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Comprehensive investigation of matrix metalloproteinases in skin cutaneous melanoma: diagnostic, prognostic, and therapeutic insights

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The dysregulation of matrix metalloproteinases (MMPs) in skin cutaneous melanoma (SKCM) represents a critical aspect of tumorigenesis. In this study, we investigated the diagnostic, prognostic, and therapeutic aspects of the MMPs in SKCM. Thirteen SKCM cell lines and seven normal skin cell lines were cultured under standard conditions for experimental analyses. RNA and DNA were extracted, followed by RT-qPCR to assess MMP expression and promoter methylation analysis to determine methylation levels. Functional assays, including cell proliferation, colony formation, and wound healing, were conducted post-MMP7 knockdown using siRNA in A375 cells. Databases like GEPIA2, HPA, MEXPRESS, and miRNet were employed for expression, survival, methylation, and miRNA-mRNA network analyses. We investigated the expression and promoter methylation landscape of MMPs in SKCM cell lines, revealing significant (p-value < 0.05) up-regulation of MMP1, MMP7, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, and MMP25, alongside down-regulation of MMP2, MMP3, and MMP21. Furthermore, our analysis demonstrated a significant (p-value < 0.05) inverse correlation between MMP expression levels and promoter methylation status, suggesting a potential regulatory role of DNA methylation in MMP dysregulation. Notably, MMP7, MMP11, and MMP14 exhibited significant (p-value < 0.05) associations with the overall survival of SKCM patients, emphasizing their prognostic significance. Additionally, Receiver operating characteristic (ROC) curve analysis highlighted the significant (p-value < 0.05) diagnostic potential of MMP7, MMP11, and MMP14 in distinguishing SKCM from normal individuals. Subsequent validation across multiple cohorts confirmed significant (p-value < 0.05) elevated MMP expression levels in SKCM tissues, particularly in advanced disease stages, further emphasizing their role in tumor progression. Furthermore, we elucidated potential regulatory pathways involving miR-22-3p, which targets MMP7, MMP11, and MMP14 genes in SKCM. Our findings also revealed associations between MMP expression and immune modulation, drug sensitivity, and functional states of SKCM cells. Lastly, MMP7 knockdown in A375 cells significantly significant (p-value < 0.05) impacted several characteristics, including cell proliferation, colony formation, and wound healing. Our findings highlight the diagnostic, prognostic, and therapeutic potential of MMP7, MMP11, and MMP14 in SKCM. These MMPs could serve as biomarkers for early detection and targets for therapy. Future efforts should focus on preclinical and clinical validation to translate these insights into personalized diagnostic and therapeutic strategies.

Keywords SKCM, MMPs, Biomarker, Treatment, Prognosis

Skin cutaneous melanoma (SKCM) stands as a formidable challenge in oncology, characterized by its aggressive nature and propensity for metastasis[1,2]. Accounting for the majority of skin cancer-related deaths, its incidence continues to rise globally, posing a significant burden on public health systems worldwide[3,4]. Despite advancements in treatment modalities, including targeted therapies and immunotherapies, the management of metastatic melanoma remains a formidable task, underscoring the urgent need for a deeper understanding of its molecular

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underpinnings. Exposure to ultraviolet (UV) radiation, genetic predisposition, and immunosuppression are among the well-established risk factors implicated in SKCM pathogenesis[5]. While early-stage melanomas are often curable through surgical excision, the prognosis dramatically worsens in advanced stages when metastatic dissemination occurs[6].

The intricate interplay between tumor cells and their microenvironment plays a pivotal role in the progression of melanoma. Among the key players orchestrating this dynamic interaction are the matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases with multifaceted roles in tissue remodeling, inflammation, and cancer progression[7,8]. MMPs exert their effects by degrading various components of the extracellular matrix (ECM), facilitating tumor invasion, angiogenesis, and metastasis. The involvement of MMPs in cancer development and progression has been extensively documented across various malignancies, including breast, lung, and colorectal cancers[9–12]. In the context of melanoma, emerging evidence suggests a nuanced role for MMPs in modulating key aspects of tumor biology, ranging from tumor initiation to metastatic dissemination[13]. Moreover, alterations in MMP expression profiles have been associated with clinicopathological parameters and patient outcomes, underscoring their potential as prognostic biomarkers and therapeutic targets in SKCM[14].

The MMP family comprises various members, each exhibiting distinct substrate specificities and cellular functions[15]. These MMPs are known to degrade various components of the ECM, including collagen, laminin, and fibronectin, thereby facilitating tumor cell invasion and metastasis[16]. In addition to their role in ECM degradation, MMPs also contribute to the modulation of signaling pathways involved in cell proliferation, survival, and angiogenesis[17]. Through the proteolytic processing of growth factors, cytokines, and cell surface receptors, MMPs exert pleiotropic effects on tumor cells and their microenvironment, creating a permissive niche for tumor growth and dissemination[18].

Furthermore, emerging evidence suggests crosstalk between MMPs and components of the immune system, including infiltrating immune cells and cytokines. MMP-mediated ECM remodeling can modulate immune cell trafficking and function within the tumor microenvironment, influencing the balance between anti-tumor immunity and immune evasion[19]. Thus, targeting MMPs may offer dual benefits by inhibiting tumor progression while enhancing the efficacy of immunotherapy approaches in melanoma treatment[20].

Despite significant advances in our understanding of MMP biology in cancer development[21–23], several challenges remain to be addressed[3,24,25]. The complex interplay between MMPs and their substrates, as well as the context-dependent nature of their functions, necessitate comprehensive approaches for dissecting their roles in SKCM progression. Moreover, current therapeutic strategies, including broad-spectrum MMP inhibitors like marimastat and selective inhibitors targeting specific MMPs, have shown limited success in clinical trials due to issues such as lack of specificity, off-target effects, and toxicity[7,26]. These limitations have hindered the translation of MMP inhibitors into effective treatments. Emerging approaches, including antibody-based therapies and novel delivery systems, aim to improve therapeutic precision, but their application in melanoma is still in its infancy[27,28]. Furthermore, while existing studies primarily emphasize the functional and therapeutic roles of MMPs, there is a notable gap in understanding their diagnostic and prognostic significance in SKCM. Addressing this gap, our study provides a focused investigation into the expression, regulation, and clinical relevance of MMPs in SKCM, aiming to bridge the divide between molecular insights and their practical application in diagnostics and personalized medicine.

In this study, employing a comprehensive methodology encompassing both in silico analyses and molecular experiments, we identified significant dysregulation of MMPs in SKCM, with MMP7, MMP11, and MMP14 emerging as key players linked to tumor progression, survival, and diagnostic potential. We demonstrated an inverse correlation between MMP expression and promoter methylation, highlighting DNA methylation as a regulatory mechanism. Functional assays confirmed the critical role of MMP7 in cell proliferation, colony formation, and wound healing, while in silico analyses revealed regulatory networks and associations with immune modulation and drug sensitivity. These findings establish MMPs as promising biomarkers and therapeutic targets in SKCM, providing a foundation for future translational research.

Methodology

Cell culture

In total 13 SKCM cell lines, including A375, SK-MEL-2, SK-MEL-5, SK-MEL-28, SK-MEL-31, SK-MEL-37, SK-MEL-119, SK-MEL-147, SK-MEL-187, SK-MEL-239, WM115, WM266-4, WM793 and 07 control skin cell lines, including HaCaT, NHDF-AD, HEK293T, HUVEC, BJ, NHEK, and HDFa were purchased from the ATCC. These cell lines were cultured under standard conditions in Dulbecco's Modified Eagle's Medium (DMEM) or RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics, and maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Nucleic acid extraction

Nucleic acid extraction were conducted as follows: DNA was extracted using the organic method outlined in references[18,19], whereas RNA isolation was performed utilizing the TRIzol method following the protocol delineated in references[20,21].

RT-qPCR-based expression analysis

The quality and purity of the isolated RNA were evaluated using an Agilent Bioanalyzer (Santa Clara, CA, USA). Subsequently, the RNA underwent reverse transcription to generate complementary DNA (cDNA) using a Reverse Transcription kit (TOYOBO, Shanghai, China). Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed using SYBR Green PCR mix (Thermo Fisher Scientific, Waltham, USA) on an ABI 7900HT FAST Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The GAPDH served as an

internal control for normalization purposes. Relative mRNA expression levels were determined utilizing the $2^{-\Delta\Delta CT}$ method. The following primers were employed for GAPDH and MMPs amplification.

GAPDH-F 5′-ACCCACTCCTCCACCTTTGAC-3′,
GAPDH-R 5′-CTGTTGCTGTAGCCAAATTCG-3′.
MMP1-F: 5′-ATGAAGCAGCCCAGATGTGGAG-3′
MMP1-R: 5′-TGGTCCACATCTGCTCTTGGCA-3′
MMP2-F: 5′-AGCGAGTGGATGCCGCCTTTAA-3′
MMP2-R: 5′-CATTCCAGGCATCTGCGATGAG-3′
MMP3-F: 5′-CACTCACAGACCTGACTCGGTT-3′
MMP3-R: 5′-AAGCAGGATCACAGTTGGCTGG-3′
MMP7-F: 5′-TCGGAGGAGATGCTCACTTCGA-3′
MMP7-R: 5′-GGATCAGAGGAATGTCCCATACC-3′
MMP9-F: 5′-GCCACTACTGTGCCTTTGAGTC-3′
MMP9-R: 5′-CCCTCAGAGAATCGCCAGTACT-3′
MMP10-F: 5′-TCCAGGCTGTATGAAGGAGAGG-3′
MMP10-R: 5′-GGTAGGCATGAGCCAAACTGTG-3′
MMP11-F: 5′-GAGAAGACGGACCTCACCTACA-3′
MMP11-R: 5′-CTCAGTAAAGGTGAGTGGCGTC-3′
MMP12-F: 5′-GATGCTGTCACTACCGTGGGAA-3′
MMP12-R: 5′-CAATGCCAGATGGCAAGGTTGG-3′
MMP13-F: 5′-CCTTGATGCCATTACCAGTCTCC-3′
MMP13-R: 5′-AAACAGCTCCGCATCAACCTGC-3′
MMP14-F: 5′-CCTTGGACTGTCAGGAATGAGG-3′
MMP14-R: 5′-TTCTCCGTGTCCATCCACTGGT-3′
MMP21-F: 5′-CGGATAGGCTTCTACCCGATCA-3′
MMP21-R: 5′-TACTCGCTGTCGTCGAAGTGGA-3′
MMP25-F: 5′-CTTTGACGATGAGGAGACCTGG-3′
MMP25-R: 5′-CTGAAGCCCAGCTCCTCATCAA-3′

The RT-qPCR assay and annealing temperatures of the primers were optimized using a serial dilution and gradient PCR methods to ensure accuracy and reliability.

