SLC7A1, *SGK1* and *HMGB2* are overexpressed in cervical cancer tissues and the miR-23b-3p/HMGB2 axis regulates cell migration and invasion

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Abstract. MicroRNA (miRNA/miR)-124-3p and miR-23b-3p are tumor suppressor miRNAs that are associated with advanced cervical cancer (CC), regulating proliferation, migration, invasion, apoptosis and metastasis; however, the identity and function of the various genes regulated by these miRNAs remain unknown. The present study predicted the specific and shared targets of miR-124-3p and miR-23b-3p, cellular processes and signaling pathways involving the predicted targets. *SLC7A1* was found among the shared targets, *SGK1* among the targets of miR-124-3p and *HMGB2* as a target of miR-23b-3p. *SLC7A1*, *SGK1* and *HMGB2* mRNA expression was markedly increased in patients with cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) and levels of SGK1 and HMGB2 were associated with CC progression. *SLC7A1*, *SGK1* and *HMGB2* interact with proteins involved in cellular processes associated with cancer progression. Overexpression of miR-124-3p decreased mRNA of *SLC7A1* in C-33A cells, and of SGK1 in both cell lines. Ectopic expression of miR-23b-3p decreased HMGB2 levels in C-33A and CaSki, and reduced cell migration and invasion. *HMGB2* knockdown experiments revealed that HMGB2 modulates migration and invasion of CC cell lines. In conclusion, the results of the present study suggest that miR-124-3p and miR-23b-3p modulate processes associated with carcinogenesis and tumor progression through their individual and shared target mRNAs and that the miR-23b-3p/HMGB2 axis is among the mechanisms that modulate migration and invasion in CC.

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Introduction

Cervical cancer (CC) ranks fourth in incidence and mortality in women worldwide (1). In patients with CC the main causes of mortality are lymph node metastasis, recurrence of ≤45% after treatment and drug resistance (2-4). The progression of CC is influenced by genetic and epigenetic alterations, post-translational modifications and by the integration of human papillomavirus (HPV). HPV-16 and HPV-18 are the most frequent high oncogenic risk viral types (HR-HPV) in CC tumors (4-6). E6 and E7 of HPV-16 modify gene methylation and alter the expression of microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), among others (7,8). miRNAs are important post-transcriptional regulators of gene expression, which perform a role in tumor progression (9). Therefore, it is important to increase the understanding of the mechanisms mediated by specific miRNA targets involved in CC progression or resistance to chemotherapy, because they may be the basis for the development of treatments that improve the response and/or survival rate of patients with this type of cancer.

In CC miR-124-3p and miR-23b-3p function as tumor suppressor miRNAs (10,11). miR-124-3p contributes to the regulation of tumor metastasis, proliferation and progression

in non-small cell lung cancer, hepatocellular carcinoma and prostate cancer, respectively (12-14). In HeLa and SiHa cells miR-124-3p decreases proliferation, migration and invasion through upregulation of astrocyte elevated gene 1 (AEG-1) mRNA (10), which promotes epithelial-mesenchymal transition (EMT) through activation of the Wnt signaling pathway (15). Moreover, miR-23b-3p has been revealed to regulate the progression of hepatocellular carcinoma, prostate carcinoma, osteosarcoma and CC (16-19). In C-33A, CaSki, SiHa and HeLa cells, overexpression of miR-23b-3p reduces migration, invasion, proliferation and increases apoptosis, via its target genes Six1, ALDH1A1 and c-Met (11,19,20). The mechanism of action of the target genes of miR-124-3p and miR-23b-3p, as well as the signaling pathways by which these miRNAs modulate cellular processes associated with carcinogenesis, maintenance or progression of CC remains to be elucidated.

Cationic amino acid transporter 1 (SLC7A1) has been reported to contribute to tumor development, promote migration and proliferation of SKOV3 and OVCAR3 ovarian epithelial cancer cells and promote cisplatin resistance (21). SLC7A1 is located in the cell membrane, and is a transporter of cationic amino acids, including arginine. In hepatocellular carcinoma SNU-398 cells, depletion of SLC7A1 with short hairpin RNAs reduces intracellular arginine levels and proliferation (22). In liver cancer Hep3B cells, upregulation of miR-122 induced decreases in its target, SLC7A1, intracellular levels of arginine and nitric oxide (NO) and increased sensitivity to the antineoplastic sorafenib, which acts by reducing angiogenesis and proliferation (23). On the other hand, serum- and glucocorticoid-induced kinase 1 (SGK1), a member of the serine/threonine kinase AGC family, regulates transcriptional and post-transcriptional gene expression and modulates multiple signal transduction pathways associated with carcinogenesis (24). SGK1 is overexpressed in cancer types such as colon cancer, gastric cancer and prostate cancer, and induces resistance to inhibitors, drugs and targeted therapies, increases survival, adhesiveness, invasiveness, motility and EMT of tumor cells (25,26). Additionally, SGK1 promotes the progression of some types of cancer through regulation of autophagy, immune response, proliferation, apoptosis, angiogenesis and metastasis (24,27). In CC, SGK1 is an antioxidant factor that promotes cancer cell survival by modulating the c-JUN/NRF2 signaling axis (28). High mobility group box 2 protein (HMGB2) is a DNA-binding protein that regulates DNA transcription, replication and repair (29). In HeLa cells, increased HMGB2 expression promotes cell proliferation by activation of the AKT signaling pathway (30). In cancer, deregulation of the PI3K/AKT signaling pathway promotes increased migration, invasion, proliferation and decreased apoptosis (31,32). Neonatal foreskin keratinocytes expressing HPV-16 E7 overexpress HMGB2 and resist the antiproliferative effect of TNFα (33). Tian *et al* (34), demonstrated that the miR-543/HMGB2 axis modulates proliferation, EMT and apoptosis of HeLa cells. In gastric cancer, miR-23b-3p was revealed to regulate chemoresistance of SGC7901 cells through regulation of ATG12 and HMGB2 (35).

Our previous study used bioinformatics analysis to predict that miR-124-3p, miR-23b-3p and miR-218-5p exert synergistic or additive functional effects through specific or shared target mRNAs that have key roles in CC progression (4). Moreover, experimental data associate SLC7A1, SGK1 and HMGB2 with progression, metastasis and resistance to therapy in different types of cancer, but the expression levels of those genes in tissues of patients with CC is unknown and the relationship between SLC7A1, SGK1 and HMGB2 with downregulation of miR-124-3p and miR-23b-3p is unknown. The SLC7A1, SGK1 and HMGB2 genes are likely overexpressed in CC tissues, and their expression is suggested to be associated with the deregulation of miR-23b-3p and miR-124-3p. Additionally, these miRNAs may influence cell proliferation, migration and invasion in CC by regulating specific and shared target genes, whose gene products perform synergistic roles in tumor progression.

The aim of the present study was to predict specific and shared targets of miR-23b-3p and miR-124-3p, the cellular processes and signaling pathways in which the predicted target genes participate, and to verify whether any of these processes and pathways contribute to cancer progression. A further purpose was to analyze the mRNA and protein expression levels of SLC7A1, SGK1 and HMGB2 from data recorded in public repositories and, finally, to examine whether the genes *SLC7A1*, *SGK1* and *HMGB2* are potentially regulated by miR-124-3p and/or miR-23b-3p in C-33A and CaSki cells.

Materials and methods

Target gene prediction of miR-124-3p and miR-23b-3p, Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. To explore whether the specific and shared target genes of miR-23b-3p and miR-124-3p are involved in the modulation of cellular processes and/or signaling pathways that contribute to cancer progression, bioinformatics analysis was performed. The mRNA and protein expression levels of SLC7A1, SGK1 and HMGB2 were examined using data from public repositories of patients with and without cancer, employing various informatics tools. Additionally, an experimental study was performed to determine whether SLC7A1, SGK1 and HMGB2 are potential targets regulated by miR-124-3p and/or miR-23b-3p in C-33A and CaSki cell lines. Target gene prediction of miR-124-3p and miR-23b-3p was performed on the TargetScan (http://www.targetscan.org/vert_80/; v.7.2; accessed on October 08, 2024) and miRDB (http://mirdb.org/; v.6.0; accessed on October 08, 2024) platforms. Probable targets of miR-124-3p, miR-23b-3p or both miRNAs were considered to be those present on both platforms. To gain insight into the signaling pathways and biological processes involving the predicted target mRNAs and selected target genes (SLC7A1, SGK1 and HMGB2), the bioinformatics resources Functional Annotation Bioinformatics Microarray Analysis (DAVID) (https://david.ncifcrf.gov/) and GO (http://www.geneontology.org/) were used. Biological processes and signaling pathways were organized according to enrichment score. A value of $P<0.5$ was considered indicative of significant enrichment.

Recognition elements (MREs) for miR-124-3p and miR-23b-3p in SLC7A1, SGK1 and HMGB2. SLC7A1 was predicted as a common target of miR-124-3p and miR-23b-3p, SGK1 as a target of miR-124-3p and HMGB2 as a target of miR-23b-3p.

Specific recognition sites for these miRNAs were identified in the 3'UTR region of each mRNA using the TargetScan platform (http://www.targetscan.org/vert_80/; v.7.2; accessed October 08, 2024).

SLC7A1, SGK1 and HMGB2 expression in TCGA- and Human Protein Atlas (HPA)-recorded CC tissues. The GEPIA server (http://gepia.cancer-pku.cn/), which includes The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression databases, was used to obtain miR-124-3p and miR-23b-3p target gene expression data in biopsies from patients with CC (36). Expression data was retrieved from 306 cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) and 13 normal cervical tissue samples. The expression of SLC7A1, SGK1 and HMGB2 were analyzed in CESC samples from patients with EMT activation, PI3K-AKT or hormone alterations. The protein level of SLC7A1, SGK1 and HMGB2 was analyzed in histological sections of cervical tissue stained by immunohistochemistry annotated in HPA. Images of non-tumorous tissue, adenocarcinoma and CESC were obtained.

Protein-protein interaction (PPI) network. A global network of direct (physical) and indirect (functional) target-specific or shared interactions of miR-124-3p and miR-23b-3p (37) were integrated using the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) v.12.0 database (https://string-db.org, accessed October 08, 2024). SLC7A1, SGK1 and HMGB2 were entered as input to STRING, for prediction. Only experimental data and curated databases with a confidence level of 0.9 were considered. A PPI network was generated with proteins that had direct or indirect interaction with SLC7A1, SGK1 and HMGB2. Cellular processes and signaling pathways involved in tumor progression, enriched by SLC7A1, SGK1 and HMGB2 and the proteins with which they interact were identified by GO and KEGG analysis.

