finally added to stain the cells at 4 ℃ away from light for 30 min. Cell cycle was then assessed by flow cytometry.

Transwell assay

Logarithmically growing cells were harvested and prepared into a single-cell suspension. The cell suspension was added to the upper chamber of a Transwell chamber pre-coated with Matrigel (BD, 356234), and the DMEM with 10% FBS was added to the lower chamber. After routine culture for 48 h, cells that invaded to the lower chamber were collected, fixed with 4% paraformaldehyde for 30 min, stained with Giemsa Staining Solution (Solarbio, G1015) for 30 min, and finally observed microscopically.

Cell transfection

One day before siRNA knockdown target gene transfection, 0.5-2 xio 5 cells were seeded in a petri dish, and 500 dishes of medium without antibody and serum were added to make the cell density reach 50% during transfection. Take an appropriate amount of Lipo3000 and serum-free DMEM, and stir at room temperature for 5 minutes. Take appropriate amount of siRNA and serum-free DMEM and mix well. Gently mix the two mixtures above (to avoid fragmentation in the RNA) and let stand at room temperature for 20 min to form a fluorescent mixture. Add the mixed solution to the well (about 400, 1) of the medium containing the cells and mix gently. Incubate for 4-6 h in a 37 °C incubator, replacing the medium with complete serum containing medium. After 6 hours of transfection, the transfection efficiency can be detected:.

scratch assay

At first, a "十 (ten in Chinese) “ marker was made on the backside of a 6-well plate. Two lines (spaced by 0.5-1 cm) were drawn in each well. Logarithmically growing A875 cells were seeded into the 6-well plate at (5-6) × 10^5 cells/well overnight until 100% coverage. Subsequently, two intersecting lines parallel to the existing lines were made using a 10 μL peptide. PBS washing was performed to remove floating cells. Serum-free medium was added, and the cells were then cultured at 37 ℃ with 5% CO_2 in an incubator. The results at 0, 6, 12 and 24 h were observed by electron microscopy and images were captured.

Western blot

Total proteins were extracted from cells treated with ZNC97018978 using RIPA lysis buffer (Solarbio, R0010), and the supernatant collected from centrifugation (13,000 rpm, 10 min) was analyzed by BCA assay kit to examine protein concentration. Electrophoresis (10% separation gel and 5% spacer gel) was performed to separate proteins, which were then transferred to a biofilm. Antigen-antibody reactions were achieved by addition of primary antibodies overnight at 4 ℃. Hybridization was obtained with secondary antibodies for 1.5 h at 37 ℃. ECL method was used to visualize the protein bands. Image J was applied to perform quantitative analysis. Primary antibodies included AURKA (GeneTex, GTX13824), Caspase-3 (GeneTex, GTX110543), Bax (Abcam, ab32503), Bcl-2 (Abcam, ab196495), CDK1 (Abcam, ab18) and β-actin (Immunoway, YM3028).

Immunohistochemistry (IHC)

The xenograft tumors were paraffin-embedded, sectioned, deparaffinized and hydrated to water. PBS washing was performed for three times (5 min per). Antigen retrieval was obtained via heating with sodium citrate. 3% H_2O_2 was added at room temperature to block endogenous peroxidase activity, followed by addition of normal goat serum. Primary antibodies were added overnight at 4 ℃. On the following day, secondary antibodies were added for 2 h at room termperature. Finally, the specimens were successively treated with DAB, hematoxylin, gradient alcohol and xylene. Microscopic observation was then performed. The results were assessed and scored by two pathologists who were blinded to the sample source. The staining intensity was analyzed using the semi-quantitative integration method.

Statistical Analysis.

After log2 transformation, all gene expression data were normalized. Wilcox-tests were used to compare the differences between the two groups, and for KaplanMeier curves, p-values and hazard ratios (HR) with 95% confidence intervals (CI) were obtained by logrank test and univariate Cox proportional hazards regression. Univariate and multivariate cox regression analysis results Forest maps were implemented by the "forestplot" R package, and Spearman or Pearson correlation analysis was performed between the two variables. The above results were achieved with the R (v4.0.3) packages ggplot2 and pheatmap. P<0.05 was considered significant.

Results

AURKA is an important core hub gene of SKCM

We identified the top 10 hub genes associated with SKCM (Figure 1 (A-E, G)) and found that their mutation frequency was low (Figure 1(F), Supplementary Figure 1 (A)), however, missense mutations were the main polymorphisms and were mainly expressed as C>T and C>A mutation (Supplementary Figure 1 (B-C)). Copy number variation (CNV) occurred in all 10 hub genes, except in *AURKA* and *UBE2C,* which were Hete. Amp, and the remaining 8 hub genes showed Hete. Amp and Hete. Del (Supplementary Figure 1 (E)). Correlation analysis revealed that of the 10 hub genes, *KIF20A* mRNA expression level was negatively correlated with CNV, while the mRNA expression levels of the remaining nine genes were positively correlated with CNV, of which *CDKN3* and *AURKA* had the strongest correlation (Supplementary Figure 1 (D)). At the same time, univariate COX regression and KM analysis revealed that *AURKA* affected the prognosis of SKCM patients (Figure 1 (HI), Supplementary Figure 1 (F-J)). The ROC diagnostic curve found that the AURKA and UBF2C AUC values were the highest at 0.9 and above (Supplementary Figure 2), Correlation analysis found that the correlation coefficients between AURKA and the other 9 hub genes were all above 0.7 except for *PBK* (0.65) and *KIF20A* (0.69) (Figure 1(J)). In conclusion, analysis of the role of the top 10 hub genes in

SKCM revealed that *AURKA* mainly affected the progression and deterioration of SKCM patients.