Promoter methylation analysis

For the construction of normal BS-seq libraries, 10 μg of genomic DNA underwent fragmentation utilizing a Covaris sonication system (Covaris S2). Subsequent to fragmentation, libraries were established following the Illumina Paired-End protocol, encompassing end repair, addition of <A> bases, and ligation of methylated adaptors. The ligated DNA underwent bisulfite conversion employing the EZ DNA Methylation-Gold kit (ZYMO) and was subsequently amplified via PCR. PCR was executed in a final reaction volume of 50 μl, comprising 20 μl of purified DNA, 4 μl of 2.5 mM dNTP, 5 μl of 10× buffer, 0.5 μl of JumpStart™ Taq DNA polymerase, 2 μl of 10 μM PCR primers, and 37.5 μl of water. The thermal cycling program included an initial step at 94 °C for 30 s, followed by 10 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 1 min. Subsequent sequencing was conducted utilizing the HighSeq2000 platform (Illumina). The methylation level was normalized as a beta value.

Receiver operating characteristic (ROC) curve

The ROC curve offers a comprehensive evaluation by integrating the continuous variables of sensitivity and specificity, providing a robust measure of diagnostic accuracy. In this study, ROC curve analysis was conducted using GraphPad Prism 7.0, using data obtained from RT-qPCR and promoter methylation analyses to assess the diagnostic potential of MMPs in SKCM. This analysis was performed to determine the ability of MMP expression and methylation status to distinguish SKCM from normal samples. A standard threshold of AUC > 0.7 was applied to identify markers with acceptable diagnostic performance, and a p-value threshold of < 0.05 was set to ensure statistical significance.

Survival analysis

GEPIA2 (http://gepia2.cancer-pku.cn/#index)[29] is an advanced web-based platform that has transformed cancer genomics and gene expression analysis. With its user-friendly interface, GEPIA2 enables researchers to explore cancer-related data comprehensively, allowing comparisons of gene expression and survival durations between normal and tumor tissues using The Cancer Genome Atlas (TCGA) datasets. This functionality is particularly relevant for survival analysis, as it integrates gene expression data with patient survival information, providing Kaplan-Meier survival plots and hazard ratio estimations. Therefore, the GEPIA2 database was utilized in this study to analyze the survival relevance of MMPs in SKCM, enabling the identification of MMPs with significant prognostic value.

Western blot analysis

Extracted proteins from SKCM and control cell lines via urea method were separated using 11% SDS-PAGE and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). Following a 1-hour blocking step with 5% non-fat milk at room temperature, the PVDF membranes underwent three 10-minute washes with phosphate-buffered saline (PBS). Subsequently, the membranes were incubated overnight at 4 °C

with primary antibodies targeting MMP7 (abcam, ab232737), MMP11 (abcam, ab53143), MMP14 (abcam, ab53712), and the control protein GAPDH (abcam, ab8245). After thorough washing, the membranes were incubated for 2 h with secondary antibodies. Following an additional three 10-minute washes with Tris-buffered saline/Tween-20 (TBST) at room temperature, the immunoreactivity was visualized using an ECL kit (Sangon Biotech), and the membranes were then exposed to Kodak XAR-5 film (Sigma-Aldrich).

Expression analysis of MMPs using TCGA and HPA sources

GEPIA2[29] is an advanced web-based platform that has transformed cancer genomics and gene expression analysis. In our study, we utilized the GEPIA2 database to analyze MMP expression at the mRNA level across SKCM TCGA datasets. GEPIA2 was chosen for this analysis because it provides a robust and user-friendly platform that allows for direct comparisons of gene expression between normal and tumor tissues using TCGA data, offering high-resolution and large-scale cancer-related gene expression data.

HPA (https://www.proteinatlas.org/)[30] is a comprehensive and openly available resource that offers valuable insights into the human proteome. This database provides detailed information on the expression patterns and subcellular localization of proteins in diverse tissues and cell types, with abundant data derived from immunohistochemistry and immunofluorescence assays. HPA was selected for this study because of its extensive protein-level expression data across different human tissues, including cancer samples. By using HPA, we were able to validate the findings from mRNA expression analysis with protein-level data across SKCM and normal samples.

Constriction of the prognostic model

Next, we employed the Lasso and multivariate Cox proportional hazard regression analysis to develop a prediction model using the "survival" package in R language. The TCGA-SKCM dataset served as the training dataset, while the GSE99898, GSE98394, GSE78220, GSE59455, GSE54467, GSE53118, GSE46517, GSE22154, GSE22153, GSE190113, GSE133713, and GSE100797 datasets were used for validation. Positive coefficients in the analysis indicated an increased risk of events such as death, while negative coefficients suggested reduced risk. The magnitude of these coefficients reflected the impact of variables on hazard rates, assisting in the construction of prognostic models for survival outcomes. The formula for the prognostic model of SKCM patients' prognosis was defined as the risk score, which was calculated as the sum of the multivariate Cox regression coefficient variation of each mRNA.

Validation of promoter methylation level and mutational analysis across TCGA datasets

MEXPRESS (https://mexpress.ugent.be/)[31] is a valuable resource for researchers and clinicians in oncology, offering a comprehensive repository of cancer-related information, including promoter methylation data. MEXPRESS was selected for this study due to its specialized focus on integrating promoter methylation data with gene expression, making it an ideal platform for exploring the methylation status of MMPs in the TCGA SKCM patient cohort.

Similarly, cBioPortal (https://www.cbioportal.org/) serves as a robust web-based platform that simplifies the exploration of complex cancer genomics data[32]. Providing an intuitive interface, cBioPortal enables researchers to interactively analyze and visualize multifaceted cancer datasets, incorporating genetic mutations and clinical information. cBioPortal was chosen for mutational analysis of MMPs across TCGA SKCM patients due to its comprehensive data integration and visualization capabilities, allowing for a detailed assessment of MMP-related genetic alterations and their potential implications in SKCM pathogenesis.

miRNA-mRNA network analysis

The miRNet (https://www.mirnet.ca/) database is a valuable resource for investigating microRNA (miRNA) interactions and their regulatory networks[33]. It integrates miRNA-target interactions from multiple repositories, enabling researchers to explore the functional roles of miRNAs in diverse biological processes, including cancer. With its user-friendly interface and comprehensive data coverage, miRNet facilitates the identification of miRNA-target interactions, pathway enrichment analysis, and network visualization. In the present study, the miRNet database was used to construct the miRNA-mRNA network of the MMPs.

Moreover, the expression analysis of has-mir-22-3p was conducted using RT-qPCR assay using the aftermentioned protocol. U6 was used as a reference gene. The relative expression of has-mir-22-3p miRNA to U6 was calculated using the $2^{-\Delta\Delta Ct}$ method. The following primers were used for the amplification of has-mir-22-3p and U6:

has-mir-22-3p-F: 5′-ATGCGGATCCATGGCCGGCAACGTGAAGAAG-3′
has-mir-22-3p-R: 5′-ATGCGTCGACCTATGGTGCTACATGGGCAGG-3′
U6-F: 5′-CTCGCTTCGGCAGCACAT-3′
U6-R: 5′-TTTGCGTGTCATCCTTGCG-3′

Correlation of MMPs with immune modulators

The TISIDB database (http://cis.hku.hk/TISIDB/) is a comprehensive resource for exploring the interactions between tumors and the immune system at the single-cell level[34]. It integrates diverse data types, including single-cell RNA sequencing, bulk RNA sequencing, and clinical information, enabling researchers to investigate immune infiltration, immune-related gene expression, and immune checkpoint profiles across different cancer types. TISIDB facilitates the identification of potential immunotherapy targets and biomarkers, enhancing our understanding of tumor immunology and informing personalized cancer treatment strategies. In the present study, the TISIDB database was used to explore correlations between MMPs and immune modulator genes in

SKCM. Correlation analysis was conducted using Spearman's correlation coefficient to assess the strength and direction of the relationship between MMP expression levels and immune modulator genes.

Drug sensitivity and immunolytic analyses

The GSCA database (https://guolab.wchscu.cn/GSCA) serves as a comprehensive resource for the analysis of drug sensitivity in various cancer types[35]. It integrates large-scale pharmacogenomic datasets, including genomic profiles of cancer cell lines and their corresponding drug response data. Researchers can explore the relationship between genetic alterations in cancer cells and their sensitivity or resistance to various anticancer drugs using the GSCA database. In this study, GSCA was utilized for drug sensitivity and immunolytic analyses of MMPs. Drug sensitivity patterns were determined using Spearman's correlation coefficient to assess the relationship between MMP expression and the response to various anticancer drugs.

The drug sensitivity data from GSCA may aid in identifying potential therapeutic targets by revealing which MMPs are associated with resistance or sensitivity to specific drugs, thus guiding the development of targeted therapies for SKCM. By correlating MMP expression with drug response data, we can pinpoint MMPs that may serve as novel biomarkers for predicting treatment efficacy and inform strategies to overcome drug resistance in SKCM patients.

CancerSEA analysis

CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/) is a comprehensive database providing a wealth of information on cancer-associated signaling pathways[36]. With curated data from various sources, CancerSEA offers insights into the intricate molecular mechanisms underlying cancer progression. Researchers used its diverse collection of signaling pathways to deepen their understanding and explore therapeutic targets. In the current work, CancerSEA was used to analyze the correlation of MMPs with 14 functional states of SKCM. The functional states analyzed in CancerSEA include apoptosis, cell cycle, DNA damage, EMT, invasion, methylation, stemness, toxins, tumorigenicity, proliferation, metastasis, immune response, hypoxia, and senescence. These states were selected based on their relevance to the processes underlying cancer progression.

siRNA transfection

MMP7 gene knockdown was achieved using MMP7-specific siRNA (Thermo Fisher Scientific, Silencer Select siRNA, Cat. No. 4390824). A375 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and transfected with 30 nM siRNA using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific, Cat. No. 13778075) according to the manufacturer's instructions. Control cells were transfected with Silencer Select Negative Control No. 1 siRNA (Thermo Fisher Scientific, Cat. No. 4390843).

RT-qPCR and Western blot analyses were performed according to the aftermentioned protocols.

