Received: 10 September 2019 | Revised: 8 January 2020 | Accepted: 14 January 2020

DOI: 10.1002/cbf.3506

RESEARCH ARTICLE

CELL BIOCHEMISTRY & FUNCTION WILEY

DGUOK-AS1 promotes cell proliferation in cervical cancer via acting as a ceRNA of miR-653-5p

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Funding information
Key Laboratory of High-Incidence Tumor Prevention and Treatment, Guangxi Medical University, Grant/Award Number: GKE2018-10

Cervical cancer (CC) holds the second highest incidence and is the fourth dominating cause of cancer-induced death in women. It has been widely accepted that long noncoding RNAs (lncRNAs) are implicated in pathological and physiological activities of CC. However, the research of lncRNAs is still in the initial stage. The biological function of lncRNA deoxyguanosine kinase antisense RNA 1 (DGUOK-AS1) in human cancers has not been reported yet. We found that DGUOK-AS1 was aberrantly upregulated in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) tissues through TCGA database. Real-time quantitative polymerase chain reaction (RT-qPCR) also verified the high expression of DGUOK-AS1 in CC cell lines. Loss-of-function assays indicated that DGUOK-AS1 silence repressed CC cell growth. In addition, dual-luciferase reporter and RNA immunoprecipitation (RIP) experiments validated the binding relation between miR-653-5p and DGUOK-AS1 or EMSY. Results of the rescue assays elucidated that EMSY overexpression or miR-653-5p downregulation reversed the suppressive function of DGUOK-AS1 knockdown on cell growth and DNA repair in CC. To sum up, this research highlighted that DGUOK-AS1 could promote CC cell proliferation via serving as a ceRNA of miR-653-5p to release EMSY, which might inspire us to discover novel strategies for CC treatment.

Significance of the study: DGUOK-AS1 knockdown hinders proliferation of CC cells. DGUOK-AS1 sequesters miR-653-5p to elevate EMSY in CC. EMSY is required for DGUOK-AS1 to induce cell proliferation and repress DNA damage in CC.

KEYWORDS
cancer, cervical cancer, DGUOK-AS1, EMSY, miR-653-5p

1 | INTRODUCTION

Despite a continuing decline of morbidity and mortality rate in recent years, cervical cancer (CC) seriously threatens women's health worldwide. CC patients in the early stages may not present observable symptoms, leading to very limited diagnosis.[1] Therefore, revealing new biologic markers of CC remains an urgent task for us.

Long noncoding RNAs (lncRNAs) belong to a group of transcripts without protein-code ability and possess over 200 nucleotides in length.[2] It has been extensively researched that lncRNAs are involved in initiation and development of various human cancers, CC included. For example, lncRNA LBCS suppresses stem cell self-renewal and chemoresistance in bladder cancer.[3] LncRNA AFAP1-AS1 contributes to promotion of cell migration in nonsmall cell lung cancer.[4] LncRNA MIR31HG facilitates cell proliferation and tumourigenesis in head and neck cancer via targeting HIF1A and P21.[5] LncRNA TUG1 promotes CC progression.[6] Further, lncRNA deoxyguanosine kinase antisense RNA 1 (DGUOK-AS1) is located in human chromosome 2p13.1. The biological function of DGUOK-AS1 in human diseases has never been unmasked till now.

Accumulative evidence has demonstrated that lncRNAs could play the role of competing endogenous RNAs (ceRNAs) to modulate downstream genes in an indirect way.[7] For instance, lncRNA PTAR accelerates cell invasion, metastasis, and epithelial-mesenchymal transition (EMT) in serous ovarian cancer via sequestering miR-101-3p to

release ZEB1 expression.[8] LncRNA PFAR facilitates the activation and fibrosis of lung fibroblast through sponging miR-138 to affect the YAP1/Twist axis.[9] LncRNA DLX6-AS1 promotes proliferation of CC cells via acting as a ceRNA to target miR-199a.[10]

The function of DGUOK-AS1 in CC proliferation was addressed here in this study. Additionally, the molecular mechanism underlying DGUOK-AS1 in CC was testified.