Cell culture. CC cell lines C-33A and CaSki were purchased from American Type Culture Collection. The HaCaT cell line was authenticated, certified and donated by the National Cancer Institute, Mexico City, Mexico. C-33A and CaSki cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc.), supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and the HaCaT cell line was cultured in DMEM-F12 medium (Invitrogen; Thermo Fisher Scientific, Inc.), supplemented with 10% FBS. All cells were incubated in humidified atmosphere with 5% CO_2, at 37˚C, until 80% confluence was obtained.

Transfection of miR-124-3p, miR-23b-3p and small interfering (si)-HMGB2. C-33A and CaSki cells were seeded in 6-well plates at a density of $4x10^5$ cells/well. Cells were transfected with 100 nM of hsa-miR-124-3p mimetic (assay ID MC10060; Ambion; Thermo Fisher Scientific, Inc.) and 100 nM of hsa-miR-23b-3p (accession no. MIMAT0000418; assay ID MC10499; Ambion; Thermo Fisher Scientific, Inc.), using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. As a negative control, mirVana miRNA Mimic Negative Control #1, Scrambled, (Invitrogen; Thermo Fisher Scientific, Inc.) was used. Cells were harvested 24 h after transfection at 37˚C. For HMGB2 silencing, siHMGB2 (assay ID s6648; Ambion; Thermo Fisher Scientific, Inc.) and a negative control siRNA (assay ID 4390843; Ambion; Thermo Fisher Scientific, Inc.) were used. C-33A and CaSki cells were transfected with 80 nM of each siRNA using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) during 48 h at 37˚C and subsequently, the assays were performed in triplicate.

Total RNA extraction and reverse transcription-quantitative PCR (RT-qPCR) for miR-124-3p, miR-23b-3p, SLC7A1, SGK1 and HMGB2. Total RNA from $4x10^5$ cells/well transfected and non-transfected cells and HaCaT cells was obtained with TRIzol® reagent (Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. RNA quantification and purity were determined on a NanoDrop 2000c UV-Vis (Thermo Fisher Scientific, Inc.) To determine the expression of miR-124-3p and miR-23b-3p by RT-qPCR, 5 ng of total RNA was subjected to reverse transcription using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Expression of miR-124-3p (assay ID 001182; Thermo Fisher Scientific, Inc.) and miR-23b-3p (assay ID 000400; Thermo Fisher Scientific, Inc.) was determined using the TaqMan microRNA assay kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. RNU6b (assay ID 001093; Thermo Fisher Scientific, Inc.) was used as a reference control for miRNAs.

To determine the expression of SLC7A1 (assay ID Hs00931450_m1; Thermo Fisher Scientific, Inc.), SGK1 (assay ID Hs00178612_m1; Thermo Fisher Scientific, Inc.) and HMGB2 (assay ID Hs01127828_g1; Thermo Fisher Scientific, Inc.), 50 ng of RNA and the TaqMan RNA-to Ct 1-Step Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) were used. RT-qPCR reactions were processed on Prism 7500 equipment (Applied Biosystems; Thermo Fisher Scientific, Inc.) and qPCR reactions were performed on the 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). GAPDH expression (assay ID Hs9999999905_m1; Thermo Fisher Scientific, Inc.) was used as a normalization control for mRNAs. The reaction conditions were established at 48˚C for 15 min, followed by a denaturation step of 95˚C for 10 min, 95˚C for 15 sec and extension at 60˚C for 1 min. The relative expression of miRNAs and target mRNAs was calculated using the $2^{-\Delta\Delta Cq}$ method (38). Experiments were performed in triplicate at independent times.

Protein collection and western blotting for HMGB2. After a 24 h transfection with the miR-23b-3p mimetic, C-33A and CaSki cells were harvested and treated with TRIzol® reagent (Thermo Fisher Scientific, Inc.). Total proteins were obtained from the organic phase following the manufacturer's instructions. Protein concentration was quantified with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Inc.). A total of 20 μg of proteins were separated by 10% SDS-PAGE and transferred to a 0.45 μm nitrocellulose membrane (Bio-Rad Laboratories, Inc.). The membrane was blocked at room temperature with 5% low-fat milk in TBS-Tween-20 (0.05% Tween 20 in TBS, pH 8.0). The membrane was incubated

overnight at 4˚C with a primary antibody directed to HMGB2 1:2,000 (cat. no. D1P9V; Cell Signaling Technology, Inc.) and GAPDH 1:3,000 (cat. no. 14C10, Cell Signaling Technology, Inc.). GAPDH was used as a loading control. The membrane was incubated with HRP-coupled secondary antibody for 2 h at room temperature. The bands were visualized using the Chemiluminescent HRP Substrate kit (MiliporeSigma) on the iBright™ CL1500 Imaging System (Invitrogen; Thermo Fisher Scientific, Inc.). Experiments were performed in triplicate.

Wound closure migration assay. A total of $4x10^5$ C-33A or CaSki cells were seeded per well in 6-well plates to 100% confluence and transfected with 100 nM of the miR-23b-3p mimetic or scrambled, which was used as a negative control for 48 h at 37˚C. After 24 h the cells were treated with 10 μM cytosine arabinoside (MilliporeSigma) for 2 h at 37˚C to inhibit proliferation. The cell monolayer was scratched with a scraper to make a uniform linear wound. The culture was washed with 1X PBS to remove unattached cells and DMEM medium added with 0.5% FBS was added. The culture medium was replaced every 24 h. Images of wound closure were captured with an inverted microscope (Eclipse TS2; Nikon Corporation) at magnification, x10 at 0, 12, 24, 24, 36 and 48 h after scratching. The percentage of wound closure was calculated with ImageJ software v1.54 (National Institutes of Health). Experiments were performed in triplicate.

Matrigel invasion assay. Cell invasion assays were performed in Transwell chambers (cat no. 354578; BioCoat; Corning Inc.), with 8 μm pore polycarbonate membrane, pre-coated with ECMatrix (MiliporeSigma) at a 1:10 concentration using FBS-free DMEM medium for 2 h at 37˚C. On top of each insert, $1x10^5$ C-33A or CaSki cells transfected with 100 nM miR-23b-3p or Scrambled were seeded, suspended in FBS-free DMEM medium. To the bottom of the Transwell chamber, 600 μl of DMEM medium supplemented with 10% FBS, as a chemoattractant, was added. The cells were incubated in a humidified atmosphere with 5% CO_2 at 37˚C for 48 h. Two washes with 1X PBS were performed to remove unattached cells and invading cells were fixed with 4% formaldehyde for 20 min and stained with 0.1% crystal violet diluted in 1X PBS for 12 min at room temperature. Images of invading cells were obtained with an inverted microscope (Eclipse TS2; Nikon Corporation) with 10X objective. Additionally, cells were indirectly counted by elution of crystal violet with 500 μl of 10% acetic acid, the absorbance of the solution was measured at 590 nm in a microplate reader (Thermo Fisher Scientific, Inc.). The assays were performed in triplicate at independent times.

Statistical analysis. TCGA data were analyzed by one-way ANOVA in the GEPIA2 platform, which allows calculation of differences in gene expression levels between normal and tumor tissues. Expression data of mRNAs and miRNAs were presented as mean ± SD of at least three independent experiments and analyzed using GraphPad Prism 5.0 software (Dotmatics). Data analysis was performed by comparing the mean relative expression levels of miRNAs, mRNAs, or proteins obtained from three technical replicates and three biological replicates per experimental condition. unpaired Student's t-test was used to determine the P-value for comparisons between two groups, and Welch's ANOVA was applied for comparisons among three or more groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

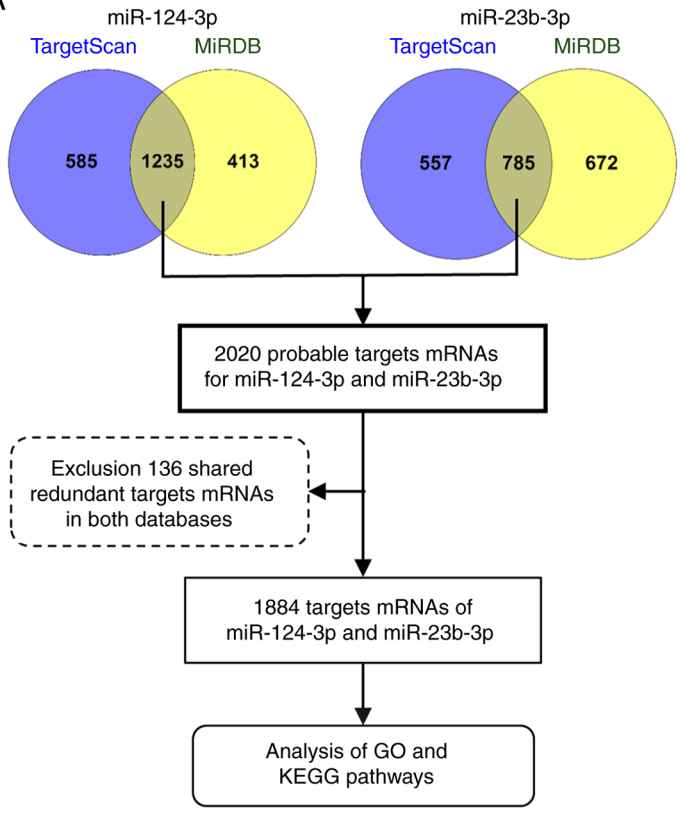
miR-124-3p and miR-23b-3p regulate specific and shared targets. Probable targets of miR-124-3p and miR-23b-3p were predicted and only mRNAs with MREs for miRNAs, highly conserved and included in TargetScan and miRDB, were considered for analysis. A total of 1,235 probable targets of miR-124-3p and 785 for miR-23b-3p were found, (Fig. 1A), of these, 136 mRNAs were shared targets of miR124-3p and miR-23b-3p (Fig. 1B).