Cell proliferation assay

Cell proliferation was assessed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) (Promega, Cat. No. G3582). Transfected A375 cells were seeded in 96-well plates at a density of 3000 cells per well. At 24, 48, and 72 h post-transfection, 20 µL of MTS reagent was added to each well, and cells were incubated for 2 h at 37 °C. Absorbance was measured at 490 nm using a microplate reader.

Colony formation assay

Transfected A375 cells were trypsinized, counted, and seeded in 6-well plates at a density of 500 cells per well. Cells were cultured for 10 days, with the medium changed every 3 days. Colonies were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. The number of colonies containing at least 50 cells was counted manually.

Wound healing assay

A375 cells were seeded in 6-well plates and grown to 90% confluence. A uniform scratch was made in the monolayer using a 200 µL pipette tip. Cells were washed with PBS to remove debris and incubated in serum-free DMEM. Images of the wound area were taken at 0 and 24 h using an inverted microscope. The wound width was measured at five different points per image, and the percentage of wound closure was calculated.

Statistics

Statistical analyses were performed using GraphPad Prism 7.0 and R language (version 4.3.1). Differences in MMP expression levels between SKCM and control groups were evaluated using unpaired Student's t-tests. Correlation analyses, including Spearman's rank or Pearson's correlation coefficient, were applied for associations between MMP expression and clinicopathological or immune-modulator variables. ROC curves and AUC values were used to assess diagnostic performance, with a threshold of $AUC > 0.7$ for acceptable markers. Kaplan–Meier survival curves were constructed for survival analysis, with hazard ratios (HR) and 95% confidence intervals (CI) computed using Cox proportional hazards models. Lasso regression and multivariate Cox models were employed to develop the prognostic risk model, validated on independent GEO datasets. Statistical significance was defined as $p < 0.05$.

Results

Expression and promoter methylation landscape of MMPs in SKCM samples

We investigated the differential expression of MMPs between SKCM and control (non-tumor) skin cell lines using RT-qPCR. The inclusion of non-tumor skin cell lines (HaCaT, NHDF-AD, HEK293T, HUVEC, BJ, NHEK, and HDFa) as controls was crucial to provide a baseline for normal MMP expression, allowing for a clear

distinction between physiological and pathological expression levels observed in SKCM cell lines. The analysis revealed significant upregulation (p-value < 0.05) of MMP1, MMP7, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, and MMP25 mRNA expression levels in SKCM cell lines compared to the control cell lines. Conversely, MMP2, MMP3, and MMP21 were significantly downregulated (p-value < 0.05) in SKCM cell lines (Fig. 1A).

To further understand the regulation of MMP expression, bisulfite sequencing analysis was performed to assess the promoter methylation status. Results showed significantly lower promoter methylation levels (p-value < 0.05) for MMP1, MMP7, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, and MMP25 in SKCM cell lines compared to controls, suggesting hypomethylation as a potential mechanism for their upregulation. In contrast, the promoter methylation levels of MMP2, MMP3, and MMP21 were significantly higher (p-value < 0.05), correlating with their reduced expression in SKCM cell lines (Fig. 1B).

Associations of the MMPs with the survival of SKCM patients

We correlated MMP mRNA expression with the survival of SKCM patients using the GEPIA2 database. For this analysis, we stratified patients into high- and low-expression groups based on the median mRNA expression

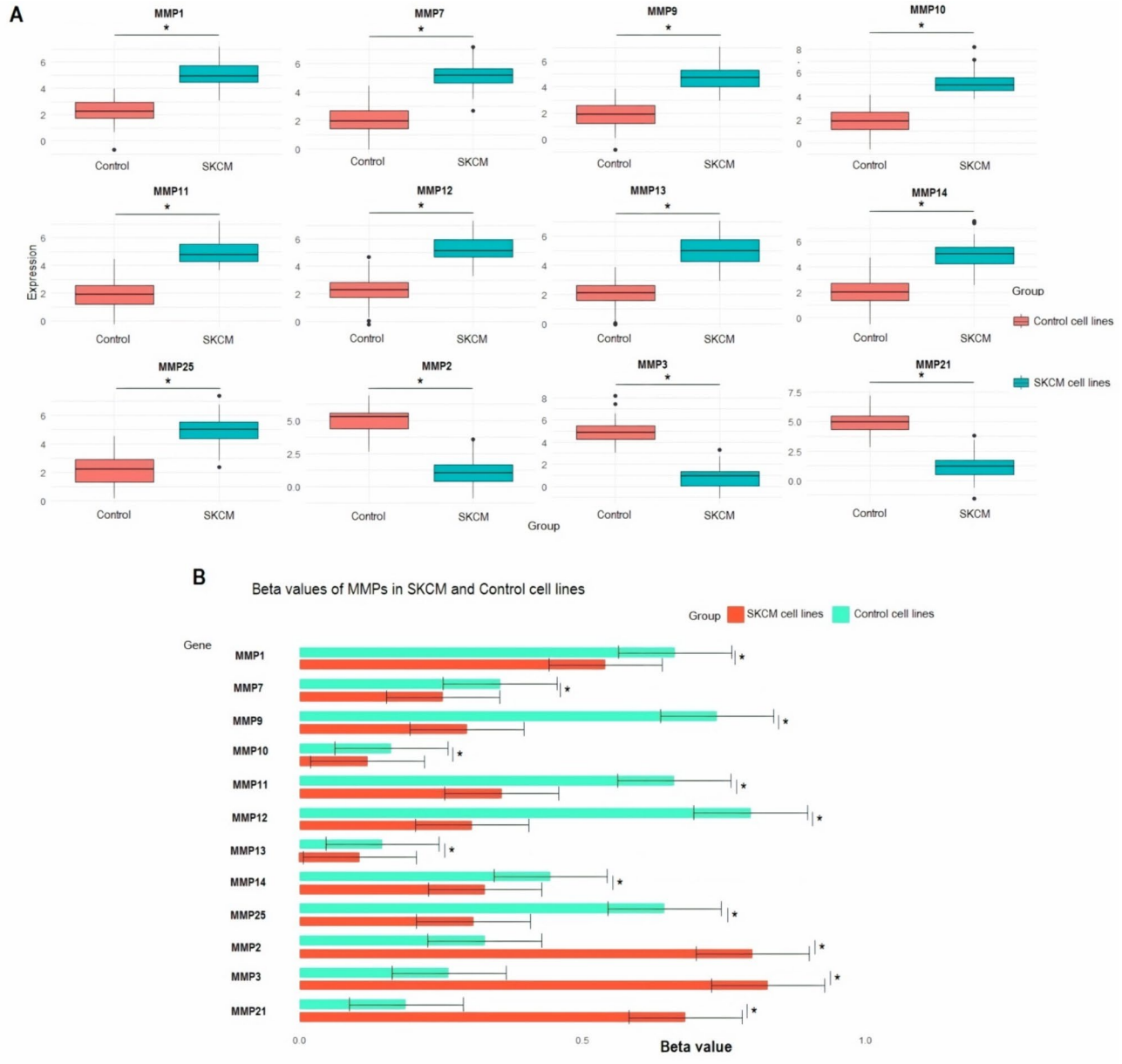

Fig. 1. This figure illustrates the expression and prompter methylation analysis of MMPs in skin cutaneous melanoma (SKCM) and control cell lines using the RT-qPCR technique. Panel (**A**) presents box plots showing the expressions of MMPS in SKCM and control cell lines. Panel (**B**) displays box plots illustrating the promoter methylation levels of the MMPs in SKCM and control cell lines. P*-value < 0.05.

levels of each MMP, as provided by the KM-plotter. Among all the MMPs analyzed, only MMP7, MMP11, and MMP14 exhibited a significant association with overall survival (OS) in SKCM patients (Fig. 2). Higher expression levels of these MMPs were associated with poorer survival outcomes, emphasizing their potential role as adverse prognostic markers. Conversely, other MMPs showed no discernible association with SKCM patient OS (Fig. 2).

These findings align with previous studies that have highlighted the prognostic significance of MMPs in various cancers. For instance, MMP7 has been linked to aggressive tumor behavior and poor prognosis in colorectal and gastric cancers[37,38], while MMP11 and MMP14 have been implicated in promoting tumor invasion and metastasis in breast and lung cancers[39,40]. In SKCM specifically, earlier studies have suggested that elevated MMP14 expression correlates with increased metastatic potential, further supporting its prognostic value[41,42]. Building on these insights, the next part of our study focused on exploring the roles of MMP7, MMP11, and MMP14 in SKCM, aiming to uncover their functional contributions to disease progression.

Diagnostic potential of the MMP7, MMP11, and MMP14

Subsequently, we employed ROC curve analysis to assess the diagnostic predictive capabilities of the MMP7, MMP11, and MMP14 genes in SKCM. The ROC curves demonstrated that the area under the curve (AUC) values for all three MMPs exceeded 0.8, indicating high diagnostic accuracy in distinguishing SKCM patients from normal individuals (Fig. 3). These findings suggest that MMP7, MMP11, and MMP14 exhibit substantial diagnostic value.

To provide context, established biomarkers for SKCM, such as S100B[43], LDH[44], MIA (Melanoma Inhibitory Activity)[45], and TYRP1 (Tyrosinase-Related Protein 1)[46], often show AUC values in the range of 0.7–0.85 for detecting melanoma at various stages. The AUC values observed for MMP7, MMP11, and MMP14 are comparable to these known markers, emphasizing their potential as reliable diagnostic tools.

Expression analysis of MMP7, MMP11, and MMP14 using additional SKCM cohorts

Subsequently, the expression patterns of MMP7, MMP11, and MMP14 were investigated in SKCM by leveraging additional cohorts. Utilizing the GEPIA2 and HPA databases along with Western blot analysis, we assessed and confirmed the MMP expression levels in both tumor and adjacent non-tumor tissues. The GEPIA2 database revealed a notable (p-value < 0.05) elevation in mRNA expression levels of MMP7, MMP11, and MMP14 in SKCM tissues compared to normal tissues (Fig. 4A). Additionally, upon analyzing the expression patterns of these genes across various stages (Stage 0, I, II, III, and IV) of SKCM, a significant (p-value < 0.05) up-regulation was noted in patients with advanced stages of the disease (Fig. 4B). Furthermore, MMP7, MMP11, and MMP14 expression levels were assessed in both SKCM and control cell lines through Western blot analysis and referencing the HPA database. Consistent with earlier findings, MMP7, MMP11, and MMP14 exhibited significantly elevated expression in SKCM samples compared to controls (Fig. 4C,D and Supplementary data Fig. 1).