Clinical significance of *AURKA*

To precisely understand the role of *AURKA* in the development of SKCM, we analyzed the expression, clinicopathological characteristics, prognosis, mutation, copy number variation, and methylation of *AURKA* in SKCM, and found that AURKA mRNA and protein expression was high in the SKCM cell lines of the CCLE database, GTEx database, SKCM-TCGA database, as well as the GSE3189 and GSE46517 datasets of the GEO database and those of the HPA database ($p<0.05$) (Figure 2(A-F). the mRNA expression of *AURKA* was investigated in 33 tumors in TCGA and revealed that the mRNA level of *AURKA* in the other 31 tumors was higher than that in the corresponding normal tissues except for TGCT and PCPG Figure 2 (I). Further IHC analysis revealed that AURKA expression in SKCM tissues was significantly higher than that in adjacent normal tissues (Figure 2(G-H)), and high AURKA expression levels were significantly correlated with T stage, radiation therapy, disease-specific survival (DSS), PFI event, melanoma ulceration, overall survival (OS), and gender in the TCGA SKCM dataset. (Figure 3(L). In addition, KM analysis revealed that AURKA, which was highly expressed in both the GSE22155 dataset and the TCGA SKCM dataset, was a risk factor associated with a poor prognosis in SKCM patients (Figure 2(J-L)). Furthermore, high expression of AURKA is a risk factor associated with a poor prognosis with multiple clinicopathological factors in SKCM patients (Figure 2(N-O)). Multivariate Cox regression analysis showed that AURKA expression, T stage, N stage, and pathological stage were independent factors affecting the prognosis of SKCM (Figure 2(M)). Further analysis revealed that missense mutations were most prevalent in *AURKA*, with a low mutation frequency of 0.43%. In SKCM patients, the most significant mutation in the AURKA-high and -low expression groups was TTN mutation with a mutation frequency of 72%. The *TP53* mutation most affected *AURKA* expression (Figure 3(A-B,D)). However, *AURKA* mutation did not affect SKCM prognosis and level of immune cell infiltration in SKCM patients (Figure 3(C,E)). However, there was a significant positive correlation between the expression of AURKA and copy number variation ($p<0.05$), and AURKA expression affected the level of immune cell infiltration (Supplementary Figure 1 (D-E), Figure 3(F)). AURKA expression was significantly associated with the methylation level of *AURKA* was negatively correlated (Figure 3(G)) and affected the prognosis of patients (Figure 3(H)) in both SKCM and the other 38 tumors. AURKA expression was strongly positively correlated with three key enzymes regulating DNA methylation, namely DNMT1, DNMT3A, and DNMT3B0 (Figure 3(I-J)). These results suggest that AURKA may mediate

tumorigenesis by regulating the methylation status in SKCM. Taken together, these findings strongly suggest that AURKA is an oncogene closely associated with the progression and metastatic deterioration of SKCM and thus, serves as a potential therapeutic target and prognostic biomarker of SKCM.

Identification of SKCM molecular typing based on AURKA expression

A total of 54 DEGs, including 52 up-regulated genes and 2 down-regulated genes, were identified in AURKA-high and -low expression groups (Figure 4(C)). According to the CDF curve, the relative change of the CDF Delta area curve, and the heat map of the consistent clustering results, the optimal number of clusters was determined to be 2 (K value = 2) (Figure 4(A-B)). Therefore, all SKCM patients were divided into two groups, namely, AURKA type I (274 cases, 58.0%) and AURKA type II (196 cases, 42.0%) (Figure 4(B)). KM survival analysis showed that compared with the AURKA type II group, AURKA type I patients had significantly improved prognosis, OS, DSS, and disease-free survival (Figure 4(J-K)). The tumor stemness index was higher in the AURKA type II than that of the AURKA type I group (Figure 4(I))。

AURKA function annotation

To further study the functional role of AURKA in SKCM, we first analyzed the skin melanoma single-cell sequencing datasets GSE72056 and GSE81383 based on the cancerSEA online database [29] and found that the expression level of AURKA was correlated with the cell cycle, proliferation, invasion, EMT and DNA damage (Figure 4(D-E)). Abnormally high expression of AURKA can promote the malignant development of melanoma. We then performed GSEA enrichment analysis on patients from TCGA skin melanoma AURKA-high and -low expression groups. It was found that the cell cycle, DNA damage and repair, mitosis, glycolysis, matrix, and oncogenic activation pathways such as the p53 and TGF-β signaling pathways, were significantly positively correlated with the AURKA-high expression group. It was negatively correlated with immune activation signaling pathways such as DC cells and MHC molecules (Figure 4(G)). Therefore, AURKA may be related to the occurrence and development of tumors. Analysis of the GASElite online database[30]revealed 54 AURKA-related genes mainly related to apoptosis, cell cycle, DNA damage and repair, and EMT activation (Figure 4(F)). Further GSEA functional enrichment analysis to explore potential mechanisms between the two subtypes of AURKA revealed that the AURKA type II group was significantly enriched in the cell cycle, DNA damage, matrix, and oncogenic activation pathways such as p53 and TGF-β signaling pathways, indicating that AURKA and its related genes may be related to tumorigenesis. The AURKA type I was significantly enriched in immune activation-related pathways, including MHC molecules, DC cells, TCL cells, and IL-2

activation signaling pathways, further suggesting that AURKA promotes the occurrence and development of skin melanoma (Figure4(H)). The effect of AURKA on melanoma was further assessed by analyzing the effect of AURKA expression on the function of melanoma cells using the small molecule compound ZNC97018978 targeting AURKA. The results revealed that a decrease in the expression level of AURKA in the A875 melanoma cell line also significantly reduced the proliferation and migration ability of the cells (Figure 9(B-D); Transwell experiment) (Figure 9(E); Scratch test). This corroborates that AURKA may be involved in the cell cycle, proliferation, apoptosis, and immune regulation during the occurrence, development, and metastasis of SKCM.

AURKA reshapes the tumor immunosuppressive microenvironment

Recent studies have demonstrated that AURKA, which is highly expressed in SKCM, inhibits effector T cell-mediated cytotoxicity [31]. At the same time, the functional annotation results indicated that AURKA influences the TME, therefore, we further investigated the correlation between AURKA and TME. Analysis of the TMER2.0 online database revealed that the increased infiltration levels of CD8+, CD4+ T cells, B cells, and DC cells in SKCM patients improved the prognosis of patients, and the increased infiltration level of MDSCs was an adverse risk factor for SKCM patients (Supplementary Figure 3 (A-P)). It is suggested that the infiltration abundance of immune cells in TME is closely related to tumor prognosis. Therefore, the differences in the infiltration abundance of various immune cells in patients in the AURKA-high and -low expression groups and the AURKA types I and II groups were analyzed. The results showed that DC cells, CD8+, CD+4, B cells, and other anti-tumor immune cells were significantly enriched in the AURKA-low expression and AURKA type I groups, while immunosuppressive cells were significantly enriched in with the AURKA-high expression and AURKA type II groups (Figure 5(H, Figure 6(G)), confirming that AURKA has an immune-suppressing effect. Using the ESTIMATE algorithm and the Xcell algorithm, we determined that AURKA was negatively correlated with all three scoring forms, and positively correlated with tumor purity (Figure 5 (F)). Analysis using the Xcell algorithm revealed that the immune score in the AURKA-low expression and AURKA type I groups were higher than that in the AURKA-high expression and AURKA type II groups (Figure 5(H), Figure 6(G)), which further confirmed the tumor-promoting function of AURKA in TME. Finally, three algorithms in the Immuneeconv package of R language combined with the ssGSEA algorithm and the online database TMER2.0 were used to further verify the relationship between AURKA and various tumor immune cells; we found that NK, T, DC, M1, activated CD4 T cells, activated CD8 T cells, and effector memory CD8 T cells were inversely correlated with the infiltration abundance of tumor-promoting immune cells

(MODS, Treg, CAF, Th2, M2) Abundance was positively correlated (Figure 5(A-D,G)). At the same time, anti-tumor immune cell marker genes was negatively correlated (Figure 5(E)), further supporting the role of AURKA in promoting tumor immune escape.