Target mRNAs of miR-124-3p and miR-23b-3p regulate cellular processes that are recognized as cancer hallmarks. To explore the potential function of both miRNAs in CC, the 1,884 probable target mRNAs of miR-124-3p and miR-23b-3p (Fig. 1A), were subjected to GO and KEGG analysis. GO analysis indicated that among the biological processes markedly enriched by miR-124-3p and miR-23b-3p targets is 'Actin cytoskeleton organization', 'Apoptotic process', 'Angiogenesis', 'Proliferation' and 'Positive regulation of cell migration'. SLC7A1, a shared target between both miRNAs is involved in 'Amino acid transport' and 'Amino acid import across plasma membrane'; SGK1, target of miR-124-3p is involved in 'Protein phosphorylation', 'Apoptotic process', 'Regulation of cell population proliferation' and 'Regulation of cell migration'. Conversely, HMGB2, target of miR-23b-3p is involved in 'Regulation of transcription by RNA polymerase II', 'Positive regulation of DNA-templated transcription' and 'Positive regulation of endothelial cell proliferation' (Fig. 2A). Pathways identified in KEGG as enriched by miR-124-3p and miR-23b-3p target genes include 'Pathways in cancer', 'mTOR signaling pathway', 'PI3K-Akt signaling pathway' and 'FoxO signaling pathway' (Fig. 2B).

SLC7A1, SGK1 and HMGB2 contain response elements for miR-124-3p and miR-23b-3p. Based on GO and KEGG pathways analysis SLC7A1, SGK1 and HMGB2 participate in processes and pathways involved in cancer progression, therefore, recognition sequences for miR-124-3p and miR-23b-3p were sought in the 3'UTR region of the mRNA of SLC7A1, SGK1 and HMGB2. Based on affinity criteria (score and hybridization type), SLC7A1 possesses a 7mer-m8 MRE for miR-124-3p and an 8mer site for miR-23b-3p, SGK1 mRNA contains a 7mer-m8 type MRE for miR-124-3p and HMGB2 mRNA contains three MREs for miR-23b-3p among which two 8mer sites stand out (Table I).

SLC7A1, SGK1 and HMGB2 interact with proteins involved in cancer-related processes. SLC7A1 is one of 136 target mRNAs shared by miR-124-3p and miR-23b-3p. To explore its interaction with cellular proteins, a PPI network was generated. The PPI network consists of 31 nodes and 159 statistically significant interactions, and analysis of GO and KEGG pathways indicates that SLC7A1 interacts with proteins involved in 'Intrinsic apoptotic signaling pathway in response to DNA damage' and 'Cell communication' (Fig. 3A). SGK1 was

A



B

136 common targets for miR-124-3p and miR-23b-3p

ADAM19	FMR1	NFIA	SMAD5
AFF1	FNBP1L	NFIB	SNX30
ALDH1L2	FNIP2	NLGN4X	SPOPL
ASF1A	FZD5	NOL4	SRGAP3
ATMIN	G3BP2	NUFIP2	STK35
BACH2	GALNT12	OSBPL8	STK4
BAZ2B	GLCE	OTUD4	STON2
BICD2	GNG2	OXSR1	STRN
BRWD1	GXYLT1	PALLD	SUCO
BTBD7	HIC1	PAPOLG	TANC2
C2orf69	HTR2C	PAQR9	TAOK1
CACUL1	INO80D	PCDH19	TEAD1
CAPN6	IPMK	PDE4B	TENM1
CCDC6	KIAA1958	PGRMC2	THAP2
CCDC71L	KLHL28	PHF19	TJP1
CEP350	LPP	PIK3C2A	TMCC3
CEP85L	LRIG1	PPIF	TMEM178B
CLEC1A	MAML1	PRPS1	TMEM87B
COL4A1	MAP3K1	PRR14L	TMOD1
CPD	MAP3K3	PTPN11	TNRC6B
CPOX	MAP7	QKI	TOX
DCUN1D5	MAPRE1	QSER1	TRPS1
DMXL1	MARK1	RAD17	TXNRD1
DYRK2	MAT2A	RAP1A	UBE20
EEA1	MED12L	RAP2B	UBN2
EFNA5	MEF2A	RCOR1	UHMK1
EGR1	MITF	REEP1	ULK2
ELK3	MTF1	RPIA	VCAN
ELL2	MTMR10	RUFY2	VKORC1L1
ENTPD5	MYNN	SEMA6D	VSNL1
EPG5	NAA15	SESN3	WNK1
ETNK1	NACC2	SH3PXD2A	YOD1
ETV1	NAP1L5	SIX4	ZBTB34
EYA1	NEGR1	SLC7A1	ZNF652

Figure 1. Target genes of miR-124-3p, miR-23b-3p and shared by both miRNAs. (A) The Venn diagram shows the target mRNAs of miR-124-3p and miR-23b-3p recorded in miRDB and TargetScan. (B) The table shows the name of the 136 genes predicted as targets shared by miR-124-3p and miR-23b-3p. miR, microRNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

selected as a likely target of miR-124-3p. The corresponding PPI network indicates that SGK1 interacts with proteins associated with 'Positive regulation of cell growth', 'Regulation of epithelial to mesenchymal transition' and 'Apoptotic process', which are associated with cancer progression (Fig. 3B). SLC7A1 and SGK1 are involved in the modulation of the 'FoxO signaling pathway'. Finally, HMGB2 mRNA was considered as a target of miR-23b-3p and the HMGB2 PPI network consisted of 31 nodes and 103 statistically significant interactions. The results of GO analysis of the PPI network suggested that HMGB2 interacting proteins are involved in 'Cell communication', 'Positive regulation of NIK/NF-κB signaling' and 'Positive regulation of MAP kinase activity' and 'Positive regulation of the JNK cascade'; process and pathways that are key in cancer progression (Fig. 3C).

SLC7A1, SGK1 and HMGB2 mRNA are markedly overexpressed in biopsies from patients with CESC. The GEPIA platform was used to analyze the expression levels of *SLC7A1*, *SGK1* and *HMGB2* in patients with CESC. In 306 patients with CESC, the mRNA levels of SLC7A1, SGK1 and HMGB2 were markedly increased compared with levels found in cancer-free cervical tissue biopsies, (Fig. 4A-C). TCGA data revealed that, in patients with CESC, there is significant increase in SLC7A1 expression, but this significance is lost in tissues with EMT, hormonal alterations or activation of the PI3K-AKT pathway compared with normal tissue, (Fig. 4A). A similar behavior is observed for SGK1 expression, although for SGK1 the significant increase is maintained in samples with EMT (Fig. 4B). Conversely, HMGB2 mRNA is markedly increased among the 306 patients with CESC and this behavior persists in biopsies from women with PI3K-AKT pathway activation, hormonal alterations or EMT (Fig. 4C). In HPA, protein levels denote high expression of SLC7A1 and SGK1 in adenocarcinoma tissues and a moderate level of HMGB2, compared with non-cancer tissue (Fig. 4D). In CESC tissues, there is high expression of SLC7A1 and HMGB2 proteins and moderate SGK1 levels (Fig. 4D). (Images available online https://www.proteinatlas.org/ENSG00000139514-SLC7A1/pathology/cervical+cancer; https://www.proteinatlas.org/ENSG00000118515-SGK1/pathology/cervical+cancer; https://www.proteinatlas.org/ENSG00000164104-HMGB2/pathology/cervical+cancer; accessed October 08, 2024).

Expression of SLC7A1, SGK1, HMGB2 in response to overexpression of miR-124-3p and miR-23b-3p in C-33A and CaSki. To verify the relationship between the level of miRNAs and *SLC7A1* mRNA and to check the consistency between the experimental results with those of computational analysis, which suggests that *SLC7A1* is shared target of miR-124-3p and miR-23b-3p, the expression of miR-124-3p, miR-23b-3p and target mRNA were determined. Analysis revealed that the level of miR-124-3p and miR-23b-3p is reduced in C-33A (***P<0.0001; ***P<0.0001, respectively) and CaSki

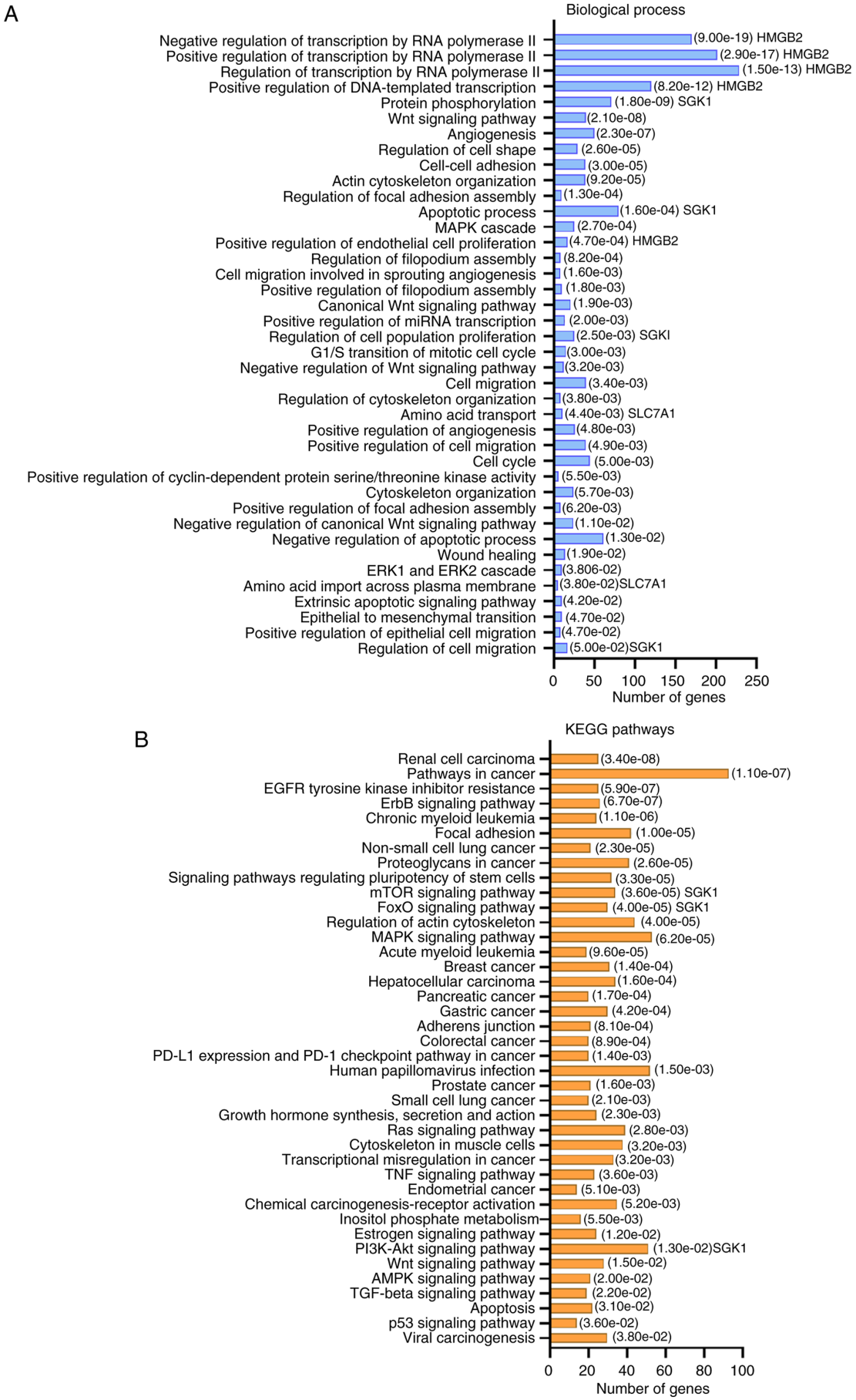


Figure 2. Functional analysis of miR-124-3p and/or miR-23b-3p target mRNAs. (A) Biological processes and (B) KEGG pathways enriched by genes included among the 1,884 target transcripts of miR-124-3p and miR-23b-3p. Terms with P<0.05 were considered statistically significant. miR, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table I. Response elements for miRNAs in HMGB2, SLC7A1, SGK1 mRNAs, likely targets of miR-124-3p, miR-23b-3p or both miRNAs.