Validation of promoter methylation level and mutational analysis across TCGA datasets

To further validate the connections between MMP7, MMP11, and MMP14 expression and DNA methylation, the methylation levels of these genes in SKCM tissue samples were assessed using the MEXPRESS database. Figure 5 illustrates the default MEXPRESS plot depicting MMP7, MMP11, and MMP14 expression in SKCM samples, organized according to their respective expression values. The findings affirm a notable (p-value < 0.5) decrease in methylation levels of these genes between SKCM and normal tissues, suggesting a potential regulatory role of DNA methylation in controlling MMP7, MMP11, and MMP14 expression.

The mutation status of MMP7, MMP11, and MMP14 genes in 467 SKCM samples from the TCGA project was investigated using the cBioPortal database. Analysis revealed that the mutation frequencies of MMP7, MMP11, and MMP14 were only 1% in SKCM samples (Fig. 6A), with the most common mutation being C > T.

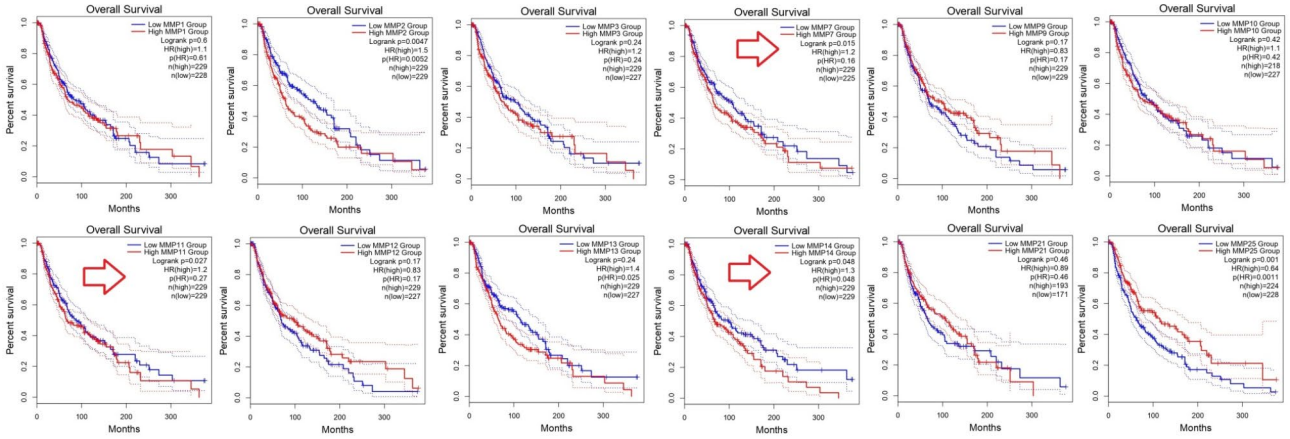

Fig. 2. Prognostic values of MMPs in skin cutaneous melanoma (SKCM) patients using GEPIA2 tool. P-value < 0.05.

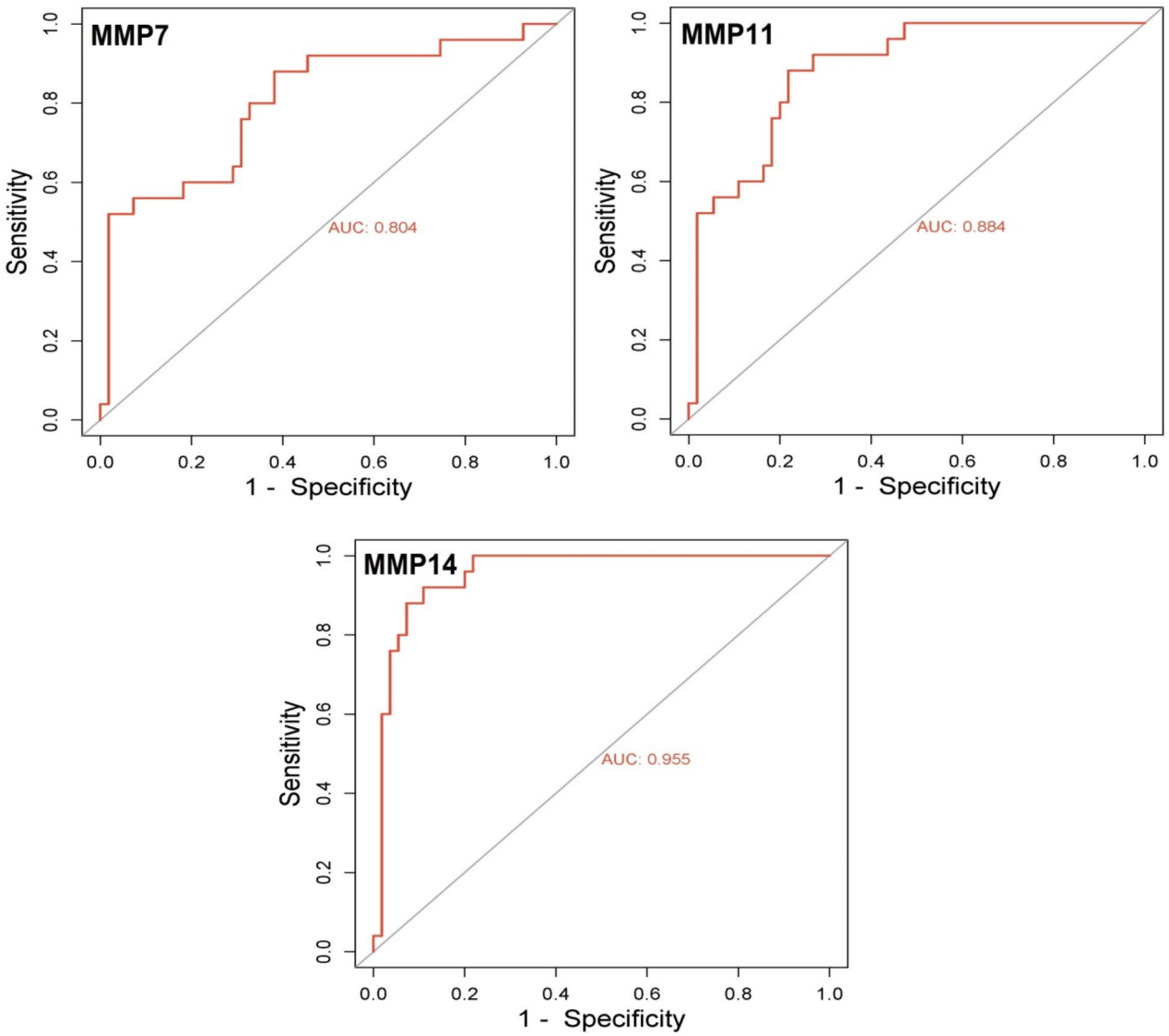

Fig. 3. The Receiver Operating Characteristic (ROC) curves of MMP7, MMP11, and MMP14 illustrate the diagnostic potential of these genes in patients with skin cutaneous melanoma (SKCM). P-value < 0.05.

This suggests that genetic mutations may not be associated with the increased expressions of MMP7, MMP11, and MMP14 genes in SKCM.

Prognostic model development

Next, the Cox regression analysis-based prognostic model incorporating MMP7, MMP11, and MMP14 genes collectively demonstrated their capability to evaluate the overall survival (OS) of SKCM patients (Fig. 6B). This comprehensive model underscores the combined impact of these four dysregulated genes in predicting the prognosis of SKCM patients, emphasizing their potential significance in clinical assessments and therapeutic interventions.

miRNA-mRNA network analysis

To further uncover significant miRNA/mRNA regulatory pathways involving MMP7, MMP11, and MMP14 genes in SKCM, we utilized the miRNet database to predict miRNAs that regulate these genes. Eight miRNAs were identified as potential regulators targeting MMP7, MMP11, and MMP14 genes in SKCM tissues. Among these, has-mir-22-3p miRNA was found to simultaneously target MMP7, MMP11, and MMP14 genes (Fig. 7A,B). This suggests that has-mir-22-3p miRNA is the most notable regulator of expression for MMP7, MMP11, and MMP14 genes.

Next, to further evaluate the role of has-mir-22-3p miRNA in the dysregulation of MMP7, MMP11, and MMP14 genes in SKCM, expression analysis of this miRNA was analyzed in SKCM cell lines using RT-qPCR