AURKA inhibits CD8+ T cell infiltration by activating the TGF-β signaling pathway

As a key component of adaptive immunity, CD8+ T cells have become the crux of immunotherapy in recent years owing to their specificity for antigen recognition and powerful tumor killing[32]. AURKA was found to be inversely correlated with CD8+ T cell infiltration based on GSEA enrichment analysis (Figure 6(C-D)), indicating that it is immunosuppressive. To verify the results of GSEA enrichment analysis, we used the TMER2.0 and GASElite online databases for further analysis and found that AURKA expression was negatively correlated with CD8+ T cell infiltration (Figure 6(J-K)), confirming that AURKA promotes the formation of immunosuppressive TME. We next explored the correlation and differential expression of T cell signature genes and AURKA and found that multiple T cell signature genes in patients in the AURKA-high expression and AURKA type II groups were significantly lower than those in the AURKA-low expression and AURKA type I groups, and multiple T-cell signature genes were found to be negatively correlated with AURKA (Figure 6(L-N)). In addition, AURKA was negatively correlated with antigen presentation and processing based on GSEA enrichment and correlation analysis (Figure 6(A-B)), MHC-related genes were negatively correlated and significantly expressed in the AURKA-low expression group and AURKA type I groups, (Figure 6(E-F,H-I))),which further confirmed that AURKA can promote the formation of immunosuppressive TME.

We then used GSEA to explore the molecular mechanism of AURKA affecting T cell infiltration and found that genes related to the TGF-β signaling pathway were highly expressed in AURKA and significantly enriched in AURKA type II patients (Figure 7 (F-I)); AURKA and TGF-β signaling is positively correlated. In addition, to verify the effect of AURKA on the TGF-β signaling pathway, we conducted a correlation analysis between AURKA and important genes of the TGF-β signaling pathway (*TGFβR1, ACVR2B, SMAD2, SMAD4, ACVR1C, TGFβ1*), and found that AURKA was positively correlated with important genes of TGF-β signaling pathway (Figure 7(C-D)), and was highly expressed in AURKA type I and AURKA type II patients (Figure 7(A-B)), suggesting that AURKA inhibited CD8+ by activating the TGF-β signaling pathway and T cell infiltration. To further verify this phenomenon, the expression of six important genes in the TGF-β signaling pathway was investigated using small interfering RNA (siRNA) under the condition of low AURKA expression. The expression of five important genes of the TGF-β signaling pathway(*TGFβR1, SMAD2, SMAD4, ACVR1C, TGFβ1*) was decreased under

low AURKA expression (Figure 7 (J), confirming that AURKA activates the TGF-β signaling pathway to inhibit T cell infiltration.

AURKA participates in the regulation of hypoxia

The functional enrichment analysis of GSEA and GSVA revealed that hypoxia-related signaling pathways were significantly enriched in the AURKA-high expression group (Figure 8(A-B)). In recent years, hypoxia has been found to play an important role in the tumor microenvironment[33] . Therefore, we further explored the correlation between AURKA and hypoxia, as well as between hypoxia and the tumor microenvironment. We found that AURKA was positively correlated with 14 hypoxia-related genes, especially in the AURKA-high expression group and AURKA subtype II; *CDKN3* was highly correlated with AURKA (Figure 8 (C, E-G)). Correlation analysis of 15 hypoxia-related genes and AURKA in pan-cancer revealed that many hypoxia-related key genes were positively correlated with AURKA in various tumors, especially *CDKN3* was significantly positively correlated with AURKA in 40 tumors (Figure 8(D)). It was revealed that the abnormally high expression of AURKA mediates hypoxia. Correlation analysis of immune cell infiltration levels in TME of 15 hypoxia-related genes based on the TMER and EPIC algorithms revealed that hypoxia characteristic genes played a role in inhibiting immune cell infiltration (Figure 8 (H-I)), suggesting that hypoxia remodels the tumor immunosuppressive microenvironment and promotes tumor immune escape. To further verify these findings, we analyzed the correlation between hypoxia signature genes and T cell signature genes and MHC molecules and found that hypoxia signature genes were negatively correlated with T cell signature genes and MHC molecules (Figure 8 (J-K)). Therefore, hypoxia remodels the tumor immunosuppressive microenvironment and promotes tumor immune escape. In conclusion, AURKA mediates hypoxic processes to reshape the tumor immunosuppressive microenvironment and promotes tumor immune escape.

Screening of small-molecule inhibitors of AURKA and their effects on skin melanoma

We screened the top 100 potential small-molecule inhibitors targeting AURKA, and preliminarily evaluated the toxic effects of the top 10 small-molecule drugs on A875 cells by MTT test. We found that the ZNC97018978 molecule has better biological activity and can significantly inhibit AURKA in A875 cells. proliferation (Figure 9(A)). The effect of ZNC97018978 on the proliferation and migration of A875 melanoma cells was then analyzed. MTT results revealed that ZNC97018978 had strong cytotoxicity to A875 and could significantly inhibit the proliferation of A875 cells in a dose- and time-dependent manner (Figure 9(G)). We also evaluated the effect of ZNC97018978 on the A875 cell cycle and apoptosis and found that ZNC97018978 led to A875 cell arrest in the G2/M

phase and promoted apoptosis (Figure 9(H,K)). In addition, we also detected the expression of AURKA, Caspase-3, Bax, Bcl-2, and CDK1 in A875 cells after ZNC97018978 treatment and found that compared with the control group, the expression of AURKA, Bcl-2, and CDK1 in A875 cells in the ZNC97018978-treated group was significantly lower, and the of Bax and Caspase-3 was significantly higher (Figure 9(J),I-L).

Discussion

AURKA is a cell cycle-regulated kinase, which has been confirmed to be abnormally expressed in a variety of tumor tissues and is associated with tumor cell proliferation, invasion, metastasis, drug resistance, and clinical prognosis [34]. In this study, we identified AURKA as a core driving hub gene in the occurrence and development of melanoma, We established AURKA as an independent prognostic factor of SKCM, and the nomogram developed based on AURKA expression also showed good prognostic prediction. We also found that AURKA can promote melanoma cell proliferation, invasion, and metastasis, as well as, remodel the tumor immunosuppressive microenvironment and participate in the treatment response of melanoma. Based on molecular docking technology and in vitro and in vivo experiments, we found that the small-molecule compound ZNC97018978 targeting AURKA has obvious anti-SKCM activity. These findings suggest that abnormally high expression of AURKA may serve as a potential tumor target and prognostic marker for the identification and treatment of melanoma patients with poor prognoses.

Mutations in genes in normal cells promote the progression of normal cells to tumors through stages of proliferation and dysplasia, and ultimately, metastatic tumors [35]. Therefore, genetic analysis of known oncogenes can provide further insights into the roles of these genes in tumor progression[36]. In the present study, we found that AURKA exhibited a lower mutation rate, and missense mutation was the most common type of mutation. We also found that 1041 genes had somatic mutations in the AURKA-high and -low expression group in SKCM patients, of which the frequency of *TTN* mutation was the highest at 72%, and the *BRAF* mutation also had a remarkable role in the pathogenesis of SKCM. Furthermore, 103 gene mutations were found to affect the expression of AURKA, of which the *TP53* mutation was the most significant. We also found that the expression level of AURKA was significantly correlated with copy number variation ($p<0.05$), and affected the level of immune cell infiltration. These findings provided a new perspective for the analysis of variations in *AURKA*.