Target gene	miRNA	Sites in untranslated region	Type of site
SLC7A1	miR-124-3p	Position 4576-4582 5' UUUCCUGCUUAAGUU**GUGCCUU**U | | | | | | | miR-124-3p 3' CCGUAAGUGGCG**CACGGAA**U	7mer-m8
	miR-23b-3p	Position 4921-4928 5' CACCACCUUUCAAAA**AAUGUGA**A | | | | | | | miR-23b-3p 3' CCAUUAGGGACCG**UUACACU**A	8mer
SGK1	miR-124-3p	Position 353-359 5' CGAAUAUUAUGAAAU**GUGCCUU**U | | | | | | | miR-124-3p 3' CCGUAAGUGGCG**CACGGAA**U	7mer-m8
		Position 64-71 5' AAUUAUUUUGCUAAG**AAUGUGA**A | | | | | | | miR-23b-3p 3' CCAUUAGGGACCG**UUACACU**A	8mer
HMGB2	miR-23b-3p	Position 1090-1097 5' AAGAAGAGGGGCUAG**AAUGUGA**A | | | | | | | miR-23b-3p 3' CCAUUAGGGACCG**UUACACU**A	8mer
		Position 3475-3481 5' ACCAUUUGAUGUCAC**AUGUGA**A | | | | | | miR-23b-3p 3' CCAUUAGGGACCGU**UACACU**A	7mer-A1

SLC7A1, cationic amino acid transporter 1; SGK1, serum- and glucocorticoid-induced kinase 1; HMGB2, mobility group box 2 protein; miR, microRNA.

(***P<0.0001; ***P<0.0001, respectively) cells compared with HaCaT (Fig. S1) and the mRNA levels of SLC7A1 are markedly decreased in CaSki cells compared with HaCaT cells (***P<0.0001; Fig. 5A), in addition, in C-33A cells we observed an increased expression of SLC7A1 mRNA compared with CaSki (***P<0.0001; Fig. 5A). To determine the effect of miR-124-3p and miR-23b-3p on SLC7A1 expression levels in C-33A and CaSki cells, mimetics of one or both miRNAs were transfected, (Fig. S2). C-33A cells with increased miR-124-3p or both miRNAs revealed significantly decreased levels of SLC7A1 compared with the control (***P<0.0001; **P<0.001, respectively; Fig. 5B). By contrast, increased SLC7A1 expression was observed in C-33A cells overexpressing miR-23b-3p compared with the control and to cells transfected with miR-124-3p or both miRNAs (*P<0.05; ****P<0.00001; ****P<0.00001, respectively; Fig. 5B). In CaSki cells, an increase in SLC7A1 expression was observed in cells transfected with miR-23b-3p or both miRNAs compared with the control (**P<0.001; ***P<0.0001, respectively; Fig. 5C). By contrast, no changes in SLC7A1 expression were observed in CaSki cells transfected with miR-124-3p compared with the control (Fig. 5C).

SGK1 expression levels were significantly increased in CaSki (*P<0.05) and C-33A cells (**P<0.001) compared with that in HaCaT cells (Fig. 6A), and increased SGK1 expression was observed in C-33A cells compared with CaSki (**P<0.001; Fig. 6A). Overexpression of miR-124-3p was associated with significantly decreased SGK1 mRNA levels in C-33A cells (*P<0.05) but not in CaSki cells compared with scrambled (Fig. 6B and C).

The mRNA levels of HMGB2 were significantly increased in C-33A (***P<0.0001) and CaSki (**P<0.001) cells compared with HaCaT cells (Fig. 7A) and the protein expression levels were also significantly increased in C-33A and CaSki cells (***P<0.0001 and **P<0.001, respectively) compared with HaCaT cells (Fig. 7B). In C-33A and CaSki cells, overexpression of miR-23b-3p resulted in decreased expression levels of HMGB2 mRNA (****P<0.00001 and *P<0.05, respectively; Fig. 7C and D) and this effect is reproduced at the protein level in C-33A and CaSki cells (**P<0.001 and *P<0.05, respectively; Fig. 7E and F).

miR-23b-3p and HMGB2 knockdown negatively regulates C-33A and CaSki cell migration and invasion. Bioinformatic analysis revealed that miR-23b-3p modulates migration and invasion through the regulation of 'Wnt signaling pathway', 'MAPK cascade', 'Cytoskeleton organization', 'FoxO signaling' pathway, 'mTOR signaling' pathway and 'Focal adhesion'. To determine the effect of ectopic expression of miR-23b-3p on C-33A and CaSki cell migration and invasion, wound closure assays were performed. In miR-23b-3p-treated C-33A cells the percentage of wound closure is markedly reduced compared with in Scrambled-treated cells at 36 and 48 h (*P<0.05 and **P<0.001, respectively Fig. 8A). Increased expression of miR-23b-3p decreased the percentage of wound closure in CaSki cells at 24 h compared with the control group (Scrambled-treated cells; *P<0.05, Fig. 8B). This trend is maintained ≤48 h post-transfection but the differences are not significant. Transwell assays demonstrated that increased expression of miR-23b-3p reduces C-33A and

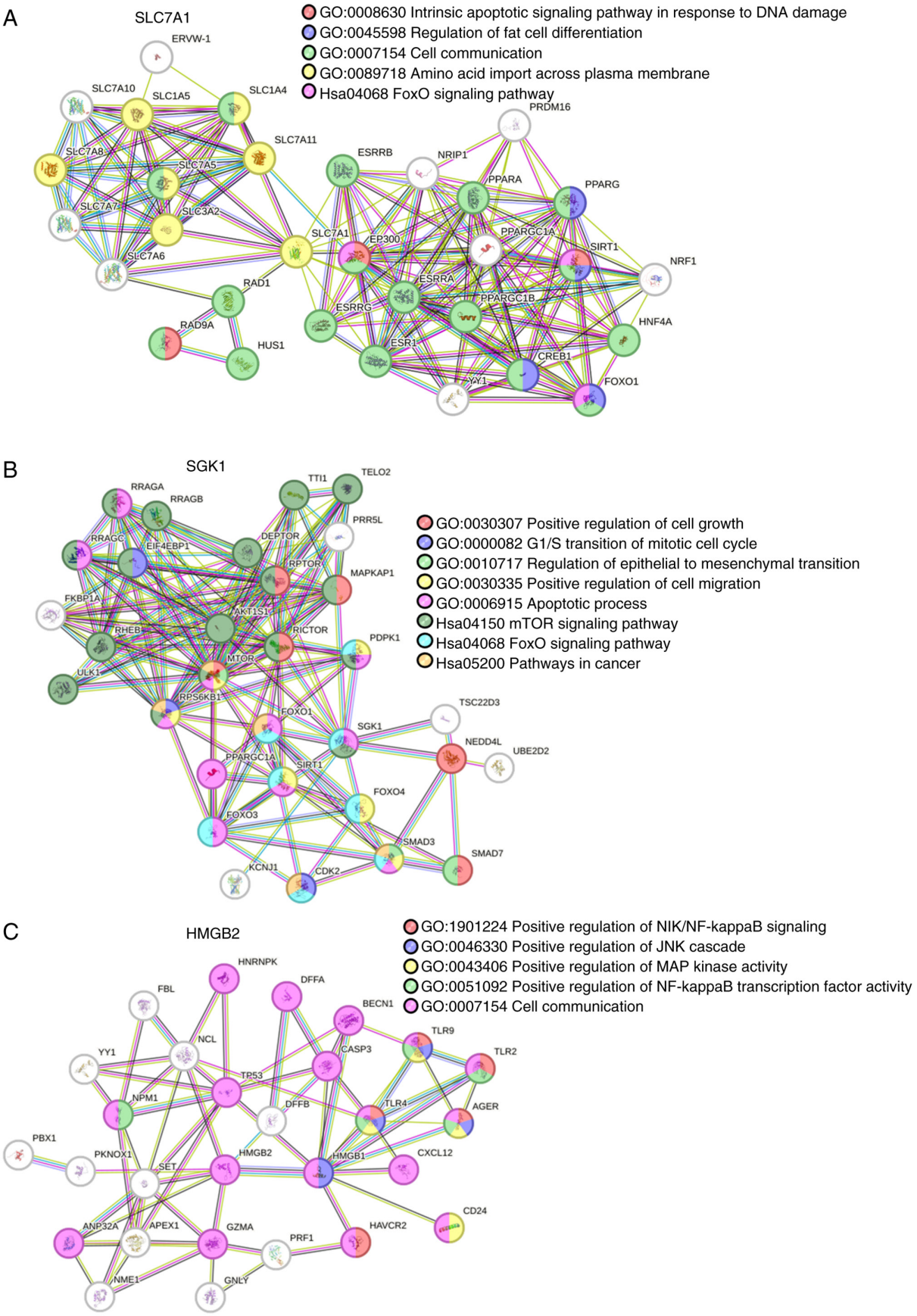


Figure 3. SLC7A1, SGK1 and HMGB2 are involved in the regulation of biological processes and signaling pathways involved in cancer progression. The PPI network represents physical and functional interactions of (A) SLC7A1, (B) SGK1 or (C) HMGB2 with cellular proteins. Each node represents a protein and the lines represent protein-protein interactions. The colors represent the biological processes and signaling pathways in which the interacting proteins participate. Blank nodes symbolize proteins that are not involved in the aforementioned processes or pathways. All interactions were statistically significant ($P<5.5x10^{-7}$). SLC7A1, cationic amino acid transporter 1; SGK1, serum- and glucocorticoid-induced kinase 1; HMGB2, mobility group box 2 protein; PPI, protein-protein interaction.