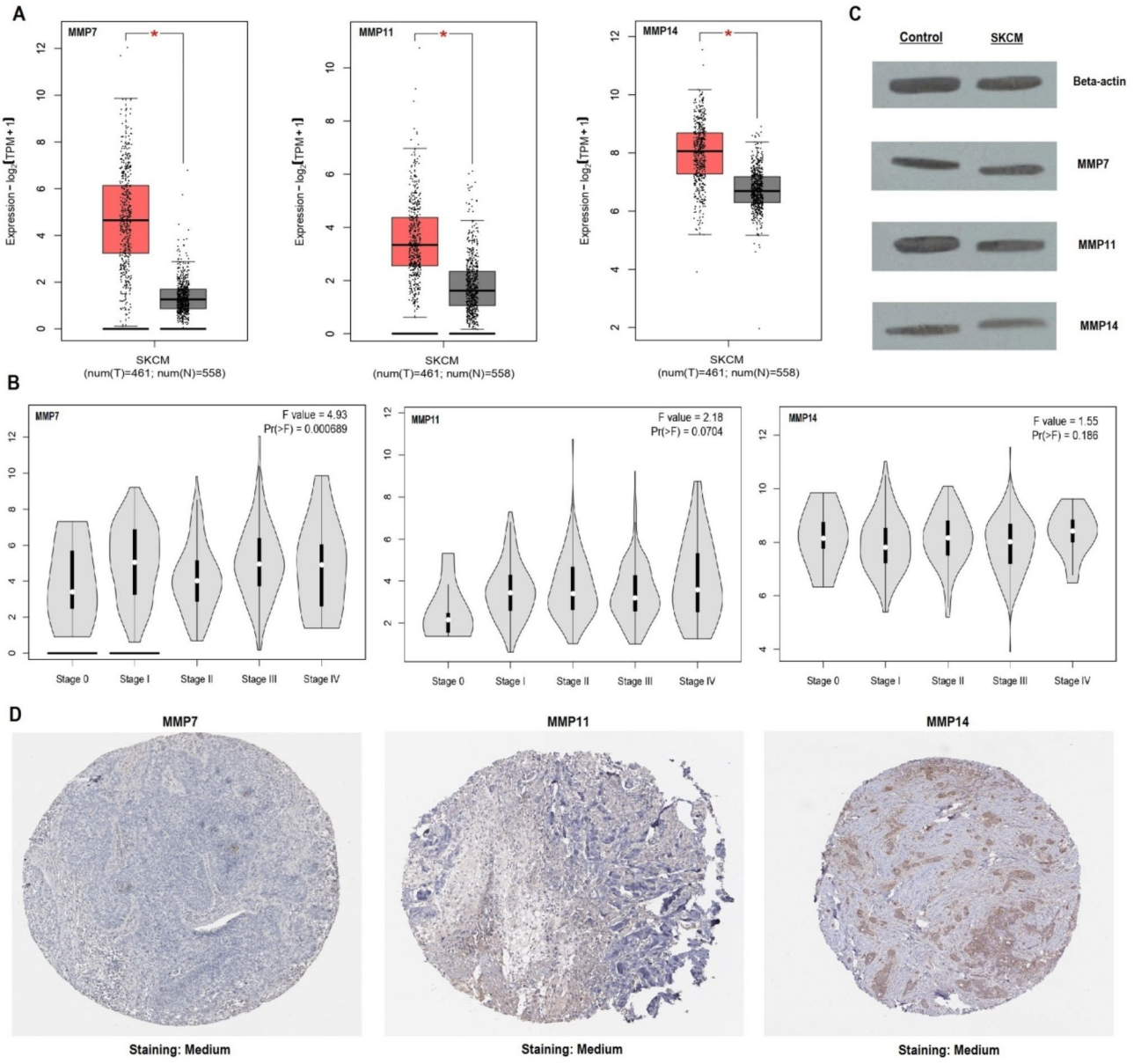

Fig. 4. This figure presents the expression profiling of MMP7, MMP11, and MMP14 genes using GEPIA2, Western blotting analysis, and HPA database. Panel (**A**) illustrates GEPIA2 based-based mRNA expression of MMP7, MMP11, and MMP14 genes in skin cutaneous melanoma (SKCM) and control tissue samples. Panel (**B**) illustrates GEPIA2 based-based mRNA expression of MMP7, MMP11, and MMP14 genes in skin cutaneous melanoma (SKCM) samples of different cancer stages and control samples. Panel (**C**) depicts expression analysis via Western blotting, demonstrating elevated levels of MMP7, MMP11, and MMP14 proteins in SKCM cell lines samples compared to control cell lines samples. Panel (**D**) presents expression analysis through the Human Protein Atlas (HPA) database, further confirming overexpression of MMP7, MMP11, and MMP14 genes in SKCM samples. P-value < 0.05.

assay. Results of the RT-qPCR assay show that the expression of has-mir-22-3p was significantly (p-value < 0.05) up-regulated in SKCM cell lines relative to control cell lines (Fig. 7C).

Correlation of MMP7, MMP11, and MMP14 with immune modulators

Using the TISIDB database, we explored how MMP7, MMP11, and MMP14 express in a correlation with 45 immune modulators. Our analysis revealed a strong inverse correlation between the expression levels of MMP7, MMP11, and MMP14 and immune inhibitor genes, including TNFSF18 and CD27 (Fig. 8A). This finding highlights a notable link between MMP7, MMP11, and MMP14 expressions and other genes involved in immune checkpoint regulation in SKCM.

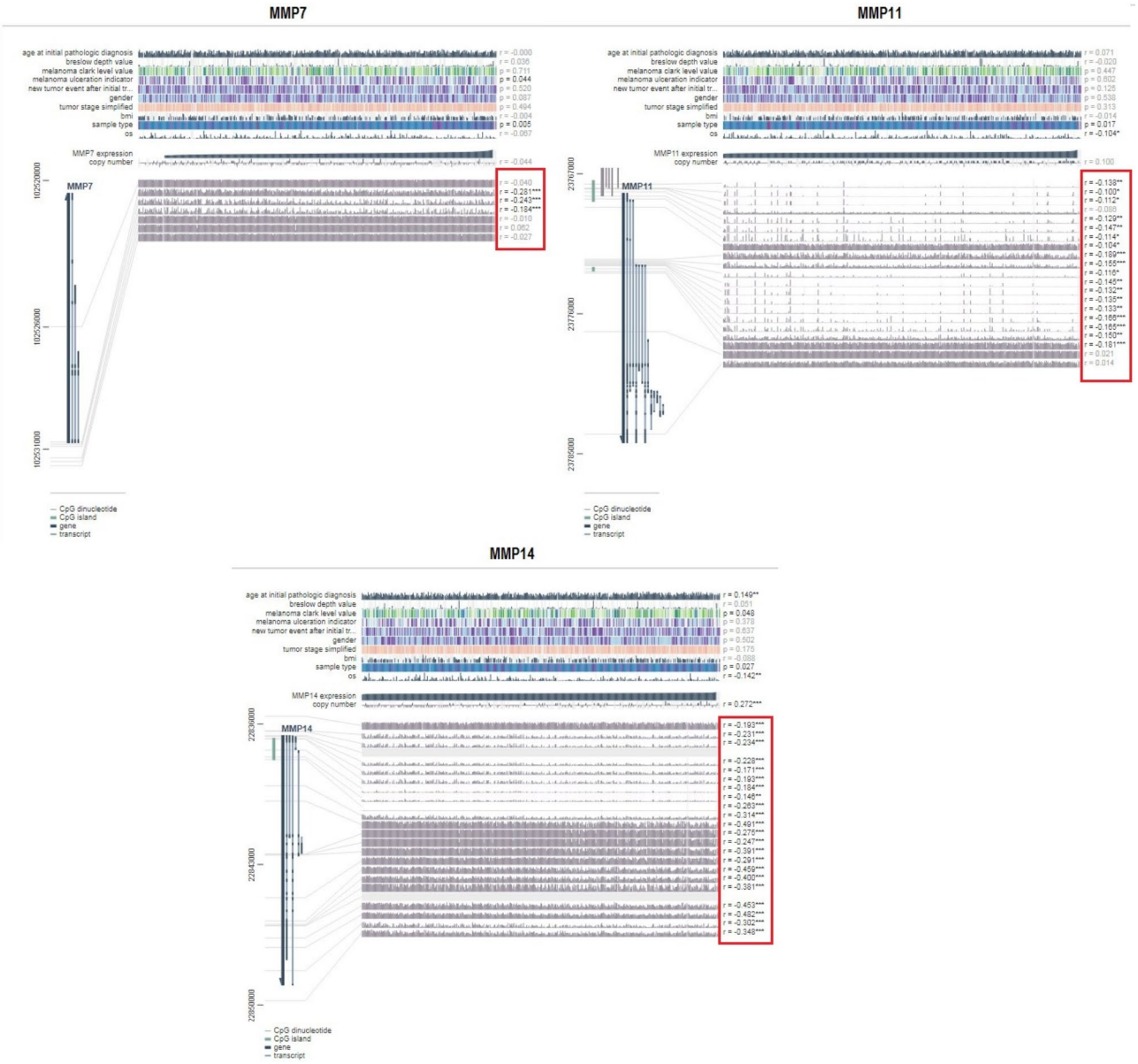

Fig. 5. Promoter methylation levels profiling of MMP7, MMP11, and MMP14 genes across skin cutaneous melanoma (SKCM) and normal control samples suing the MEXPRESS platform. P-value < 0.05.

Drug sensitivity and immunolytic analyses of MMP7, MMP11, and MMP14

In order to investigate the impact of MMP7, MMP11, and MMP14 genes on tumor immune infiltration and the microenvironment in SKCM, we employed the GSCA database to assess the association between their expression levels and the infiltration of immune cells. The findings validated that the expression of MMP7, MMP11, and MMP14 exhibited a predominantly negative correlation with the majority of immune cells (Fig. 8B).

In examining the correlation between MMP7, MMP11, and MMP14 expression in SKCM and the sensitivity of SKCM cells to drug resistance, we employed the GDSC IC50 drug data sourced from the GSCA database (Fig. 8C). Our analysis revealed that SKCM cell lines exhibiting MMP7, MMP11, and MMP14 overexpression demonstrated both resistance and sensitivity to a broad spectrum of drugs, encompassing those utilized in malignancies, both targeted and non-targeted (Fig. 8C).

Correlation of MMP7, MMP11, and MMP14 genes with different functional states of SKCM

In order to gain deeper insights into the role and potential mechanisms underlying MMP7, MMP11, and MMP14 expression in SKCM, we examined their functional status in the CancerSEA database. The analysis revealed distinct associations: MMP7 expression showed positive correlations with "EMT, DNA damage, and invasion" (Fig. 9A). MMP11 expression exhibited positive associations with "angiogenesis, differentiation, and inflammation" (Fig. 9B). Meanwhile, MMP14 expression demonstrated positive correlations with "angiogenesis, differentiation, EMT, and metastasis" (Fig. 9C).