Recruiting tumor-activated CD8+ T cells is the focus of immunotherapy. Dendritic cells (DC) play a central role in the uptake and presentation of cancer antigens, and the initiation

and activation of CD8+ T cell anti-tumor immunity [37]. In contrast, CD4+ T cells secrete a variety of cytokines with direct effector functions and activate other immune cells such as B cells and CD8 T cells[38].In the present study, we also observed that AURKA inhibited the immune-infiltrating levels of anti-tumor immune cells (DC, B cells, CD4+ T cells, and CD8+ T cells) in the TME, while increasing the infiltration level of tumor immune escape-promoting cells (MDOS,). Our findings are consistent with that of previous reports [31,39,40]suggesting that AURKA can inhibit the increased level of anti-tumor immune cell infiltration in the TME. We postulate that the formation of the immunosuppressive microenvironment is promoted for the following. First, AURKA inhibits the function of dendritic cells by activating the TGF-β signaling pathway[41] and negatively regulates T lymphocytes [42]. It also inhibits the transcription of cytotoxicity-related genes such as perforin, granzyme A, and granzyme B[43,44] which can significantly inhibit the adaptive immune surveillance ability of T lymphocytes and play a role in promoting tumorigenesis [43-45]. Second, AURKA inhibits the cytotoxic function of CD8+ T cells through the ferroptosis pathway, thereby weakening the anti-tumor function of CD8+ T cells; AURKA mediates the development of a hypoxic microenvironment, thereby remodeling the immunosuppressive microenvironment. Therefore, our findings suggest that AURKA may play an immunosuppressive role by remodeling the tumor immunosuppressive microenvironment and impairing tumor immune surveillance function, however, the mechanism underlying its immunosuppressive effect is not known and must be further studied.

Hypermethylation of promoter regions often leads to silencing or inactivation of tumor suppressor genes in cancer cells[46,47]. In the present study, we found that AURKA overexpression may be associated with AURKA hypomethylation. Interestingly, *AURKA* methylation was associated with SKCM prognosis, and patients with *AURKA* hypermethylation had lower overall survival, which was consistent with the prognosis of mRNA expression of this gene. Moreover, the expression of AURKA in 39 kinds of tumors was found to be closely related to the expression of DNMT1, DNMT3A, and DNMT3B, and *AURKA* methylation was found to promote the increased expression of EMT immune-related factors. Therefore, our findings suggest a role of AURKA methylation in SKCM and can be further investigated.

Since AURKA is the core hub gene in the occurrence and malignant development of cutaneous melanoma, it is closely related to the stage, treatment, and prognosis of cutaneous melanoma. This finding has important clinical implications, as it reveals that targeting AURKA may benefit not only patients with early-stage primary melanoma but also patients with advanced-stage melanoma. In recent years, several attempts have been

made to develop AURKA inhibitors, some of which have entered the clinical trial stage. However, these AURKA inhibitors that are currently under investigation have limitations, such as poor selectivity, which can cause serious adverse reactions in patients (neutropenia, hypertension, hepatotoxicity, somnolence, fatigue, etc.) [48,49]. Therefore, the development of efficient AURKA inhibitors with a clear mechanism of action, strong specificity, and less toxic side effects is essential. Therefore, we used molecular docking technology to screen for small molecule inhibitors that target AURKA and identified ZNC97018978 from the ZINC database that can bind *AURKA* and block its expression. In vitro experiments confirmed that ZNC97018978 could significantly inhibit the proliferation and invasion of A875 cells and promote G2/M phase arrest. In vivo experiments further showed that ZNC97018978 can inhibit the growth of CM xenografts without obvious toxic and side effects on other organs. Further, MTT assay and flow cytometry confirmed that after ZNC97018978 treatment, the proliferation rate of A875 cells decreased significantly, the expressions of proapoptotic Bax and Caspase 3 increased, and that of antiapoptotic Bcl-2 and CDK1 decreased. It is suggested that the anti-melanoma activity of ZNC97018978 may induce apoptosis by blocking the cell cycle, and thereby, inhibit cell proliferation.

This study improves our understanding of the relationship between AURKA and SKCM, but some limitations remain. First, although we investigated the correlation between AURKA and immune infiltration in SKCM patients, interpretation of immune analyses of different SKCM subgroups was lacking. Second, most analyses in this study were based on AURKA mRNA levels. A deeper analysis based on protein level will further improve the validity of the data. Finally, the effect of ZNC97018978 on skin melanoma needs to be tested in vivo.

In conclusion, our results suggest that AURKA can serve as a potential therapeutic target for SKCM, and the role of epigenetic regulation in AURKA function. Thy also suggests that AURKA likely regulates TME immune cell infiltration in SKCM patients as well as via ferroptosis regulation. Our study may help clarify the role of AURKA in the development of SKCM, and offer insights into developing more precise and personalized immunity against AURKA in the future.

Ethics Statement

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Lanzhou University.Patients/participants provided their written informed consent to participate in this study.

Conflicts of Interest

The authors declare that they do not have any conflicts of interest.

Author contributions

SY designed this study and XF oversaw it. SY was analyzed data.. XF examines statistical methods. The manuscript was written by SY. All authors in this study contributed to the article and approved the submitted version.

Acknowledgments

We thank the TCGA database and the GEO database, We gratefully acknowledge contributions from the CASElite and TMER2.0 platforms

reference

1. Schadendorf D, van Akkooi ACJ, Berking C, Griewank KG, Gutzmer R, Hauschild A, Stang A, Roesch A, Ugurel S (2018) Melanoma. Lancet (London, England) 392 (10151):971-984. doi:10.1016/s0140-6736(18)31559-9

2. Linares MA, Zakaria A, Nizran P (2015) Skin Cancer. Primary care 42 (4):645-659. doi:10.1016/j.pop.2015.07.006

3. Siegel RL, Miller KD (2019) Cancer statistics, 2019. 69 (1):7-34. doi:10.3322/caac.21551

4. Flaherty KT, Hodi FS, Fisher DE (2012) From genes to drugs: targeted strategies for melanoma. Nature reviews Cancer 12 (5):349-361. doi:10.1038/nrc3218

5. Bajor DL, Mick R, Riese MJ, Huang AC, Sullivan B, Richman LP, Torigian DA, George SM, Stelekati E, Chen F, Melenhorst JJ, Lacey SF, Xu X, Wherry EJ, Gangadhar TC, Amaravadi RK, Schuchter LM, Vonderheide RH (2018) Long-term outcomes of a phase I study of agonist CD40 antibody and CTLA-4 blockade in patients with metastatic melanoma. Oncoimmunology 7 (10):e1468956. doi:10.1080/2162402x.2018.1468956

6. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144 (5):646-674. doi:10.1016/j.cell.2011.02.013

7. Binnewies M, Roberts EW, Kersten K, Chan V (2018) Understanding the tumor immune microenvironment (TIME) for effective therapy. 24 (5):541-550. doi:10.1038/s41591-018-0014-x

8. Pandey PR, Young KH, Kumar D (2022) RNA-mediated immunotherapy regulating tumor immune microenvironment: next wave of cancer therapeutics. 21 (1):58. doi:10.1186/s12943-022-01528-6

9. Kalaora S, Nagler A, Wargo JA (2022) Mechanisms of immune activation and regulation: lessons from melanoma. doi:10.1038/s41568-022-00442-9

10. D'Assoro AB, Liu T, Quatraro C, Amato A, Opyrchal M, Leontovich A, Ikeda Y, Ohmine S, Lingle W, Suman V, Ecsedy J, Iankov I, Di Leonardo A, Ayers-Inglers J, Degnim A, Billadeau D, McCubrey J, Ingle J, Salisbury JL, Galanis E (2014) The mitotic kinase Aurora--a promotes distant metastases by inducing epithelial-to-mesenchymal transition in ERα(+) breast cancer cells. Oncogene 33 (5):599-610. doi:10.1038/onc.2012.628

11. Yan M, Wang C, He B, Yang M, Tong M, Long Z, Liu B, Peng F, Xu L, Zhang Y, Liang D, Lei H, Subrata S, Kelley KW, Lam EW, Jin B, Liu Q (2016) Aurora-A Kinase: A Potent Oncogene and Target for Cancer Therapy. Medicinal research reviews 36 (6):1036-1079. doi:10.1002/med.21399

12. Guo M, Lu S, Huang H, Wang Y, Yang MQ, Yang Y, Fan Z, Jiang B, Deng Y (2018) Increased AURKA promotes cell proliferation and predicts poor prognosis in bladder cancer. BMC systems biology 12 (Suppl 7):118. doi:10.1186/s12918-018-0634-2

13. Umene K, Yanokura M, Banno K, Irie H, Adachi M, Iida M, Nakamura K, Nogami Y, Masuda K, Kobayashi Y, Tominaga E, Aoki D (2015) Aurora kinase A has a significant role as a therapeutic target and clinical biomarker in endometrial cancer. International journal of oncology 46 (4):1498-1506. doi:10.3892/ijo.2015.2842

14. Xu J, Wu X, Zhou WH, Liu AW, Wu JB, Deng JY, Yue CF, Yang SB, Wang J, Yuan ZY, Liu Q (2013) Aurora-A identifies early recurrence and poor prognosis and promises a potential therapeutic target in triple negative breast cancer. PloS one 8 (2):e56919. doi:10.1371/journal.pone.0056919

15. Liu ZG, Yi W, Tao YL, Chan HC, Zeng MS, Xia YF (2012) Aurora-A is an efficient marker for predicting poor prognosis in human nasopharyngeal carcinoma with aggressive local invasion: 208 cases with a 10-year follow-up from a single institution. Oncology letters 3 (6):1237-1244. doi:10.3892/ol.2012.660

16. Scarpini S, Rouprêt M, Renard-Penna R, Camparo P, Cussenot O, Compérat E (2012) Impact of the expression of Aurora-A, p53, and MIB-1 on the prognosis of urothelial carcinomas of the upper urinary tract. Urologic oncology 30 (2):182-187. doi:10.1016/j.urolonc.2009.12.003

17. Caputo E, Miceli R, Motti ML, Taté R, Fratangelo F, Botti G, Mozzillo N, Carriero MV, Cavalcanti E, Palmieri G, Ciliberto G, Pirozzi G, Ascierto PA (2014) AurkA inhibitors enhance the effects of B-RAF and MEK inhibitors in melanoma treatment. Journal of translational medicine 12:216. doi:10.1186/s12967-014-0216-z

18. Puig-Butille JA, Vinyals A, Ferreres JR, Aguilera P, Cabré E, Tell-Martí G, Marcoval J, Mateo F, Palomero L, Badenas C, Piulats JM, Malvehy J, Pujana MA, Puig S, Fabra À (2017) AURKA Overexpression Is Driven by FOXM1 and MAPK/ERK Activation in Melanoma Cells Harboring BRAF or NRAS Mutations: Impact on Melanoma Prognosis and Therapy. The Journal of investigative dermatology 137 (6):1297-1310. doi:10.1016/j.jid.2017.01.021

19. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS (2020) TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 48 (W1):W509-w514. doi:10.1093/nar/gkaa407

20. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, Selves J, Laurent-Puig P, Sautès-Fridman C, Fridman WH, de Reyniès A (2016) Erratum to: Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome biology 17 (1):249. doi:10.1186/s13059-016-1113-y

21. Aran D, Hu Z, Butte AJ (2017) xCell: digitally portraying the tissue cellular heterogeneity landscape. 18 (1):220. doi:10.1186/s13059-017-1349-1

22. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, Bruneval P, Fridman WH, Becker C, Pagès F, Speicher MR, Trajanoski Z, Galon J (2013) Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in

human cancer. Immunity 39 (4):782-795. doi:10.1016/j.immuni.2013.10.003

23. Sturm G, Finotello F, Petitprez F, Zhang JD, Baumbach J, Fridman WH, List M, Aneichyk T (2019) Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology. Bioinformatics (Oxford, England) 35 (14):i436-i445. doi:10.1093/bioinformatics/btz363

24. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, Treviño V, Shen H, Laird PW, Levine DA, Carter SL, Getz G, Stemke-Hale K, Mills GB, Verhaak RG (2013) Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun 4:2612. doi:10.1038/ncomms3612

25. Malta TM, Sokolov A, Gentles AJ, Burzykowski T, Poisson L, Weinstein JN, Kamińska B, Huelsken J, Omberg L, Gevaert O, Colaprico A, Czerwińska P, Mazurek S, Mishra L, Heyn H, Krasnitz A, Godwin AK, Lazar AJ, Stuart JM, Hoadley KA, Laird PW, Noushmehr H, Wiznerowicz M (2018) Machine Learning Identifies Stemness Features Associated with Oncogenic Dedifferentiation. Cell 173 (2):338-354.e315. doi:10.1016/j.cell.2018.03.034

26. Doncheva NT, Morris JH (2019) Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. 18 (2):623-632. doi:10.1021/acs.jproteome.8b00702

27. Nagy Á, Győrffy B (2021) muTarget: A platform linking gene expression changes and mutation status in solid tumors. International journal of cancer 148 (2):502-511. doi:10.1002/ijc.33283

28. Modhukur V, Iljasenko T, Metsalu T, Lokk K, Laisk-Podar T, Vilo J (2018) MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. Epigenomics 10 (3):277-288. doi:10.2217/epi-2017-0118