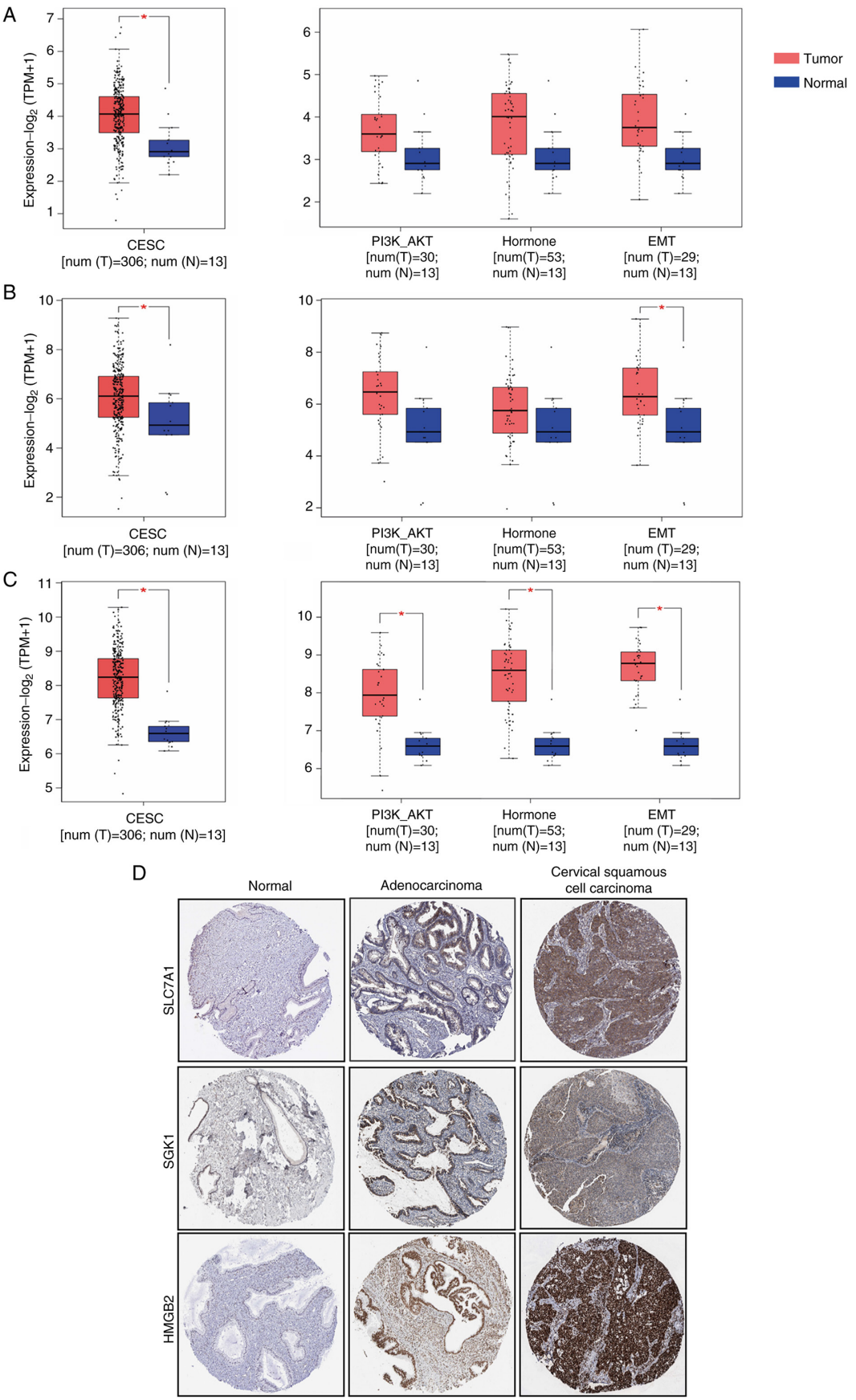


Figure 4. Expression of SLC7A1, SGK1 and HMGB2 in tissues with CESC or adenocarcinoma. (A) SLC7A1, (B) SGK1 and (C) HMGB2 mRNA levels in tissues with CESC (red; n=306) and normal (blue; n=13) recorded on the GEPIA platform (P<0.0.5. (D) Expression of SLC7A1, SGK1 and HMGB2 proteins in HPA-included tissues. Images of histological sections of normal tissue biopsies, adenocarcinoma and CESC stained by immunohistochemistry are shown. Staining intensity was classified as negative/weak, moderate or strong, magnification, x100. *P<0.05. SLC7A1, cationic amino acid transporter 1; SGK1, serum- and glucocorticoid-induced kinase 1; HMGB2, mobility group box 2 protein; CESC, cervical squamous cell carcinoma; HPA, Human Protein Atlas; EMT, epithelial-mesenchymal transition.

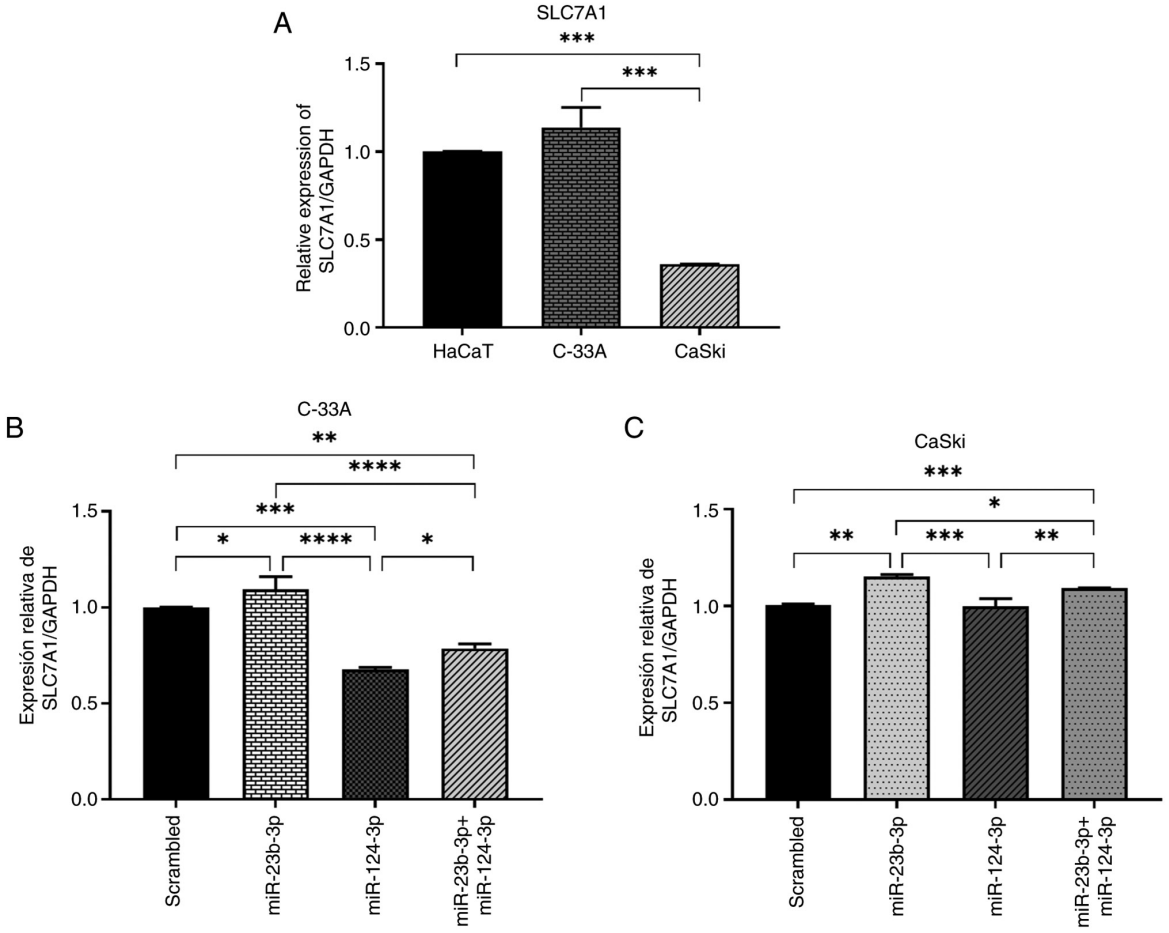


Figure 5. Relative expression of *SLC7A1* in response to overexpression of miR-124-3p, miR-23b-3p or miR-124-3p + miR-23b-3p. *SLC7A1* expression levels in (A) HaCaT, C-33A and CaSki cells without transfection; (B) C-33A and (C) CaSki cells transfected with 100 nM of miR-124-3p, miR-23b-3p or miR-124-3p + miR-23b-3p mimetic for 24 h. *SLC7A1* mRNA expression was normalized to GAPDH mRNA and calculated by the $2^{-\Delta\Delta Cq}$ method. Data are presented as mean ± SD. Statistical significance was determined by Welch's ANOVA (Games-Howell test) *P<0.05; **P<0.001; ***P<0.0001; ****P<0.00001. SLC7A1, cationic amino acid transporter 1; miR, microRNA.