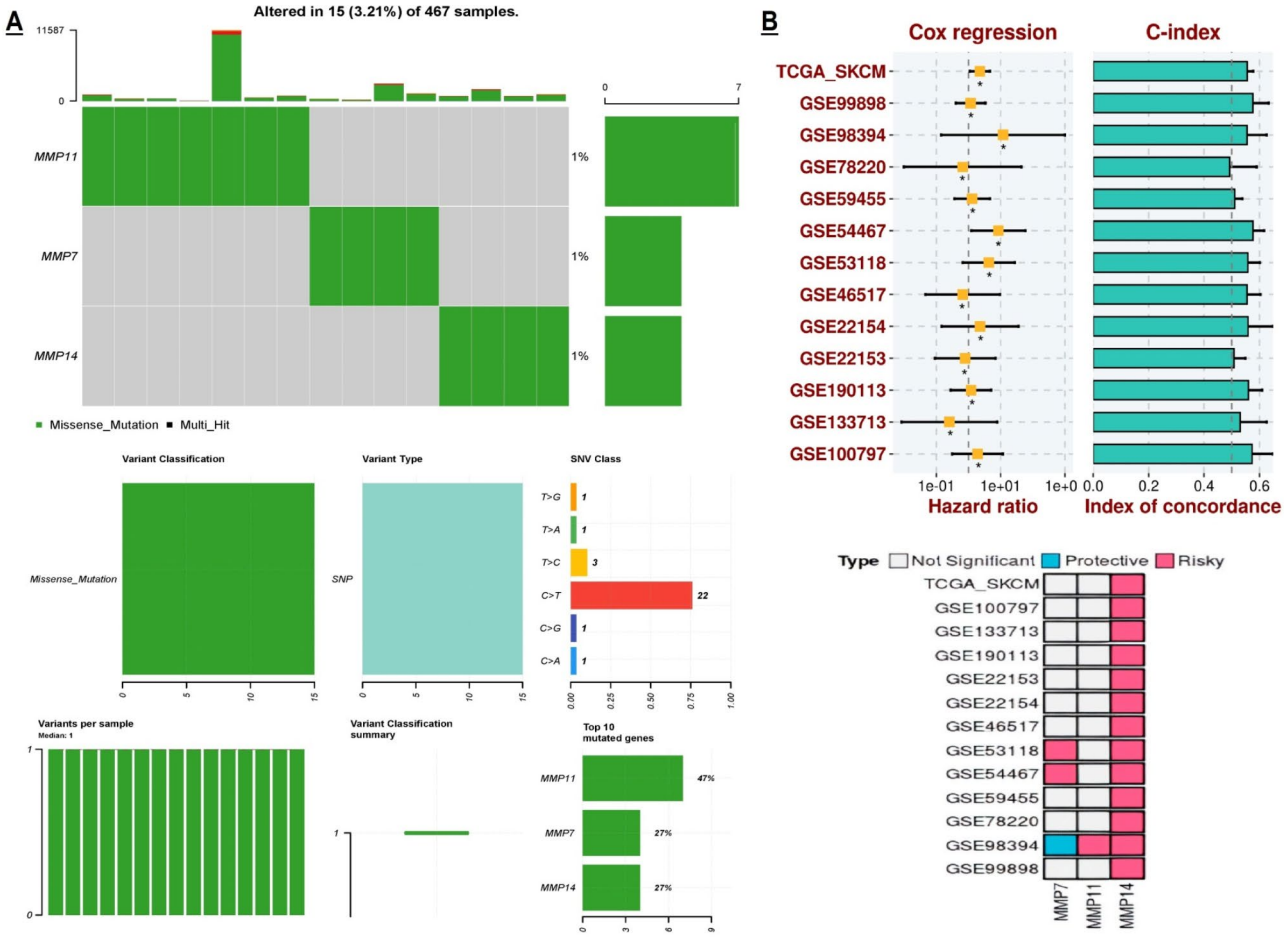

Fig. 6. This figure showcases the exploration of mutational profiles and construction of prognostic model of MMP7, MMP11, and MMP14 genes across skin cutaneous melanoma (SKCM) samples using the TCGA cohort. Panel (**A**) presents the frequencies and types of genetic mutations observed in SKCM samples, providing insights into the alteration landscape of these genes. Panel (**B**) illustrates the prognostic model of MMP7, MMP11, and MMP14 genes in SKCM patients. P-value < 0.05.

MMP7 knockdown and functional analyses

To explore the functional role of overexpressed MMP7 in SKCM, we performed siRNA-mediated knockdown of MMP7 in A375 melanoma cells. The efficiency of MMP7 knockdown was confirmed by a significant reduction in both mRNA and protein expression levels, as demonstrated by RT-qPCR and Western blot analyses (Fig. 10A–C and Supplementary Data Fig. 1). Functionally, MMP7 knockdown led to a pronounced decrease in the proliferative capacity of A375 cells. The cell proliferation assay showed significantly lower growth rates in si-MMP7-A375 cells compared to control cells, underscoring MMP7's role in promoting melanoma cell proliferation (Fig. 10D). Additionally, the colony formation assay revealed a substantial reduction in the number and size of colonies formed by si-MMP7-A375 cells compared to Ctrl-A375 cells (Fig. 10E,F). This finding suggests that MMP7 contributes to the clonogenic potential of melanoma cells, and its suppression might reduce the metastatic capacity, as colony formation is often associated with the ability of cancer cells to survive and proliferate under anchorage-independent conditions. Interestingly, the wound healing assay results revealed a paradoxical effect on cell migration. Despite the reduced proliferation and clonogenic potential, si-MMP7-A375 cells exhibited accelerated wound closure compared to control cells (Fig. 10G,H). Time-lapse analysis confirmed a steeper wound closure curve in MMP7 knockdown cells (Fig. 10I). This finding suggests that while MMP7 supports growth and survival, its knockdown may activate compensatory pathways that enhance cell motility. Possible mechanisms include alterations in cytoskeletal dynamics or compensatory activation of other matrix-modulating enzymes or migration-promoting signaling pathways.

Discussion

Skin cutaneous melanoma (SKCM) represents a significant challenge in oncology due to its aggressive nature and resistance to conventional treatments[47,48]. Understanding the molecular mechanisms underlying its progression and identifying reliable biomarkers is crucial for developing effective diagnostic and therapeutic strategies[49–54]. In recent years, significant strides have been made in elucidating the molecular landscape of SKCM, unveiling

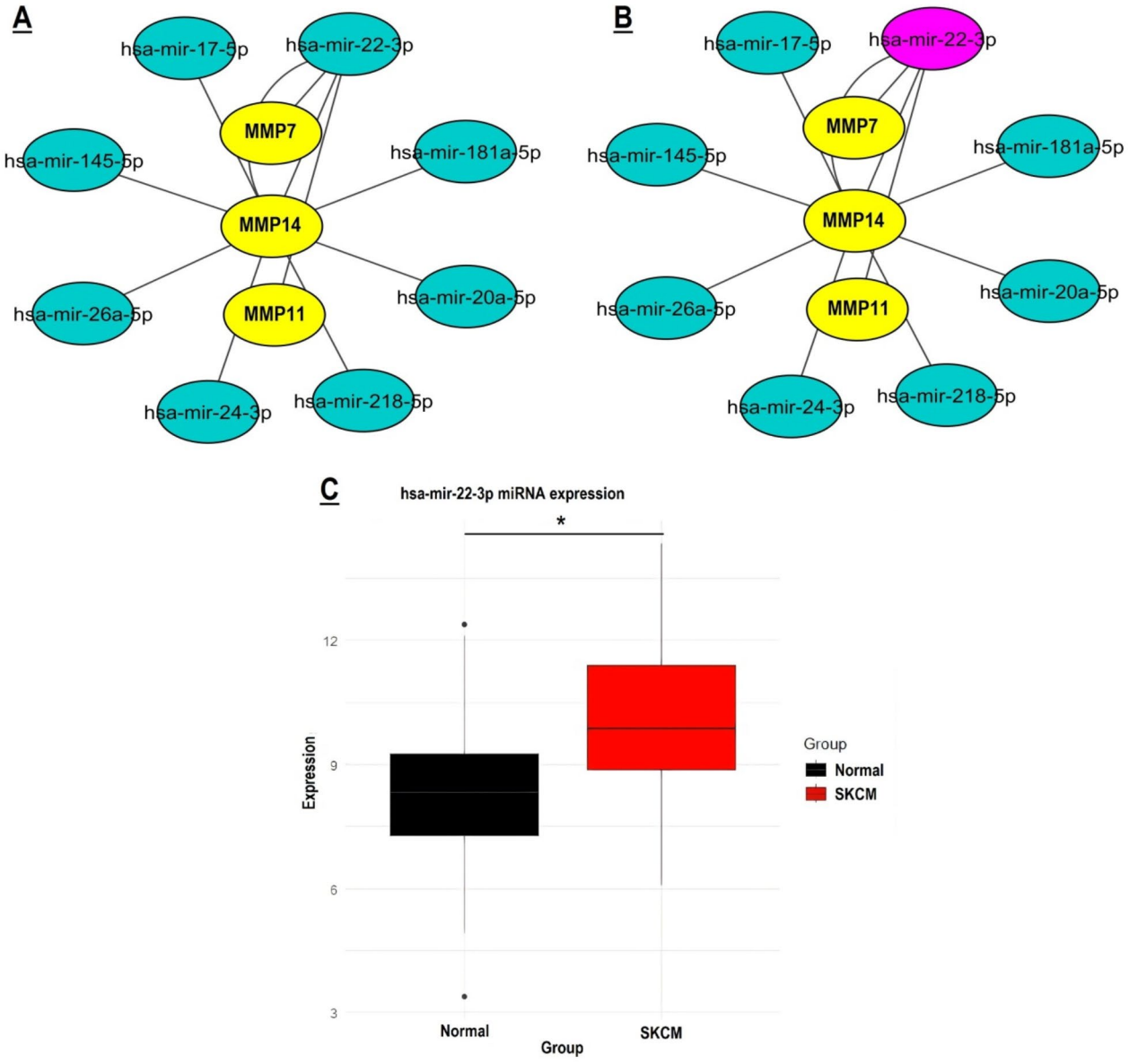

Fig. 7. This figure illustrates the miRNA-mRNA network construction analysis of MMP7, MMP11, and MMP14 genes in skin cutaneous melanoma (SKCM), conducted using the miRNet database and RT-qPCR assay. Panel (**A**) presents the miRNA-mRNA network, highlighting the interactions between MMP7, MMP11, and MMP14 genes and 08 associated miRNAs. Panel (**B**) displays the expression profiling of has-mir-22-3p miRNA across SKCM and normal control samples. P*-value < 0.05.

key genetic alterations and dysregulated signaling pathways implicated in its pathogenesis. However, despite advances in genomic profiling and targeted therapies, the heterogeneity of SKCM poses a formidable obstacle to effective treatment strategies. Moreover, the tumor microenvironment and immune evasion mechanisms further contribute to therapeutic resistance and disease progression. In this study, we conducted a comprehensive analysis of MMPs in SKCM, incorporating multiple layers of investigation, including their expression profiles, promoter methylation status, prognostic and diagnostic significance, regulatory networks, functional roles, and associations with immune modulation and drug sensitivity. By using non-tumor skin cell lines as controls, we established a baseline for normal MMP expression, thereby enabling a clearer understanding of tumor-specific alterations in SKCM.