2 | MATERIALS AND METHODS

2.1 | Tissue samples

One hundred tissues comprising 50 CC tissues and 50 adjacent nontumour tissues were provided by Guangxi Medical University Cancer Hospital. All tissues were collected during surgical resection of patients diagnosed with primary CC at Guangxi Medical University Cancer Hospital. Adjacent nontumour tissues were acquired from region at least 6 cm away from the CC tumour site. All of the patients did not receive radiation or chemotherapy treatment before receiving surgeries. Tissues were kept in liquid nitrogen.

Data on selected clinical and related parameters including age (<45 and ≥45 years), tumour size (<4 and ≥4 cm), FIGO stage (I-II and III-IV), stromal metastasis (yes and no), and lymph node metastasis (yes and no) are presented in Table 1.

This research got approval from the Ethical Committee of the Guangxi Medical University Cancer Hospital. All study participants provided written informed consent.

2.2 | Cell culture

One normal cervical epithelial cell line H8 and four human CC cell lines HeLa, SiHa, CaSki, and C33A (ATCC, VA, USA) were incubated in Dulbecco's modified Eagle's medium (DMEM) added by 10% fetal bovine serum (FBS) (Invitrogen, MA, USA) in a moist environment of 5% carbon dioxide at 37°C.

2.3 | Cell transfection

Three short hairpin RNAs (shRNAs) targeting DGUOK-AS1 (sh-DGUOK-AS1-1/2/3), another shRNA against EMSY (sh-EMASY), and the corresponding scrambles were all provided by GenePharma (Shanghai, China). For DGUOK-AS1 or EMSY overexpression, full sequences of DGUOK-AS1 or EMSY were cloned into pcDNA3.1 plasmids provided by GeneCopoecia (Guangzhou, China). For miR-653-5p interference or overexpression, miR-653-5p mimics, NC mimics, miR-653-5p inhibitor, and NC inhibitor were manufactured by GenePharma. Transfection was performed with utilization of Lipofectamine 2000 (Invitrogen). And the sequences of above plasmids were presented in Table 2.

2.4 | Real-time quantitative polymerase chain reaction

Total RNA was extracted with TRIzol reagent (Intvitrogen) for first-strand cDNA synthesis using M-MLV Reverse Transcriptase XL (Promega, WI, USA). The cDNA was utilized for polymerase chain reaction (PCR) with the help of SYBRGreen PCR Master Mix (Applied Biosystems, MA, USA). The expression levels were calculated normalized to GAPDH or U6. The primers used were as follows: DGUOK-AS1, forward 5′-TGCTCCCAGAACTCTAACCC-3′ and reverse 5′-CACCCACTCTTGAG CCACTT-3′; miR-653-5p, forward 5′-TTGAAACATTCTCTACTGAAC-3′ and reverse 5′-GAACATGTCTGCGTATCTC-3′; miR-499a-5p, forward 5′-ATGTAG CGTGCGACCG-3′ and reverse 5′-CAGGCTGACGCACTC TGTGCT-3′; EMSY, forward 5′-TCAGATGACCCAGGAAAAGAG-3′ and reverse 5′-CTCTGTCCCCTCATCAGTGC-3′; PPP1CC, forward 5′-GTTG GTCACTCTGTTTTCTGCGC-3′ and reverse 5′-GCGTTACAGGTCTCG TGGCATT-3′; GAPDH, forward 5′-TATGATGATATCAAGAGGGTAGT-3′ and reverse 5′-TGTATCCAAACTCATTGTCATAC-3′; and U6, forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTCA CGAATTTGCGTGTCAT-3′.

2.5 | Western blot

RIPA lysis buffer (Thermo Fisher Scientific, MA, USA) was applied to extract total proteins, which were subjected to SDS-polyacrylamide gel electrophoresis. Afterwards, the proteins were preserved on skim milk–blocked Hybond ECL membranes (GE Healthcare Life Sciences, Shanghai, China) and cultured with antibodies of EMSY (ab123, Abcam, MA, USA) and GAPDH (ab9485, Abcam) overnight at 4°C. The membranes washed

TABLE 1 Relationship between DGUOK-AS1 expression and clinical features in cervical cancer patients (n = 50)

	DGUOK-AS1 Expression		
Clinical Features	Low (n = 25)	High (n = 25)	*P* Value
Age, y			
<45	16	19	.538
≥45	9	6	
Tumour size, cm			
<4	18	7	.004*
>4	7	18	
FIGO stage			
I-II	15	4	.003*
III-IV	10	21	
Stromal metastasis			
No	4	6	.725
Yes	21	19	
Lymph node metastasis			
No	13	15	.776
Yes	12	10	

*$P < .05$.