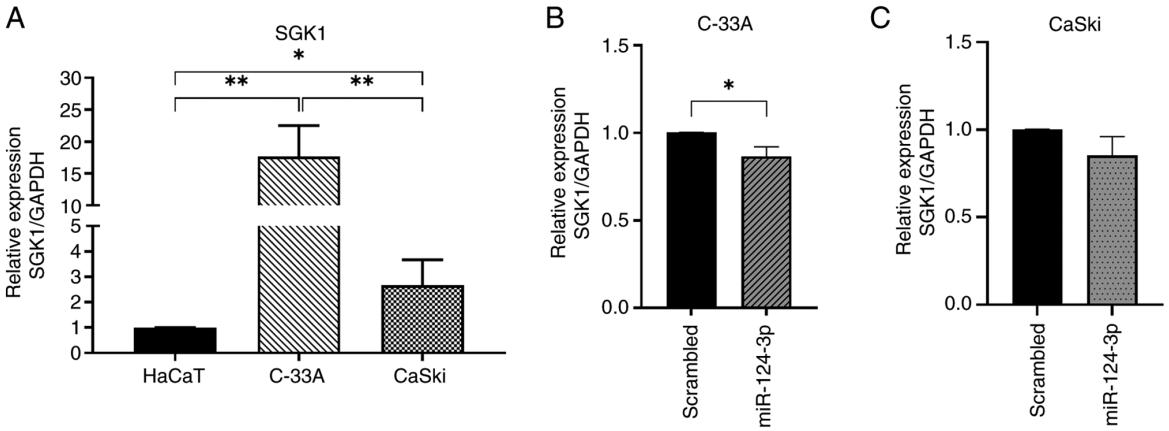


Figure 6. Relative expression of SGK1 mRNA. SGK1 mRNA levels in (A) untransfected C-33A and CaSki cells compared with HaCaT cells and in (B) C-33A and (C) CaSki cells transfected with 100 nM of miR-124-3p mimetic for 24 h. SGK1 mRNA expression was normalized to GAPDH mRNA and calculated by the $2^{-\Delta\Delta Cq}$ method. Data are presented as mean ± SD. Statistical significance was determined using unpaired Student's t-test or Welch's ANOVA (Games-Howell test). *P<0.05; **P<0.001. SGK1, serum- and glucocorticoid-induced kinase 1 miR, microRNA.

CaSki cell invasion of CC (*P<0.05 and *P<0.05, respectively, Fig. 8C and D).

To explore the mechanisms by which miR-23b-3p regulates migration and invasion of C-33A and CaSki cells and based on the fact that HMGB2 mRNA contains two MRE sites for that miRNA, the effect of HMGB2 knockdown on migration and invasion of C-33A and CaSki cells was investigated. HMGB2 mRNA expression was decreased in C-33A and CaSki cells

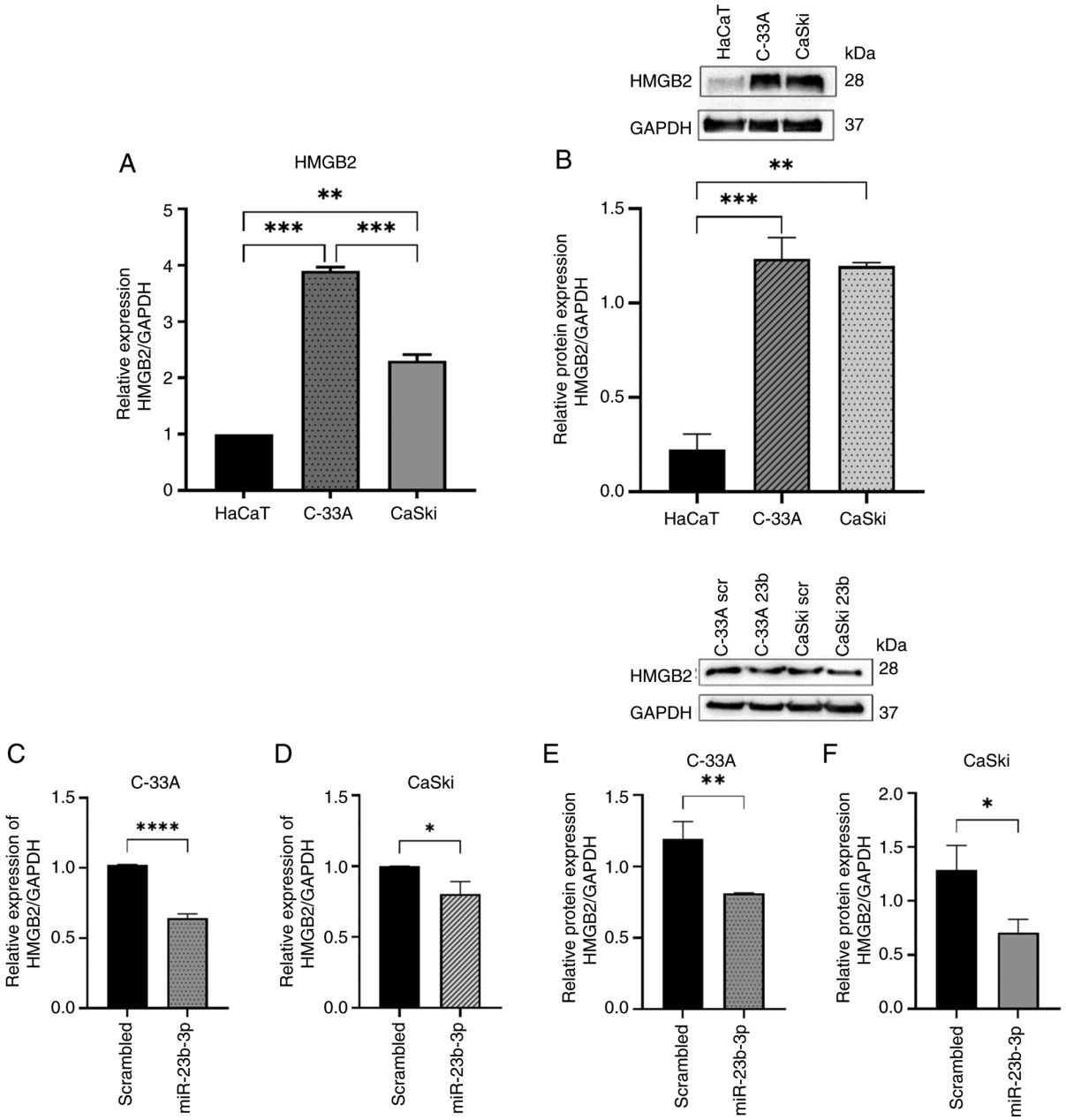


Figure 7. HMGB2 mRNA and protein expression in C-33A and CaSki cells with miR-23b-3p overexpression. (A) mRNA and (B) protein expression levels of HMGB2 in HaCaT, C-33A and CaSki cells. mRNA expression in (C) C-33A and (D) CaSki cells transfected with 100 nM of the miR-23b-3p mimic. Protein expression in (E) C-33A and (F) CaSki cells transfected with 100 nM of the miR-23b-3p mimic. mRNA and protein expression levels of HMGB2 were normalized with GAPDH and calculated by the $2^{-\Delta\Delta Ct}$ method. Data are presented as mean ± SD. Statistical significance was determined using Student's t-test unpaired or Welch's ANOVA (Games-Howell test) *P<0.05; **P<0.001; ***P<0.0001; ****P<0.00001. HMGB2, mobility group box 2 protein; miR, microRNA.

transfected with HMGB2 knockdown (***P<0.0001 and *P<0.05, respectively Fig. 8E and F). In C-33A and CaSki cells transfected with si-HMGB2 migration decreases starting at 12 h and the effect is maintained ≤48 h compared with the control (Fig. 8G and H). The results of the Transwell assay indicate that si-HMGB2 decreases the invasion of C-33A and CaSki cells compared with scrambled (**P<0.001 and **P<0.001, respectively Fig. 8I and J).

Discussion

Previous studies have revealed that tumor suppressor miRNAs promote changes in the expression of genes that regulate processes associated with the maintenance and progression of CC (4,39). miR-124-3p and miR-23b-3p function as tumor suppressors in cervical carcinoma and modulate the expression of numerous genes (10,11,19,40). miR-124-3p has been proposed as a prognostic biomarker in gastric cancer (41), CC (10), prostate cancer (14) and breast cancer (42). Conversely, miR-23b-3p has been suggested as a prognostic biomarker in ovarian cancer (43), colon cancer (44) and CC (11). In the present study, the effect of exogenous overexpression of miR-23b-3p and miR-124-3p on the expression of SGK1, SLC7A1 and HMGB2 was analyzed, although bioinformatics analysis indicated that SGK1 is a target gene of miR-124-3p and SLC7A1 is a shared target of miR-23b-3p