Earlier, various biomarkers have been identified to aid in its diagnosis, prognosis, and treatment response prediction[55–58]. Our analysis revealed dysregulated expression and promoter methylation patterns of MMPs in SKCM cell lines as compared to control cell lines. Specifically, MMP7, MMP11, and MMP14 were up-regulated and correlated with the poor OS of the SKCM patients. The up-regulation of MMP7, MMP11, and MMP14 in cancer is a multifaceted phenomenon, intricately linked to various aspects of tumor progression. These MMPs contribute to cancer development through their involvement in extracellular matrix (ECM) remodeling,

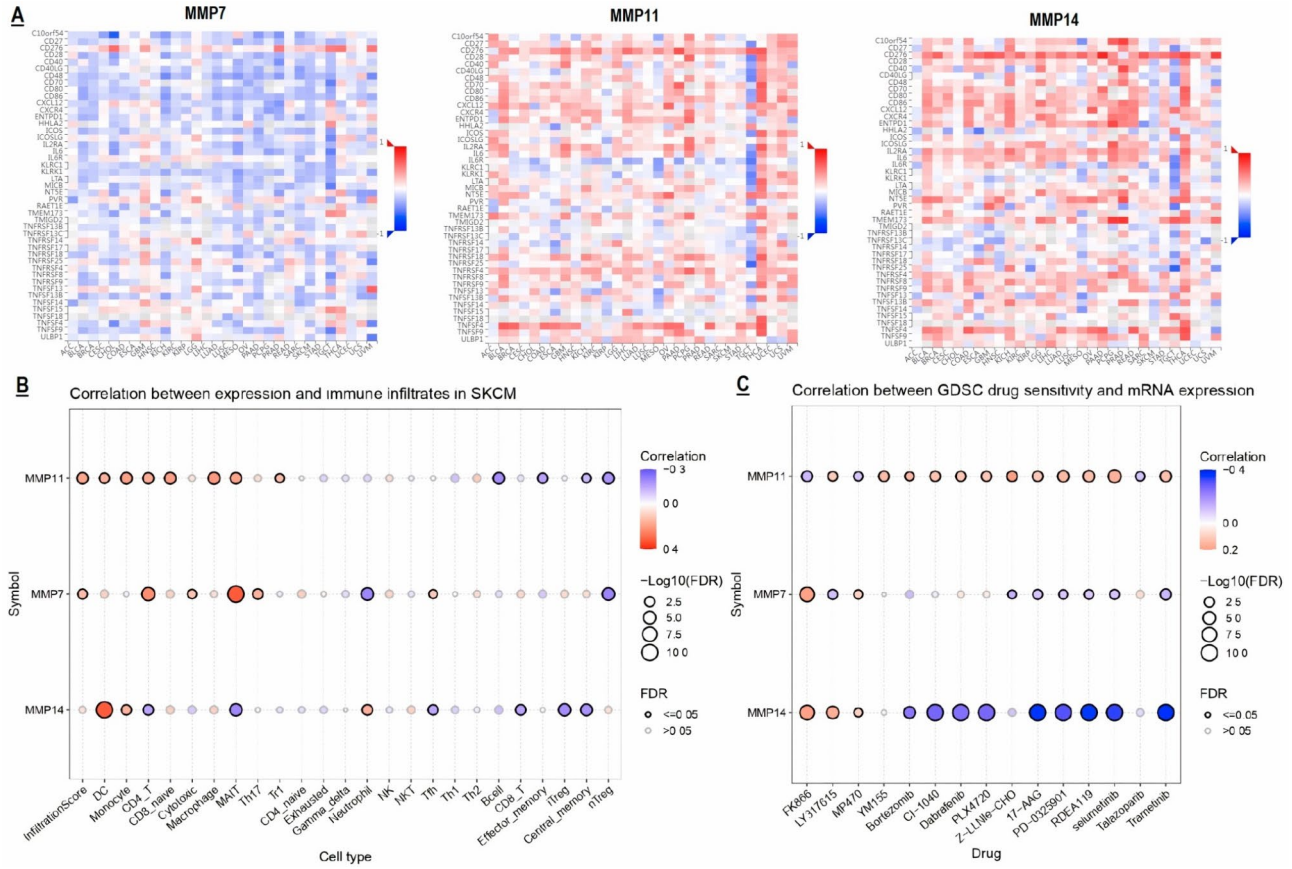

Fig. 8. This presents the correlation, immunological and drug sensitivity analysis of MMP7, MMP11, and MMP14 genes in skin cutaneous melanoma (SKCM) samples, utilizing the TISIDB and Gene Set Cancer Analysis (GSCA) database. Panel (A) Correlation analysis of MMP7, MMP11, and MMP14 with 45 immune modulator genes in SKCM via the TISIDB database. Panel (B) depicts the correlations of these MMP7, MMP11, and MMP14genes with different immune cells in SKCM, highlighting their associations with immune cell infiltration. Panel (C) illustrates the correlations of MMP7, MMP11, and MMP14 genes with various drugs in SKCM, indicating their potential roles in drug sensitivity and resistance mechanisms. P-value < 0.05.

epithelial-mesenchymal transition (EMT), angiogenesis, immune modulation, cell signaling, and interactions with tumor-associated stromal cells (TASCs)[59,60]. Mechanistically, MMPs degrade ECM components, facilitating tumor invasion and metastasis, while also promoting EMT by cleaving cell-cell adhesion molecules and inducing transcription factors that drive the acquisition of mesenchymal-like properties in cancer cells[61,62]. Furthermore, MMPs play pivotal roles in angiogenesis by releasing pro-angiogenic factors and cleaving endothelial basement membrane components, promoting the formation of new blood vessels to support tumor growth and metastasis[17,63]. Additionally, MMPs modulate immune responses within the tumor microenvironment, creating an immunosuppressive milieu that facilitates immune evasion and tumor progression[64]. Through the activation of various cell signaling pathways and crosstalk with stromal cells, MMPs enhance cancer cell survival, proliferation, migration, and interaction with the surrounding microenvironment[65]. Moreover, ROC curve analysis serves as a powerful tool to assess the diagnostic performance of biomarkers, offering insights into their sensitivity and specificity in discriminating between disease and non-disease states. In the case of MMP7, MMP11, and MMP14, the ROC curve analysis demonstrated their robust diagnostic potential in distinguishing SKCM patients from normal individuals. The AUC values exceeding 0.8 indicate high accuracy in discriminating between the two groups, underscoring the efficacy of these MMPs as novel biomarkers for early detection of SKCM. The high sensitivity and specificity exhibited by MMP7, MMP11, and MMP14 suggest their potential utility in clinical settings for identifying individuals at high risk of developing SKCM or for monitoring disease progression. Therefore, the incorporation of MMP7, MMP11, and MMP14 into diagnostic panels or algorithms alongside established biomarkers may further enhance their predictive value and clinical utility.

To elucidate the molecular mechanisms underlying MMP7, MMP11, and MMP14 dysregulation in SKCM, we investigated their methylation status, mutation profiles, and regulatory networks. Our findings suggest that DNA methylation may contribute to MMP7, MMP11, and MMP14 dysregulation, although genetic mutations appear to be rare events in SKCM. This data is consistent with prior research that reinforces the notion that alterations in promoter methylation play a significant role in the dysregulation observed in cancer patients[66–69].

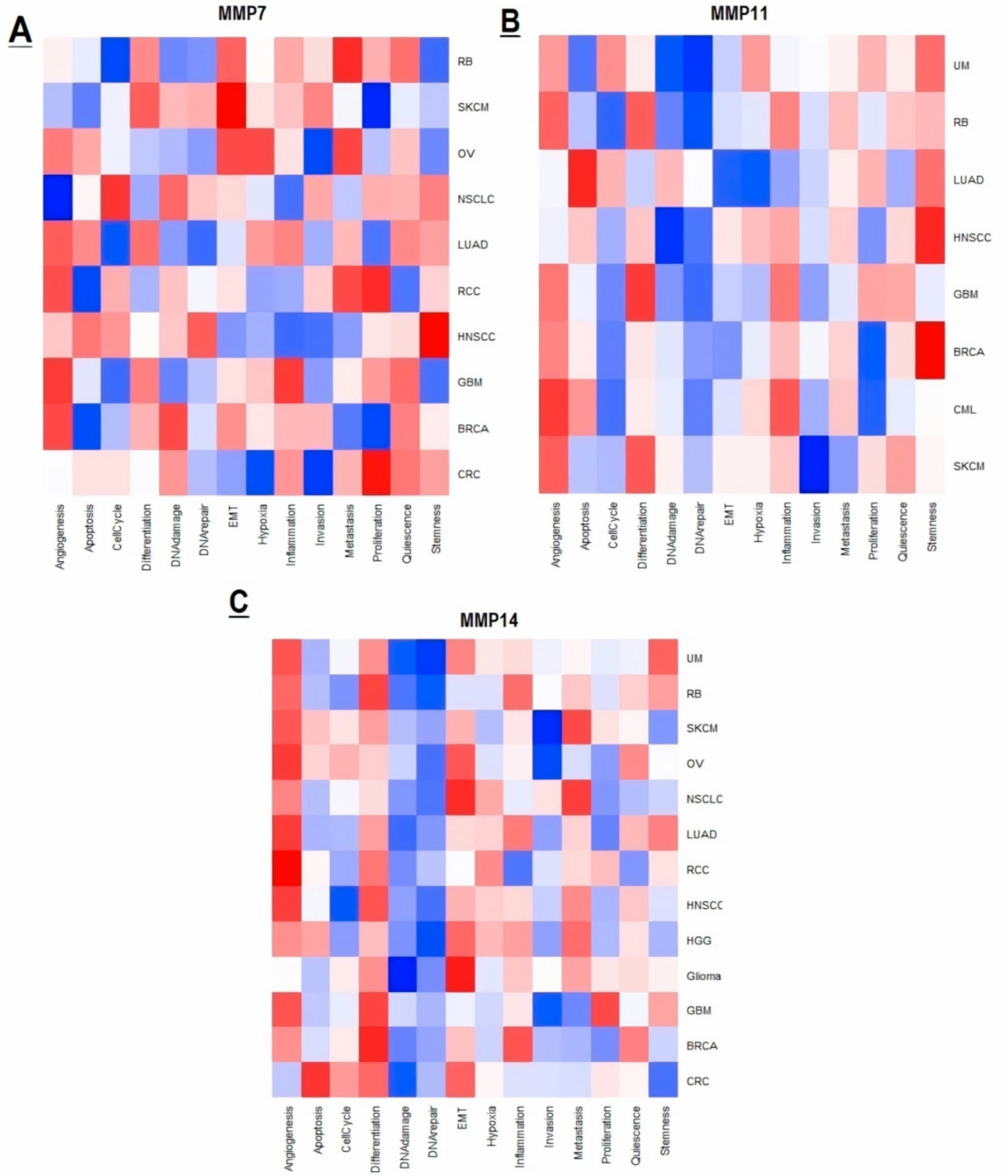

Fig. 9. Correlation of MMP7, MMP11, and MMP14 with numerous functional states and expression validation analyses in skin cutaneous melanoma (SKCM) and control cell lines. (**A**–**C**) Correlation of MMP7, MMP11, and MMP14 with numerous functional states in SKCM via the CancerSEA database. Red indicates a positive correlation, blue represents a negative correlation, and white signifies no correlation.