TABLE 2 Interference or overexpression sequences

sh-NC	CCGCGGGAATTTCAAAATGCTTTACCTCGAGGTAAAGCATTTTGAAATTCCCTTTTTG
sh-DGUOK-AS1-1	CCGCGTAGGAAAGCCTCCTTATTTCCTCGAGGAAATAAGGAGGCTTTCCTACTTTTTG
sh-DGUOK-AS1-2	CCGCGGAGATTTCCCTTTCTAGTCCCTCGAGGGACTAGAAAGGGAAATCTCCTTTTTG
sh-DGUOK-AS1-3	CCGCCAGTTTCTTTGAAAAGTCATGCTCGAGCATGACTTTTCAAAGAAACTGTTTTTG
pcDNA3.1/DGUOK-AS1	GCGACTGCTCCCGCGAGGTCAGAGCCGGGCTGTGCCGCTGCCCCCGGGACTAGAAAGGGAAATCTCCTTG
NC mimics	GACAACUUACAAUCUCUACUG
miR-653-5p mimics	GUGUUGAAACAAUCUCUACUG
NC inhibitor	CAGUAGAGAUUGUAAGUUGUC
sh-NC	CCGCCATTTTAAAATCAGATAATTCCTCGAGGAATTATCTGATTTTAAAATGTTTTTG
sh-EMSY#1	CCGCGTCAGCTAAACAGCAGAAACTCTCGAGCAGTAGAGATTGTAAGTTGTCTTTTTG
sh-EMSY#2	CCGCGCTTCTTCAGAGAAACAGACGCTCGAGCGTCTGTTTCTCTGAAGAAGCCTTTTTG
pcDNA3.1/EMSY	ATGCCTGTTGTGTGGCCAACCCTTTTGGATCTCAGCAGGGATGAATGCAAACGGATTCTTCGAAAATTGGAACTGGAGGCATATGCTGG AGTTATCAGTGCACTTCGGGCACAAGGGGACCTCACCAAGGAAAAGAAAGATCTTCTTGGAGAGCTCTCAAAAGTCCTTAGCATCT CAACAGAGCGCCACCGTGCTGAAGTTCGGAGAGCAGTAAACGATGAACGGTTAACAACAATTGCACATAAAAT GAATTTATCCTTATATTTGGGTGAAAGACCAAGTTACAGTATGTCTGGACCTAATAGTTCTTCAGAGTGGTCCATCGAAGGACGTAGATTGGTACCTC TGATGCCCAGGCTGGTTCCCCAGACTGCCTTCACTGTAACAGCGAATGCTGTGGCCAATGCAGCTGTCCAGCACAATGCGTCCCTTCCTGTGCCTGCAG AGACAGCAAGCAAAGACGGCGTTAGCTGCTCAGATGAAGATGAAAAACCCAGAAAACGCAGGCGAACAAACTCCTCTAGCTCCTCC CCTGTTGTCCTCAAGGAAGTTCCAAAGGCTGTTGTTCCGGTCTCAAAGACGATCACTGTGCCTGTGAGTGGAAGTCCCAAGATGAGC AACATTATGCAGAGCATTGCCAACTCCTTACCACCCCATATGTCACCTGTGAAAATAACGTTTACCAAACCATCAACACAGACAACA AACACAACAACACAGAAGGTTATTATAGTGACCACATCACCAAGTTCAACCTTTGTGCCCAACATTCTCTCCAAATCTCATAACTATGC AGCAGTCACTAAGCTTGTACCAACATCAGTCATTGCCTCCACGACTCAGAAGCCACCTGTTGTTATAACTGCGTCCCA GGCCTCTCTGGTCACCAGTAGCAGCAATGGCAACAGCAGTTCTACGTCATCACCTATTTCTAGTACAGTTGCAGTAACAACGGTGGTATCTTCCACACCGTCCG TGGTCATGTCAACAGTAGCACAAGGTGTATCCACATCAGCAATCAAAGTGGCATCGACAAGACTTCCTTCCCCCAAAAGCTTAGTGAGTGGCCCTACCCAGATTCTTGCACAGTTTCCT AAACAGCATCAGCAGTCTCCTAAGCAGCAGTTACAGCAAGTACAACAACAGACACAGCAGCCAGTGGCCCAGCCTTCTTCAGTGTCTCAGCAGCAGCAGCCTCAGCAG TCAGCATTGCCGCCTGGTATCAAACCTACCATCCAGATCAAACAGGAGTCAGGTGTTAAAATCATCACACAGCAGGTTCAACCAAGTA AAATCTTACCCAAACCAGTGACTGCAACTCTACCCACCAGTAGCAATTCCCCAATTATGGTGGTTAGCAGTAATGGTGCAATTATGACAACTAAACTGGTAACCACTCCTACCGGTACTCA GGCAACCTATACCCGGCCAACAGTGAGCCCATCCTTAGGTCGTGTGGCTACAACCCCTGGAGCTGCAACCTATGTGAAAACTACCAGTGGCAGCATCATCACTGTAGTACCCAAA