29. Yuan H, Yan M, Zhang G, Liu W, Deng C, Liao G, Xu L, Luo T, Yan H, Long Z, Shi A, Zhao T, Xiao Y, Li X (2019) CancerSEA: a cancer single-cell state atlas. Nucleic Acids Res 47 (D1):D900-d908. doi:10.1093/nar/gky939

30. Liu CJ, Hu FF, Xia MX, Han L, Zhang Q, Guo AY (2018) GSCALite: a web server for gene set cancer analysis. Bioinformatics (Oxford, England) 34 (21):3771-3772. doi:10.1093/bioinformatics/bty411

31. Punt S, Malu S, McKenzie JA, Manrique SZ, Doorduijn EM, Mbofung RM, Williams L, Silverman DA, Ashkin EL, Dominguez AL, Wang Z, Chen JQ, Maiti SN, Tieu TN, Liu C, Xu C, Forget MA, Haymaker C, Khalili JS, Satani N, Muller F, Cooper LJN, Overwijk WW, Amaria RN, Bernatchez C, Heffernan TP, Peng W, Roszik J, Hwu P (2021) Aurora kinase inhibition sensitizes melanoma cells to T-cell-mediated cytotoxicity. Cancer immunology, immunotherapy : CII 70 (4):1101-1113. doi:10.1007/s00262-020-02748-9

32. Zhang Y, Zhang Z (2020) The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. Cellular & molecular immunology 17 (8):807-821. doi:10.1038/s41423-020-0488-6

33. Scharping NE, Rivadeneira DB, Menk AV, Vignali PDA, Ford BR, Rittenhouse NL, Peralta R, Wang Y, Wang Y, DePeaux K (2021) Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. 22 (2):205-215. doi:10.1038/s41590-020-00834-9

34. Goos JA, Coupe VM, Diosdado B, Delis-Van Diemen PM, Karga C, Beliën JA, Carvalho B, van den Tol MP, Verheul HM, Geldof AA, Meijer GA, Hoekstra OS, Fijneman RJ (2013) Aurora kinase A (AURKA) expression in colorectal cancer liver metastasis is associated with poor prognosis. British journal of cancer 109 (9):2445-2452. doi:10.1038/bjc.2013.608

35. Garnis C, Buys TP, Lam WL (2004) Genetic alteration and gene expression modulation during cancer progression. Molecular cancer 3:9. doi:10.1186/1476-4598-3-9

36. Hahn WC, Weinberg RA (2002) Rules for making human tumor cells. The New England journal of medicine 347 (20):1593-1603. doi:10.1056/NEJMra021902

37. Jhunjhunwala S, Hammer C (2021) Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. 21 (5):298-312. doi:10.1038/s41568-021-00339-z

38. Rakhra K, Bachireddy P, Zabuawala T, Zeiser R, Xu L, Kopelman A, Fan AC, Yang Q, Braunstein L, Crosby E, Ryeom S, Felsher DW (2010) CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. Cancer cell 18 (5):485-498. doi:10.1016/j.ccr.2010.10.002

39. Vilgelm AE, Johnson CA, Prasad N, Yang J, Chen SC, Ayers GD, Pawlikowski JS, Raman D, Sosman JA, Kelley M, Ecsedy JA, Shyr Y, Levy SE, Richmond A (2016) Connecting the Dots: Therapy-Induced Senescence and a Tumor-Suppressive Immune Microenvironment. Journal of the National Cancer Institute 108 (6):djv406. doi:10.1093/jnci/djv406

40. Gide TN, Wilmott JS, Scolyer RA, Long GV (2018) Primary and Acquired Resistance to Immune Checkpoint Inhibitors in Metastatic Melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research 24 (6):1260-1270. doi:10.1158/1078-0432.ccr-17-2267

41. Keirsse J, Van Damme H, Van Ginderachter JA, Laoui D (2017) Exploiting tumor-associated dendritic cell heterogeneity for novel cancer therapies. Journal of leukocyte biology 102 (2):317-324. doi:10.1189/jlb.4MR1116-466R

42. Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. Annual review of immunology 27:485-517. doi:10.1146/annurev.immunol.021908.132710

43. Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weissleder R, von Boehmer H, Khazaie K (2005) Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. Proceedings of the National Academy of Sciences of the United States of America 102 (2):419-424. doi:10.1073/pnas.0408197102

44. Seoane J, Gomis RR (2017) TGF-β Family Signaling in Tumor Suppression and Cancer Progression. Cold Spring Harbor perspectives in biology 9 (12). doi:10.1101/cshperspect.a022277

45. Li MO, Flavell RA (2008) TGF-beta: a master of all T cell trades. Cell 134 (3):392-404. doi:10.1016/j.cell.2008.07.025

46. Wang M, Ngo V, Wang W (2021) Deciphering the genetic code of DNA methylation. Briefings in bioinformatics 22 (5). doi:10.1093/bib/bbaa424

47. Mehdi A, Rabbani SA (2021) Role of Methylation in Pro- and Anti-Cancer Immunity. 13 (3).

doi:10.3390/cancers13030545

48. de Souza VB, Kawano DF (2020) Structural basis for the design of allosteric inhibitors of the Aurora kinase A enzyme in the cancer chemotherapy. Biochimica et biophysica acta General subjects 1864 (1):129448. doi:10.1016/j.bbagen.2019.129448

49. Panicker RC, Coyne AG, Srinivasan R (2019) Allosteric Targeting of Aurora A Kinase Using Small Molecules: A Step Forward Towards Next Generation Medicines? Current medicinal chemistry 26 (13):2234-2242. doi:10.2174/0929867324666170727120315

Figure 1: Identification of SKCM hub genes (A-C) Volcano plots of DEGs in GSE3189, GSE15605, and TCGA. (D-E) Venn diagram showing upregulated and downregulated DEGs across the three datasets. (F-G) Mutation status and visualization of the top 10 hub genes. (HI) Univariate Cox regression analysis of the overall survival and disease-specific survival of the top 10 hub genes. (J) Correlation analysis of the top 10 hub genes.

Figure 2. The relationship between AURKA expression and prognosis in SKCM.

(A) Expression levels of SKCM cell lines in the CCLE database. (B, D) *AURKA* mRNA levels in SKCM tissues and normal skin tissues in GSE3189 (B)and TCGA (D) datasets. (C) *AURKA* mRNA levels in primary and metastatic SKCM tissues in GSE46517. (E-F) Protein expression levels of AURKA in normal skin tissue and melanoma. (G-H) Immunohistochemical staining of AURKA in adjacent normal lung tissue. Representative images are shown. (I *AURKA* mRNA differential expression in pan-cancer. (J) Overall survival curve of GSE22155 data; (K-L) Kaplan–Meier survival analysis of TCGA data of OS, DSS survival curve. (M) Multivariate Cox regression analysis. (N-O) Forest plot showing the correlation between *AURKA* expression and clinicopathological parameters in SKCM patients. *P<0.05, **P<0.01, ***P<0.001.