Moreover, our miRNA-mRNA network analysis identified up-regulated miR-22-3p as a potential regulator of MMP7, MMP11, and MMP14 expression, implicating post-transcriptional regulation in MMP-mediated processes in SKCM. Earlier studies have revealed both up-regulation and down-regulation of hsa-miR-22-3p in various cancer types, indicating its context-dependent role in carcinogenesis. Hsa-miR-22-3p dysregulation has been associated with aberrant cell proliferation, migration, invasion, and metastasis, highlighting its involvement

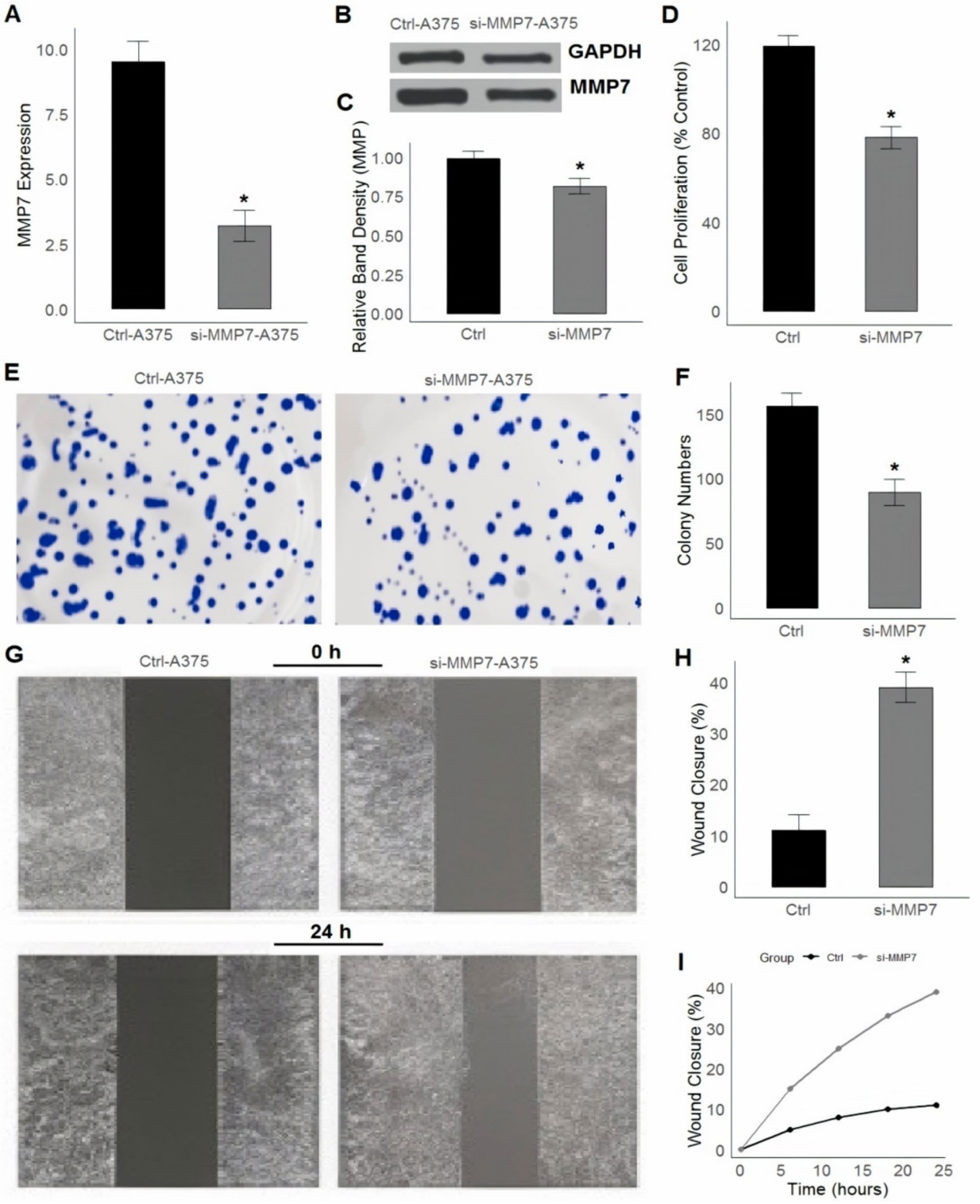

Fig. 10. The effects of MMP7 knockdown on A375 cells. (**A**) RT-qPCR-based expression of MMP7 in Ctrl-A375 cells compared to si-MMP7-A375 cells. (**B**) Western blot analysis confirming the knockdown of MMP7 in si-MMP7-A375 cells. (**C**) Densitometric analysis of MMP7 protein levels normalized to GAPDH. (**D**) Cell proliferation assay indicated that si-MMP7-A375 cells exhibited significantly lower proliferation rates compared to Ctrl-A375 cells. (**E**,**F**) Colony formation assay shows that the number of colonies formed by si-MMP7-A375 cells was significantly lower than that of Ctrl-A375 cells. (**G**) Wound healing assay images at 0 and 24. (**H**) Quantification of wound healing at 24 hours. (**I**) Time-lapse analysis of wound healing over 24 hours. P*-value < 0.05.

in key oncogenic processes[70,71]. Furthermore, hsa-miR-22-3p has been implicated in modulating the expression of genes involved in crucial signaling pathways, such as the PI3K/Akt, MAPK, and Wnt pathways, thereby influencing tumor growth and survival[72,73]. Moreover, the dysregulated hsa-miR-22-3p expression has been correlated with clinical outcomes in cancer patients, serving as a potential prognostic biomarker and therapeutic target[74,75].

Our analysis revealed significant associations between MMP7, MMP11, and MMP14 expression and key aspects of SKCM biology, including immune modulation, drug sensitivity, and functional states of SKCM cells. Specifically, MMP7, MMP11, and MMP14 exhibited inverse correlations with immune modulators and immune cell infiltration, suggesting a potential role in shaping the tumor immune microenvironment. This finding aligns with the hypothesis that MMPs may contribute to immune evasion by degrading extracellular matrix components or releasing bioactive molecules that suppress immune cell recruitment and activation. For instance, MMP-mediated cleavage of chemokines or cytokines could alter the immune cell composition within the tumor, creating an immunosuppressive microenvironment conducive to tumor progression[76,77]. Additionally, SKCM cell lines with overexpression of MMP7, MMP11, and MMP14 showed altered sensitivity to a broad spectrum of anticancer drugs. This highlights the potential role of these MMPs in driving therapeutic resistance. Mechanistically, MMP overexpression may contribute to resistance through remodeling of the extracellular matrix, which can limit drug penetration, or by activating survival signaling pathways that confer resilience against therapeutic agents. Furthermore, MMP-induced changes in the tumor microenvironment, such as increased interstitial pressure or altered stromal interactions, may further impact drug efficacy. Functionally, MMP7, MMP11, and MMP14 were associated with key processes implicated in SKCM progression, including EMT, angiogenesis, inflammation, differentiation, DNA damage, invasion, and metastasis. These findings corroborate previous reports on the multifaceted roles of MMPs in cancer biology and highlight their potential as therapeutic targets in SKCM[78–81].

The study provided detailed insights into the expression and promoter methylation landscape of MMPs in SKCM, highlighting their diagnostic, prognostic, and therapeutic potential. However, several limitations should be noted. While RT-qPCR and bisulfite sequencing analyses are robust methods for quantifying gene expression and promoter methylation, integrating additional omics approaches such as RNA-seq and whole-genome bisulfite sequencing could yield a more comprehensive understanding of MMP dysregulation. Furthermore, the reliance on bioinformatics tools for survival analysis and miRNA-mRNA network predictions is subject to the inherent limitations of database accuracy, sample heterogeneity, and the retrospective nature of many datasets. The in vitro validation, though insightful, has its own constraints. The use of A375 cells, while relevant for SKCM studies, may not fully capture the diverse behaviors of SKCM across different subtypes or patient populations. Additionally, the sample size of cell lines used could limit the generalizability of findings. Expanding the study to include multiple SKCM cell lines and patient-derived xenograft (PDX) models could provide a more representative analysis. Moreover, potential off-target effects of the MMP knockdown experiments need to be addressed. Off-target effects can lead to unintended gene silencing or activation, which could confound the interpretation of the results. Further validation with additional methods, such as CRISPR/Cas9-mediated knockout or rescue experiments, could help confirm the specific role of MMP7, MMP11, and MMP14 in SKCM. Future studies should prioritize in vivo validations to address the complexities of the tumor microenvironment, which cannot be fully replicated in vitro. The use of genetically engineered mouse models or PDX systems could provide insights into the dynamic interplay between MMPs and the tumor microenvironment, as well as their impact on immune modulation and therapeutic resistance. Clinical studies using larger patient cohorts are also necessary to validate these findings and explore MMP-based biomarkers or therapeutic targets across different SKCM subtypes. Finally, translating these findings to clinical settings poses challenges, such as variability in MMP expression among patient subtypes and differences in responses to targeted therapies. Tailored clinical study designs incorporating stratification by molecular profiles could help overcome these hurdles, paving the way for the effective clinical application of MMP-targeted interventions in SKCM.

Conclusion

In conclusion, our study provides comprehensive insights into the expression, promoter methylation, diagnostic potential, prognostic significance, and functional roles of MMPs in SKCM. Our findings reveal significant dysregulation in the expression levels and promoter methylation patterns of various MMPs, highlighting their potential as biomarkers for SKCM diagnosis and prognosis. Notably, MMP7, MMP11, and MMP14 emerged as key players, exhibiting significant associations with overall survival and diagnostic value in SKCM. Furthermore, our study uncovered potential regulatory mechanisms involving miRNAs and immune modulators, shedding light on the intricate molecular pathways underlying MMP-mediated tumorigenesis and immune evasion in SKCM. Importantly, our findings suggest the therapeutic potential of targeting MMPs and associated regulatory networks for SKCM treatment. However, it is essential to acknowledge the limitations of our study, including the reliance on bioinformatics analyses and the need for further validation in larger cohorts and experimental models. Overall, our study underscores the importance of MMPs in SKCM pathogenesis and highlights avenues for future research aimed at improving diagnosis, prognosis, and therapeutic strategies for this aggressive malignancy.

Data availability

Data can be provided by corresponding author on the appropriate request.

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L.H., C.L., and W.H. did the analyses and contributed equally to writing, editing and reanalysis of the results of the paper.

Declarations

Competing interests
The authors declare no competing interests.

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