TCATTAGCTACCTTGGGAGGCAAGATAATTAGCAGTAATATTGTTTCTGGAACGACTACCAAAATCACTACAATCCCAATGACTTCCAAGCCCAATGTGATTGTTGTACAAAAGACTACAGGA AAAGGAACGACCATTCAAGGCCTCCCTGGCAAAAACGTTGTCACAACCTTGCTAAATGCTGGAGGAGAAAAGACTCTTCAGACAGTGCCAGCTGGAGCCAAGCCAGCTATAATTACTGCTACAAG ACCCATCACCAAAATGATTGTAACACAGCCCAAAGGAATAGGTTCCGCAGTACAGCCAGCAGCTAAAATCATTCCAACAAAAATTGTGTATGGACAACAAGGGAAAACACAGGTTCTAATTAA ACCCAAACCAGTGACATTTCAAGCTACAGTTGTCAGTGAACAAACAAGGCAGCTGGTAACAGAAACATTACAGCAAGCATCCAGGGTAGCAGATGCCAGTAATTCATCTGCTCAGGAAGGAAAAGA GGAGCCACAGGGTTACACAGACAGTAGTTCTTCTTCCACAGAGTCCTCCCAGAGTTCTCAAGATTCCCAGCCTGTAGTTCATGTAATTGCTTCCCGGCGTCAGGATTGGTCAGAACATGAG ATTGCAATGGAGACTAGCCCCACCATAATATATCAGGATGTATCCAGTGAATCACAATCAGCCACTTCAACAATCAAAGCTCTGTTAGAACTCCAACAGACAACAGTAAAGGAAAAATTG GAATCTAAACCAAGACAACCCACTATTGACTTGAGTCAAATGGCAGTACCTATTCAGATGACCCAGGAAAAGAGACATTCTCCTGAGAGTCCATCCATTGCTGTGGT AGAGTCAGAACTAGTTGCTGAATACATCACCACGGTCAGCCATCGCTCCCAGCCCCAACAGCCTTCCCAGCCCCAGCGGACCCTGCTTCAACATGTGGCTCAGTCACAGACTGCG ACGCAGACTTCGGTGGTAGTAAAGTCCATCCCAGCGTCTTCCCCTGGAGCAATCACCCACATCATGCAGCAGGCATTAAGCAGTCACACCGCTTTTACGAAA CACAGTGAGGAACTTGGAACTGAGGAGGGTGAGGTTGAAGAGATGGACACATTGGATCCTCAGACAGGTCTGTTTTACCGCTCTGCCCTGACACAATCGCAGTCAACCAAACAGCAGAAA CTTAGCCAGCCCCAGCTGGAACAGACTCAGCTGCAAGTGAAAACTCTGCAGTGCTTCCAGACCAAACAGAAGCAGACCATCCACCTGCAGGCAGACCAACTCCAGCACAAACTCACAC AGATGCCCCAGCTCTCTATCAGGCATCAGAAACTGAATCCTCTCCAGCAGGAACAAGCACAGCCCAAGCCAGATGCACAGCACACACAGCATACTGTGGTGGCCAAAGAC AGGCAGCTTCCTACCTTAATGGCACAGCCCCCGCAAACTGTAGTACAGGTGCTTGCCGTGAAAACCACACAGCAGCTTCCTAAACTGCAGCAAGCTCCGAACCAACCAAAAATCTACGT GCAACCCCAAACTCCTCAGAGCCAGATGGCACTCCCCTCTTCAGAGAAACAGCCTGCAAGCCAGGTGGAGCAGCCAATTATAACTCAAGGATCCTCTGTTACAAAGATAACATTTGAGGGGCGCC AGCCTCCCACAGTTACAAAGATAACTGGTGGCAGTTCTGTTCCTAAGCTGACATCACCAGTTACAAGCATATCTCCCATTCAGGCCTCTGAGAAGACAGCAGTGTCAGACATTTTGCAAATG TCTTTGATGGAAGCTCAGATTGATACAAATGTAGAACATATGGTAGTGGATCCCCCAAAGAAGGCTCTAGCCACCAATGTGCTCACTGGTGAGGCAGGAGCTCTACCCTCCACCCATGTGGT GGTGGCAGGGATGACGAAATGTAGAGAGTCCTGTTCAAGTCCATCTGCTGTTGGCCCTCCCCTAACGACCAGAAAAATCGAAGCAGCAGGAGTGCCTACGACAGGCCAGTTCATGCGTATTCAGA ATGTAGGCCAAAAGAAAGCTGAAGAGAGCCCAACAGAAATTATCATCCAGGCCATTCCCCAGTATGCTATTCCTTGTCACTCCAGCTCCAATGTGGTGGTGGAGCCCAGTGGGCTTCTAGA GTTGAACAACTTCACCAGTCAGCAGCTAGATGACGACGAGACAGCCATGGAGCAGGACATAGACAGCAGCACGGAGGACGGGACTGAGCCCAGCCCCTCTCAGA GCGCTGTAGAACGGTCC