Figure 3. *AURKA* gene mutation, methylation,and clinicopathological features analysis in SKCM patients. (A) Lollipop plot showing the distribution of AURKA gene mutations. (B) Lollipop plot showing the top 10 somatic mutated genes in the AURKA-high and -low expression groups in SKCM. (C) Association of *AURKA* gene mutation with prognosis in SKCM patients. (D) Lollipop plot showing the top 5 mutated genes affecting AURKA expression. (E) Effects of *AURKA* mutation on six types of immune infiltrating cells (F) The correlation between *AURKA* copy number variation and infiltration abundance of six types of immune infiltrating cells. (G) The relationship

between *AURKA* methylation and its expression (H) *AURKA* promoter methylation prognostic analysis (I) The correlation between *AURKA* expression and three methyltransferases (DNMT1, DNMT3A, and DNMT3B). (J) Correlation analysis between AURKA expression and three methyltransferases (DNMT1, DNMT3A, and DNMT3B) in pan-cancer. (K) Visualization between methylation levels and AURKA

expression. (L) Relationship between *AURKA* expression and clinicopathological features.

Figure 4. DEGs and their functional enrichment analysis (A) GSE46517 and GSE15605 validation cluster analysis results. (B) Based on TCGA database photos of that SKCM dataset for 54 AURKA-related genes Cluster analysis. (C) Volcano plot showing DEGs between the AURKA-high and -low groups. (D-E) AURKA is functionally enriched in single-cell GSE81383 and GSE72056. (F) Pathway analysis of 54 AURKA-related genes. (G) GSEA enrichment analysis betweFen AURKA-high and -low groups. (H) Functional enrichment analysis between AURKA type I and AURKA type II group patients. (I) Tumor stemness index score between the AURKA type I and AURKA type II group patients. (J-K) Kaplan–Meier curve showing survival between the AURKA type I and AURKA type II groups.

Figure 5. Immune correlation analysis of AURKA in SKCM. (A-D) Analysis of the correlation between AURKA expression and immune cell infiltration abundance using the TIME, MCP-counter, xCell, and ssGSEA algorithms. (E) Correlation of AURKA with immune cell marker genes. (F) Analysis of the correlation between AURKA expression and stromal score, immune score, and ESTIMATE score using the ESTIMATE algorithm. (G) The correlation between AURKA and the infiltration level of four kinds of immunosuppressive cells in SKCM patients. (H) Differences between immune infiltrating cells between the AURKA type I and AURKA type II group patients.

Figure 6. Correlation between AURKA expression and CD8+ T cell infiltration level and T cell characteristic genes, MHC molecular analysis. (A-B) The MHC signaling pathway was significantly enriched in the AURKA-low expression and AURKA type I groups. (C-D) GSEA analysis of the correlation between AURKA expression and CD8+ T cell infiltration level. (E-F) Molecular correlation analysis of AURKA and MHC. (F-G) Differential expression of MHC molecules in the AURKA-high and -low expression groups and between the AURKA types I and II groups. (G) Immune scores in the AURKA-high and -low groups. (H-I) Differential expression of MHC molecules in the AURKA-high and -low expression groups and between the AURKA types I and II

groups. (J) GASElite database analysis of the correlation between AURKA expression and CD8+ T cell infiltration level. (K) TIMER2.0 database analysis of the correlation between AURKA expression and CD8+ T cell infiltration level. (L-M) Difference in the expression of T cell characteristic genes between the AURKA-high and -low expression groups and AURKA type I and type II groups. (N) Correlation between AURKA expression and the expression of T cell signature genes.

Figure 7. Correlation between AURKA expression and the TGF-β signaling pathway. (A-B) Difference in the expression of six important genes of the TGF-β signaling pathway in the AURKA-high and -low expression groups and the AURKA type I and type II groups. (C) Correlation between AURKA expression and the expression of important genes in the TGF-β signaling pathway analyzed by TMER2.0 database. (D) Correlation between AURKA expression and the expression of important genes in the TGF-β signaling pathway. (E) TMER2.0 database analysis of the correlation between AURKA expression and the expression of six important genes of the TGF-β signaling pathway in pan-cancer(F-I) GSEA analysis showing high AURKA expression and enrichment of the TGF-β signaling pathway in AURKA type II patients. (J) Difference in the expression of six important genes of the TGF-β signaling pathway in the AURKA-high and -low expression groups and the AURKA type I and type II groups.

Figure 8. AURKA participates in the regulation of hypoxia ,Relationship between 15 hypoxia signature genes and the tumor microenvironment.. (A-B) GSVA and GSEA analysis of AURKA expression showing positive correlation of AURKA with hypoxia signaling pathways. (C) Correlation analysis of AURKA and 15 hypoxia signature genes. (D) Pan-cancer analysis of the association of AURKA with 15 hypoxia signature genes. (E) TMER2.0 analysis of the association of 15 hypoxia signature genes with AURKA in SKCM patients.(F-G) Differential expression of 15 hypoxia signature genes in AURKA-high and -low expression groups and between AURKA type I and type II. (H-I) TMER and EPIC algorithm analysis of the correlation between 15 hypoxia signature genes and immune cell infiltration levels. (J-K) Correlation of 15 hypoxia signature genes with MHC molecules and T cell signature genes.

Figure 9. Effects of small-molecule inhibitors ZNC97018978 of AURKA on A875 cells.

(A) Schematic diagram of the docking of AURKA with ZNC97018978. (B-D) Transwell detection of changes in migration ability in the A875 and HaCaT cells. (E) Scratch assay detection of changes in migration ability in A875 cells. (F) RT-PCR detection of apoptosis and mRNA expression of cell cycle-related-factors in cells. (G) MTT detection of viability changes in A875 cells. (H) Cell cycle changes as detected by flow cytometry. (J,I-L) Western blot detection of AURKA, Caspase-3, Bax, Bcl-2, and CDK1 expression changes in the A875 and HaCaT cells. (K) Apoptosis as detected by flow cytometry.

Supplementary Figure 1. Details on the top10 hub genes. (A) Single nucleotide variations in the top 10 HUB genes. (B-C) Copy number variations in the top10 HUB genes and their correlation with expression. (D-H) The top five hub genes with prognostic value.

Supplementary Figure 2. ROC diagnostic curves (A-J) of the top 10 hub genes.

Supplementary Figure 3. The relationship between the level of immune cell infiltration and prognosis in SKCM patients (A-P). The relationship between the infiltration abundance of common immune-activating cells and immunosuppressive cells in SKCM patients and prognosis. (Q) The differential expression of the key genes of antigen presentation and processing in the AURKA-high and -low expression groups. (R) Correlation of AURKA with genes key to antigen presentation and processing.

Figures

Figure 1

Identification of SKCM hub genes (A-C) Volcano plots of DEGs in GSE3189, GSE15605, and TCGA. (D-E) Venn diagram showing upregulated and downregulated DEGs across the three datasets. (F-G) Mutation status and visualization of the top 10 hub genes. (HI) Univariate Cox regression analysis of the overall survival and disease-specific survival of the top 10 hub genes. (J) Correlation analysis of the top 10 hub genes.