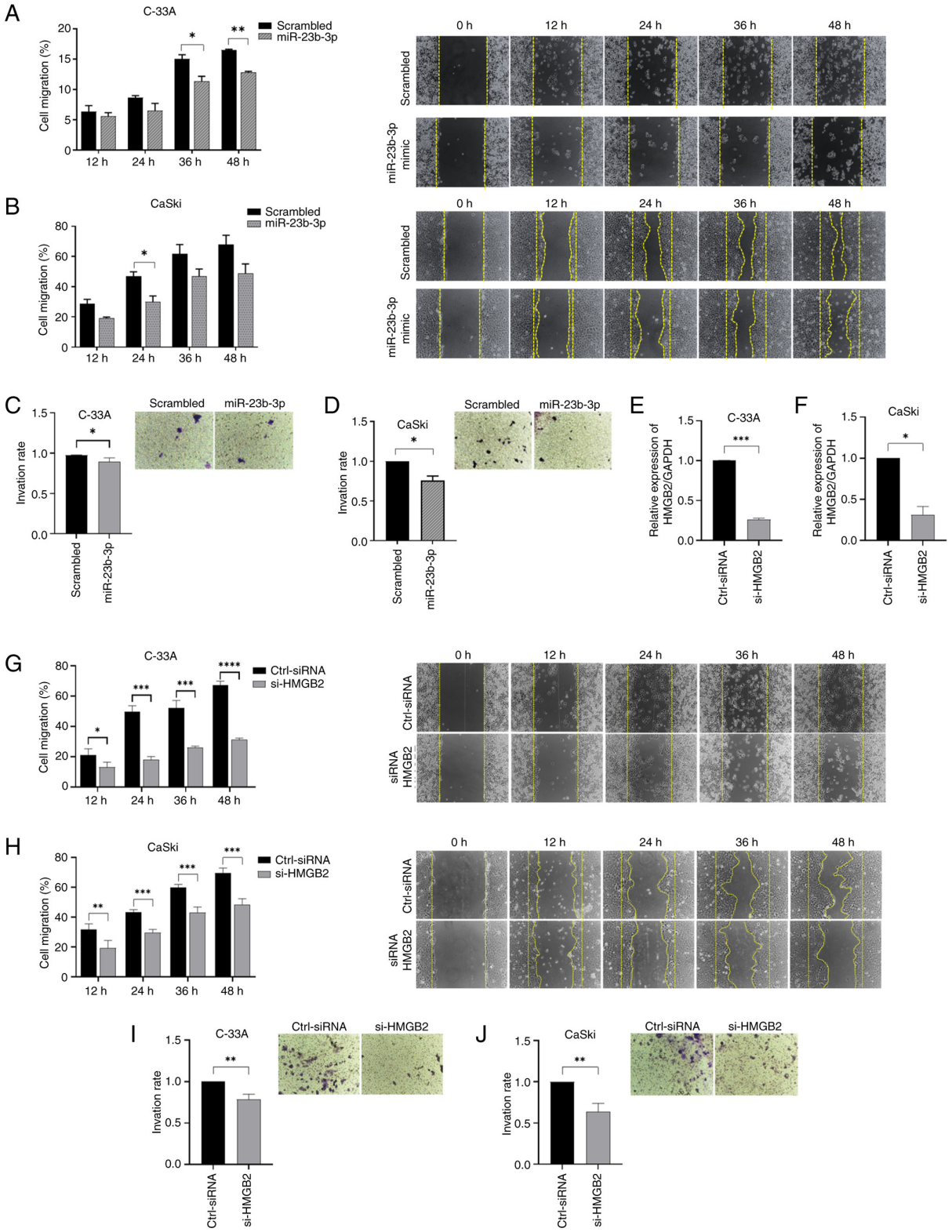


Figure 8. Overexpression of miR-23b-3p and HMGB2 knockdown decreases migration and invasion of C-33A and CaSki cells. Migration of (A) C-33A and (B) CaSki cells overexpressing miR-23b-3p. Invasion of (C) C-33A and (D) CaSki cells overexpressing miR-23b-3p. HMGB2 expression in (E) C-33A and (F) CaSki cells transfected with 80 nM si-HMGB2. Migration of (G) C-33A and (H) CaSki cells with HMGB2 knockdown. Invasion of (I) C-33A and (J) CaSki cells transfected with si-HMGB2. Migration data are expressed as percent wound closure ± SD in three independent experiments. Migration and invasion results are shown at magnification, x20. *P<0.05, **P<0.001, ***P<0.0001, ****P<0.00001. miR, microRNA; HMGB2, mobility group box 2 protein; si, small interfering; ctrl, control.

and miR-124-3p, experimental analysis revealed that increased levels of miRNAs did not alter SGK1 and SLC7A1 mRNA expression. By contrast, increased miR-23b-3p was associated with decreased HMGB2 expression in C-33A and CaSki cells. Additionally, the knockdown of HMGB2 reduced migration and invasion in both cell lines.

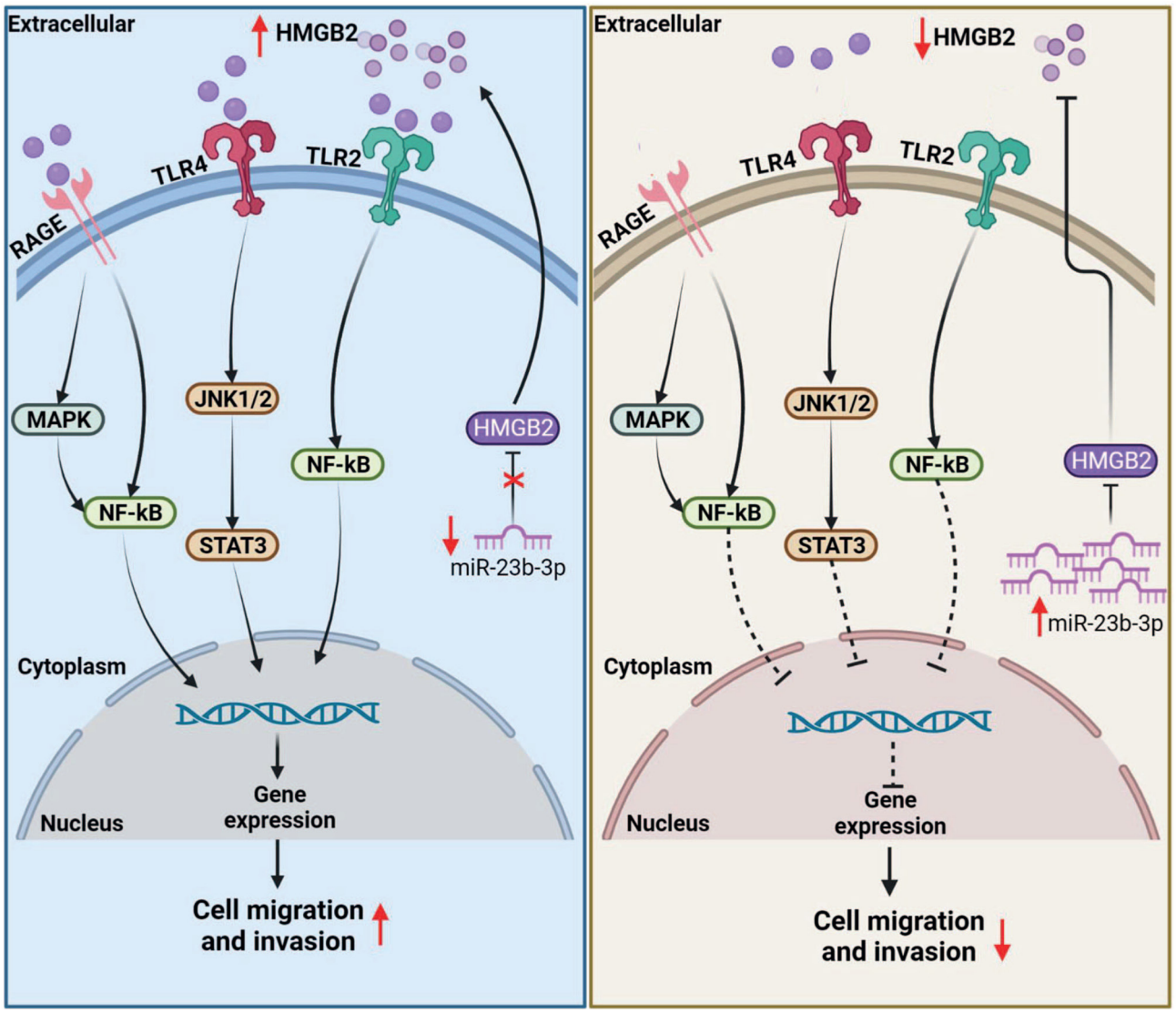


Figure 9. Predicted mechanism of regulation of cell migration and invasion through the miR-23b-3p/HMGB2 axis. HMGB2, high mobility group box 2; RAGE, receptor for advanced glycation endproducts; TLR, toll like receptor; miR, microRNA.

The results of the bioinformatics analysis performed in the present study indicated that among the 1,884 predicted target genes of miR-124-3p and miR-23b-3p there are specific and shared targets involved in biological processes and pathways associated with cancer, including 'Angiogenesis', 'Regulation of cell proliferation', 'Apoptotic process', 'Pathways in cancer' and the mTOR, FoxO, MAPK and PI3K-Akt signaling pathways that regulate tumor cell migration and invasion (32,45,46). These data suggest that miR-124-3p and miR-23b-3p have a cooperative effect in modulating cellular processes involved in tumor progression through post-transcriptional regulation of specific and shared targets.

The present study revealed that SLC7A1 i) is one of the 136 shared targets of miR-124-3p and miR-23-3p predicted; ii) participates in amino acid transport (GO) and amino acid import across the plasma membrane (GO; PPI network); iii) contains a 7mer-m8 site for miR-124-3p and an 8mer site for miR-23b-3p; iv) interacts with proteins involved in cell communication (PPI network); v) SLC7A1 mRNA and protein are markedly increased in CESC compared with non-cancer tissue, but the transcript increase is not significant in tissues with PI3k/AKT activation, hormonal alterations or EMT; vi) SLC7A1 mRNA is markedly increased in C-33A cells compared with HaCaT, but is decreased in CaSki; vii) overexpression of miR-124-3p and miR-124-3p + miR-23b-3p is associated with decreased SLC7A1 mRNA levels in C-33A cells but not in CaSki; and viii) overexpression of miR-23b-3p induces an increase in SLC7A1 mRNA in CaSki cells and does not modify its level in C-33A cells. Increased SLC7A1 expression is associated with worse prognosis in patients with CC (47); a relationship that may be linked to the role of SLC7A1 in modulating the mTORC1 signaling pathway, which regulates cell proliferation, migration and invasion in cancer (48). The PI3K/Akt/mTOR pathway has been revealed to be altered in different types of human cancer and to be involved in tumor progression (49). The significant upregulation of SLC7A1 mRNA and protein in CESC suggests that it performed a significant role in this type of cancer that remains to be revealed. Wu *et al* (47) revealed that SLC7A1 is one of

the 5 genes that integrate a prognostic model linked to inflammatory response in CC and that high levels of SLC7A1 mRNA and protein are associated with worse prognosis in patients with this type of cancer. In Huh7 cells, increased SLC7A1 is associated with resistance to chemotherapy (23). These findings highlight the importance of further investigating the role of SLC7A1 in hepatocellular carcinoma progression and response to chemotherapy.

Under basal conditions, the mRNA levels of SLC7A1 are increased in C-33A cells and decreased in CaSki compared with HaCaT cells. There are no reports regarding SLC7A1 expression in CC cell lines, but low levels of SLC7A11, a member of the SLC7 family, have been revealed in HPV-positive head and neck squamous cell carcinoma (HNSCC) samples (50). It is likely that in CaSki cells HPV-16 is dysregulating SLC7A1 expression, but little is known about the molecular mechanisms leading to this dysregulation. Conversely, analysis of *in vitro* data corroborate findings from tissues from patients with CESC, because, although SLC7A1 expression is markedly increased, there is large variation in mRNA levels and, in some cases, lower than that found in normal tissue. These variations may be influenced by factors such as the time of evolution and stage of CC, the genetic background and age of the patients, the type and variants of HPV in the samples. Additionally, it has been reported that in high-grade serous ovarian cancer biopsies, SLC7A1 is overexpressed in the stroma, specifically in fibroblasts (51). Therefore, future work should investigate the variables that modify SLC7A1 expression in CC, including the effect of HPV genotype, origin and function of the protein in the tumor microenvironment. In C-33A cells, overexpression of miR-124-3p and miR-124-3p + miR-23b-3p is associated with decreased SLC7A1 mRNA, however, this effect is not reproduced in CaSki cells. These results may be due to the specific characteristics of each cell line, C-33A was isolated from a primary tumor site and CaSki was derived from a metastatic site, moreover while C-33A is HPV-negative, CaSki cells contain HPV-16 DNA integrated into their genome. Despite predictions, the mRNA level of SLC7A1 is only associated with the overexpression of miR-124-3p in both cell lines and is not modified by the increase of miR-23b-3p.