with PBST were next cultivated with secondary antibodies, which lasted for 2 hours at room temperature. An enhanced chemiluminescence (ECL) (EMD Millipore, MA, USA) was used to capture the immunoreactive proteins.

2.6 | Cell proliferation assays

Cell proliferation was detected indirectly by CCK-8 assay and directly by EdU assay. In the CCK-8 assay, cells were harvested at 0, 1, 2, 3, and 4 days after transduction. Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was employed in line with the manufacturer's guidelines. A microplate reader (Molecular Devices, CA, USA) was used to measure absorbance at 450 nm.

In the EdU assay, cells plated in 96-well plates at 1000 cells per well were adhered overnight. EdU solution was then added for 2 hours of incubation. Next, the cells were fixed using 4% paraformaldehyde for half an hour and stained using Cell-Light EdU Apollo488 In Vitro Imaging Kit (RioBio, Guangzhou, China).

2.7 | Nuclear/cytoplasmic fractionation

Take the same amount of cells, total RNA is extracted with TRIzol. Subcellular localization of DGUOK-AS1 in HeLa and SiHa cells was confirmed by using a Cytoplasmic and Nuclear RNA Purification Kit (Norgenbiotek, ON, Canada) in line with the producer's guide. Cytoplasmic and nuclear RNAs were measured by