Figure 2

The relationship between AURKA expression and prognosis in SKCM. (A) Expression levels of SKCM cell lines in the CCLE database. (B, D) AURKA mRNA levels in SKCM tissues and normal skin tissues in GSE3189 (B)and TCGA (D) datasets. (C) AURKA mRNA levels in primary and metastatic SKCM tissues in GSE46517. (E-F) Protein expression levels of AURKA in normal skin tissue and melanoma. (G-H) Immunohistochemical staining of AURKA in adjacent normal lung tissue. Representative images are shown. (I AURKA mRNA differential expression in pan-cancer. (J) Overall survival curve of GSE22155 data; (K-L) Kaplan–Meier survival analysis of TCGA data of OS, DSS survival curve. (M) Multivariate Cox regression analysis. (N-O) Forest plot showing the correlation between AURKA expression and clinicopathological parameters in SKCM patients. *P<0.05, **P<0.01, ***P<0.001.

Figure 3

AURKA gene mutation, methylation,and clinicopathological features analysis in SKCM patients. (A) Lollipop plot showing the distribution of AURKA gene mutations. (B) Lollipop plot showing the top 10 somatic mutated genes in the AURKA-high and -low expression groups in SKCM. (C) Association of AURKA gene mutation with prognosis in SKCM patients. (D) Lollipop plot showing the top 5 mutated genes affecting AURKA expression. (E) Effects of AURKA mutation on six types of immune infiltrating cells (F) The correlation between AURKA copy number variation and infiltration abundance of six types of immune infiltrating cells. (G) The relationship between AURKA methylation and its expression (H) AURKA promoter methylation prognostic analysis (I) The correlation between AURKA expression and three methyltransferases (DNMT1, DNMT3A, and DNMT3B). (J) Correlation analysis between AURKA expression and three methyltransferases (DNMT1, DNMT3A, and DNMT3B) in pan-cancer. (K) Visualization between methylation levels and AURKA expression. (L) Relationship between AURKA expression and clinicopathological features.

Figure 4

DEGs and their functional enrichment analysis (A) GSE46517 and GSE15605 validation cluster analysis results. (B) Based on TCGA database photos of that SKCM dataset for 54 AURKA-related genes Cluster analysis. (C) Volcano plot showing DEGs between the AURKA-high and -low groups. (D-E) AURKA is functionally enriched in single-cell GSE81383 and GSE72056. (F) Pathway analysis of 54 AURKA-related genes. (G) GSEA enrichment analysis betweFen AURKA-high and -low groups. (H) Functional enrichment analysis between AURKA type I and AURKA type II group patients. (I) Tumor stemness index score between the AURKA type I and AURKA type II group patients. (J-K) Kaplan–Meier curve showing survival between the AURKA type I and AURKA type II groups.

Figure 5

Immune correlation analysis of AURKA in SKCM. (A-D) Analysis of the correlation between AURKA expression and immune cell infiltration abundance using the TIME, MCP-counter, xCell, and ssGSEA algorithms. (E) Correlation of AURKA with immune cell marker genes. (F) Analysis of the correlation between AURKA expression and stromal score, immune score, and ESTIMATE score using the ESTIMATE algorithm. (G) The correlation between AURKA and the infiltration level of four kinds of immunosuppressive cells in SKCM patients. (H) Differences between immune infiltrating cells between the AURKA type I and AURKA type II group patients.

Figure 6

Correlation between AURKA expression and CD8+ T cell infiltration level and T cell characteristic genes, MHC molecular analysis. (A-B) The MHC signaling pathway was significantly enriched in the AURKA-low expression and AURKA type I groups. (C-D) GSEA analysis of the correlation between AURKA expression and CD8+ T cell infiltration level. (E-F) Molecular correlation analysis of AURKA and MHC. (F-G) Differential expression of MHC molecules in the AURKA-high and -low expression groups and between the AURKA types I and II groups. (G) Immune scores in the AURKA-high and -low groups. (H-I) Differential expression of MHC molecules in the AURKA-high and -low expression groups and between the AURKA types I and II groups. (J) GASElite database analysis of the correlation between AURKA expression and CD8+ T cell infiltration level. (K) TIMER2.0 database analysis of the correlation between AURKA expression and CD8+ T cell infiltration level. (L-M) Difference in the expression of T cell characteristic genes between the AURKA-high and -low expression groups and AURKA type I and type II groups. (N) Correlation between AURKA expression and the expression of T cell signature genes.

Figure 7

Correlation between AURKA expression and the TGF-β signaling pathway. (A-B) Difference in the expression of six important genes of the TGF-β signaling pathway in the AURKA-high and -low expression groups and the AURKA type I and type II groups. (C) Correlation between AURKA expression and the expression of important genes in the TGF-β signaling pathway analyzed by TMER2.0 database. (D) Correlation between AURKA expression and the expression of important genes in the TGF-β signaling pathway. (E) TMER2.0 database analysis of the correlation between AURKA expression and the expression of six important genes of the TGF-β signaling pathway in pan-cancer(F-I) GSEA analysis showing high AURKA expression and enrichment of the TGF-β signaling pathway in AURKA type II patients. (J) Difference in the expression of six important genes of the TGF-β signaling pathway in the AURKA-high and -low expression groups and the AURKA type I and type II groups.

Figure 8

AURKA participates in the regulation of hypoxia ,Relationship between 15 hypoxia signature genes and the tumor microenvironment.. (A-B) GSVA and GSEA analysis of AURKA expression showing positive correlation of AURKA with hypoxia signaling pathways. (C) Correlation analysis of AURKA and 15 hypoxia signature genes. (D) Pan-cancer analysis of the association of AURKA with 15 hypoxia signature genes. (E) TMER2.0 analysis of the association of 15 hypoxia signature genes with AURKA in SKCM patients.(F-G) Differential expression of 15 hypoxia signature genes in AURKA-high and -low expression groups and between AURKA type I and type II. (H-I) TMER and EPIC algorithm analysis of the correlation between 15 hypoxia signature genes and immune cell infiltration levels. (J-K) Correlation of 15 hypoxia signature genes with MHC molecules and T cell signature genes.

Figure 9

Effects of small-molecule inhibitors ZNC97018978 of AURKA onA875 cells. (A) Schematic diagram of the docking of AURKA with ZNC97018978. (B-D) Transwell detection of changes in migration ability in the A875 and HaCaT cells. (E) Scratch assay detection of changes in migration ability in A875 cells. (F) RT-PCR detection of apoptosis and mRNA expression of cell cycle-related-factors in cells. (G) MTT detection of viability changes in A875 cells. (H) Cell cycle changes as detected by flow cytometry. (J,I-L) Western blot detection of AURKA, Caspase-3, Bax, Bcl-2, and CDK1 expression changes in the A875 and HaCaT cells. (K) Apoptosis as detected by flow cytometry.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.tif
- SupplementaryFigure2.tif
- SupplementaryFigure3.tif