These results suggested that miR-23b-3p has no effect on the posttranscriptional regulation of SLC7A1 even if its 3'UTR region contains an MRE for that miRNA. In this regard it is known that an mRNA can be regulated by multiple miRNAs and that the hybridization between a miRNA and its target mRNA depends on the distance between the MREs; on the possibility of physical and functional interaction of the effector complexes and on the spatial arrangement of the transcript molecule (52). Although SLC7A1 does not appear to be directly regulated by miR-23b-3p, further studies are needed.

Investigations into SGK1 revealed that SGK1 i) is a predicted target of miR-124-3p; ii) is involved in 'Protein phosphorylation', 'Apoptotic process', 'Regulation of cell population proliferation' and 'Regulation of cell migration' (GO) and mTOR, FOXO, and PI3K/Akt signaling pathways (KEGG; PPI network); iii) contains an MRE for miR-124-3p; iv) SGK1 mRNA and protein are markedly increased in CESC and CC tissues with EMT compared with non-cancer tissue, v) under basal conditions, SGK1 mRNA is increased in C-33A and CaSki cells compared with HaCaT, and vi) overexpression of miR-124-3p is associated with decreased SGK1 mRNA levels in both cell lines. The predictions of GO, KEGG and PPI network are in agreement with the results of other studies in different cancer types (24-27). In CC, E6 of HPV-16 promotes activation of the PI3K pathway that induces SGK1 expression in HPV-16-positive CaSki cells (53). In patients with alterations in the PI3K-AKT pathway, SGK1 expression increases and the PI3K/AKT/mTOR pathway modulates proliferation, cell growth and migration, important processes in malignant progression (54). The significant increase of SGK1 mRNA in data from patients with CESC and CESC with alterations in EMT deposited in TCGA suggests that this protein is associated with the progression of CC. These findings are in agreement with that reported in patients with lung adenocarcinoma, in whom markedly increased levels of SGK1 were revealed in tissues with mesenchymal gene signature (55). In exosomes of HPV-positive and negative HNSCC cells, SGK1 mRNA was revealed to be among the abundant transcripts and SGK1 overexpression is associated with poor survival of patients with HNSCC (56). These data support the importance of studying the role of SGK1 in CC.

Overexpression of miR-124-3p is associated with <0.5-fold decrease in SGK1 mRNA in C-33A and CaSki cells. It is likely that the recognition site for miRNA is poorly accessible in SGK1 mRNA and that miR-124-3p has hybridized to other targets with more accessible or higher affinity sites. In CC cell lines, luciferase assays revealed that SIRT1, GRB2, AEG-1 and STAT3 are targets of miR-124-3p (10,40,57,58). Moreover, in an L02 liver cell regeneration model, miR-124-3p was revealed to negatively regulate SGK1 expression levels (59).

The present study revealed that HMGB2 i) is one of 785 predicted targets for miR-23b-3p and contains three MRE sites for that miRNA; ii) positively regulates transcription, proliferation, cell communication and NIK/NF-kB, JNK cascade and MAPK (GO and PPI network) pathways; iii) mRNA and protein levels are markedly increased in tissues from CESC patients, CESC patients with PI3K/Akt pathway activation, EMT or with hormonal alterations; iv) mRNA levels are 1-3 times higher and protein levels are 5-6 times higher in CaSki and C-33A cells, respectively, compared with HaCaT; v) mRNA and protein levels are decreased in response to overexpression of miR-23b-3p; and vi) knockdown and miR-23b-3p overexpression decrease C-33A and CaSki cell migration. Target prediction, GO and PPI network analysis are in agreement with previous experimental results. HMGB2 is a DNA-binding protein that regulates DNA transcription, replication and repair (29), and in HeLa cells increased HMGB2 expression promotes proliferation through the AKT signaling pathway (30). The MAPK pathway regulates migration, invasion, EMT and metastasis, thus favoring tumor progression (30,60,61). In gastric and pancreatic cancer cell lines, luciferase assay analysis revealed that HMGB2 targets miR-23b-3p (35,62). The present study demonstrated that, miR-23b-3p decreases HMGB2 expression in C-33A and CaSki cells and reduces migration and invasion in both cell lines. Analysis of si-HMGB2 transfection data demonstrated that HMGB2 mediates the effect of miR-23b-3p on migration and invasion of CaSki and C-33A cells. This result is in agreement with the high level of HMGB2 in patients with CESC and altered EMT and with that reported in pancreatic cancer, where

the kinectin 1 antisense RNA 1/miR-23b-3p/HMGB2 axis was revealed to promote tumor progression (62). In renal tumor ACHN cells, reduced HMGB2 expression decreases migration and invasion (63). This indicates that miR-23b-3p has a prominent role in promoting CC progression through the regulation of several target genes, including HMGB2 which fulfills its oncogenic role by modulating migration and invasion.

Although the increase in the expression levels of SLC7A1 and SGK1 are not significant in CC tissues, it is evident that the average value is increased in cancer-free tissues, and the expression of SGK1 is markedly increased in tumor biopsies with evidence of EMT. By contrast, the increased level of HMGB2 remains significant in patients with evidence of PI3K/AKT pathway activation, hormonal alterations and EMT. Previous studies have revealed that EMT, hormonal alterations or activation of the PI3K-AKT signaling pathway are associated with cancer progression, decreased patient survival and response to treatment (31,32,64,65). EMT is the first step in tumor progression because it marks the beginning of migration, invasion and cancer metastasis (64), determining the prognosis for patients with CC (66,67). Hormonal signaling favors the development of hormone-sensitive neoplasias (65). Although the human cervical epithelium is known to be hormone-sensitive, little is known about the function of estrogen receptor α (Erα) and progesterone receptor in CC (65), but estrogen is known to be involved in carcinogenesis. In CC cells, Erα is frequently deficient, while its variant Erα-36 is overexpressed. ERα and ERα-36 have antagonistic functions in CC. CC cell lines and tissues express ERα-36, and the estrogen-Erα-36 interaction was revealed to activate the MAPK/ERK signaling pathway that promotes the proliferation, migration and invasion of HeLa and CaSki cells (68). Furthermore, in HeLa and CaSki cells, the estrogen-Erα-36 interaction was revealed to induce an increase in HPV E6/E7 expression (69). Meanwhile, the PI3K/AKT signaling pathway positively regulates migration, invasion and proliferation, thereby contributing to CC progression (31,32). Thus, patients with evidence of EMT, hormonal alterations and/or PI3K/AKT pathway activation with high levels of SLC7A1, SGK1 and HMGB2 mRNA may be at greater risk of metastasis.

For the analysis of SLC7A1, SGK1 and HMGB2 expression in tissues from patients with CESC, data from TCGA were used. This repository did not contain data on factors influencing CC progression, such as HPV infection, viral genotype(s) present in cancer tissues and disease progression time. These factors may be influencing the high variability in the mRNA expression data. Conversely, SLC7A1 and SGK1 were predicted to be targets of miR-124-3p and/or miR-23b-3p; however, the expression results in C-33A and CaSki cells disagree with this prediction. SLC7A1, with MREs for both miRNAs, was only downregulated in C-33A cells overexpressing miR-124-3p or both miRNAs, but in CaSki cells the mRNA level increased when miR-23b-3p was overexpressed and did not change in response to miR-124-3p or to both miRNAs. SGK1 mRNA with MREs for miR-124-3p, was markedly downregulated in C-33A cells overexpressing the miRNA but not in CaSki cells. To resolve the discrepancies between prediction and experimental results, it was necessary to confirm the miRNA-mRNA interaction and to perform HPV 16 E6/E7 silencing assays to verify whether the oncoproteins interfered with hybridization. Additionally, considering that CC types are diverse, and that tumor stage influences outcomes, the level of SLC7A1 and SGK1 expression should be confirmed in other cell lines and tissues with different histological types of CC.

In conclusion, miR-124-3p and miR-23b-3p target mRNA prediction, GO analysis, KEGG, PPI network and published data indicated that these miRNAs regulate specific and shared targets with functions that promote CC progression. The expression data of SLC7A1, SGK1 and HMGB2 in patient tissue were in agreement with computational predictions and suggested that these proteins have notable functions in CC and the differential upregulation of SGK1 and HMGB2 in EMT-disrupted CESC supported the involvement of these genes in cancer progression. The abundance of miR-23b-3p-specific targets in CC may explain the contradiction between experimental results pointing to SLC7A1 being regulated by miR-124-3p, but not miR-23b-3p, while computational prediction indicated that it is a shared target. Although miR-124-3p does not appear to have a pronounced effect on SGK1 regulation in C-33A and CaSki cells the existing data supported the importance of further study of this protein in CC. miR-23b-3p decreases cell migration and invasion through HMGB2 (Fig. 9). These results underlined the importance of investigating the expression and function of SLC7A1, SGK1 and HMGB2 in patients with different histological types of CC with and without HPV and with different viral types of high oncogenic risk, complemented by confirmatory experiments *in vitro* and in animal models. The results of this research supported the hypothesis that miR-124-3p and miR-23b-3p levels may be useful for differentiating invasive cancer *in situ*, estimating the risk of metastasis, assessing patient prognosis and monitoring recurrence. Restoring miR-124-3p and miR-23b-3p levels may represent a useful therapeutic strategy for enhancing response to conventional treatments.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Conceptualization was performed by GWVN, HJW and GFT. Methodology was performed by GWVN, MAMC, OPZ, CPP and GFT. Validation was performed by HJW, MAMC, OPZ, CPP, DHS and GFT. Formal analysis was performed by GWVN, HJW, DHS and MAMC. The study was designed by GWVN,

JAM, MJRL, HJW, DHS and GFT. Resources were provided by JAM, MJRL, CPP and GFT. Writing of the original draft was performed by GWVN, HJW, MAMC, CPP and GFT. Review and editing was performed by HJW, MAMC, DHS, OPZ and GFT. Visualization was performed by JAM, MJRL, HJW and DHS. Supervision was performed by HJW, MAMC and GFT. project administration was performed by JAM, HJW and GFT. Funding was acquired by HJW and GFT. GWVN and GFT confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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