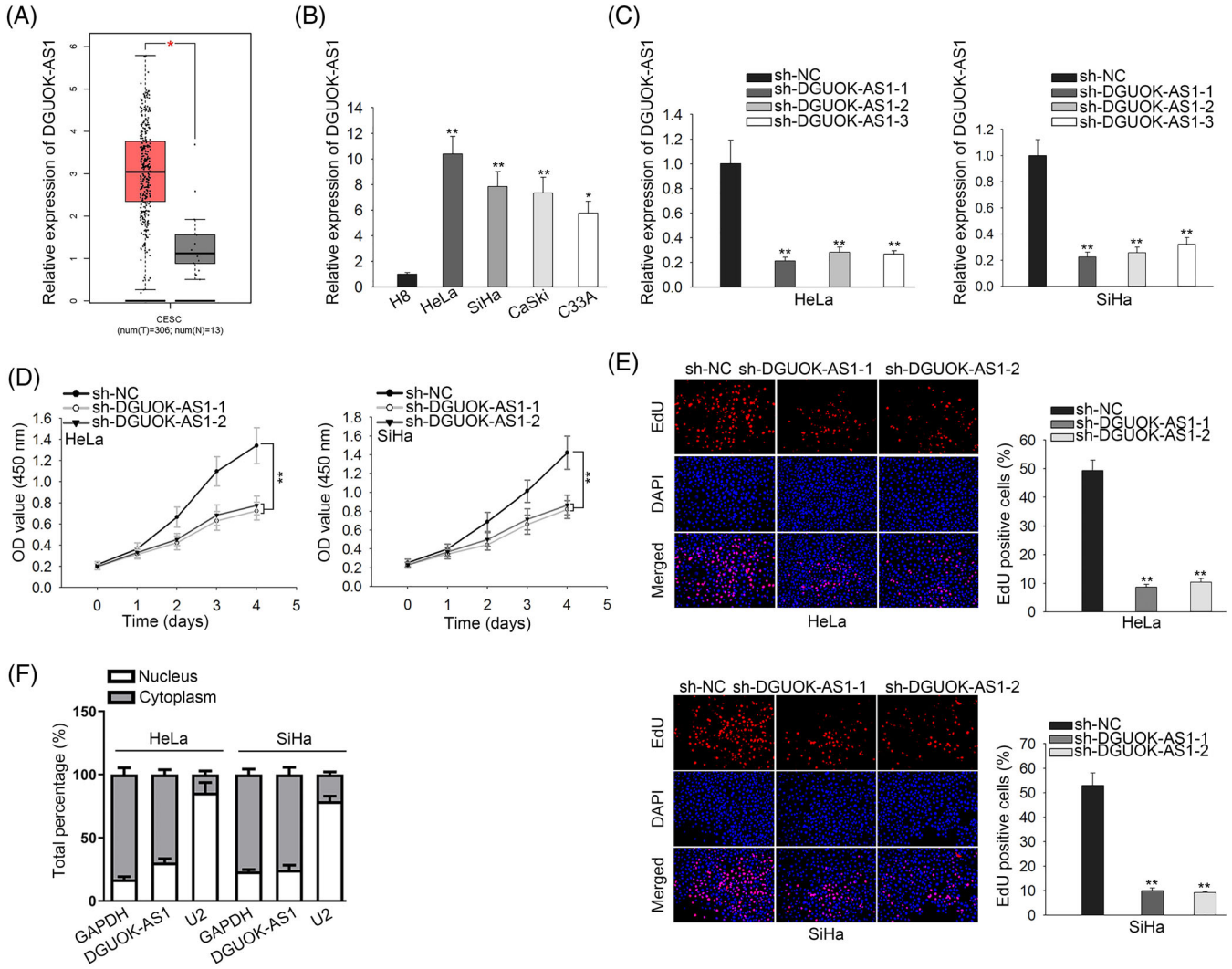

FIGURE 1 DGUOK-AS1 knockdown hinders proliferation of cervical cancer (CC) cells. A, Database of TCGA indicated the expression of DGUOK-AS1 in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC). B, Real-time quantitative polymerase chain reaction (RT-qPCR) measured the expression level of DGUOK-AS1 in CC cell lines (HeLa, SiHa, CaSki, and C33A) and the normal cervical epithelial cell line H8. C, Knockdown efficiency of sh-DGUOK-AS1-1/2/3 was validated through RT-qPCR. D,E, CCK-8 and EdU assays demonstrated the viability and proliferation of HeLa and SiHa cells. F, Cytoplasmic/nuclear fractionation revealed the cellular localization of DGUOK-AS1. Experiments were performed at least three times. *$P < .05$, **$P < .01$

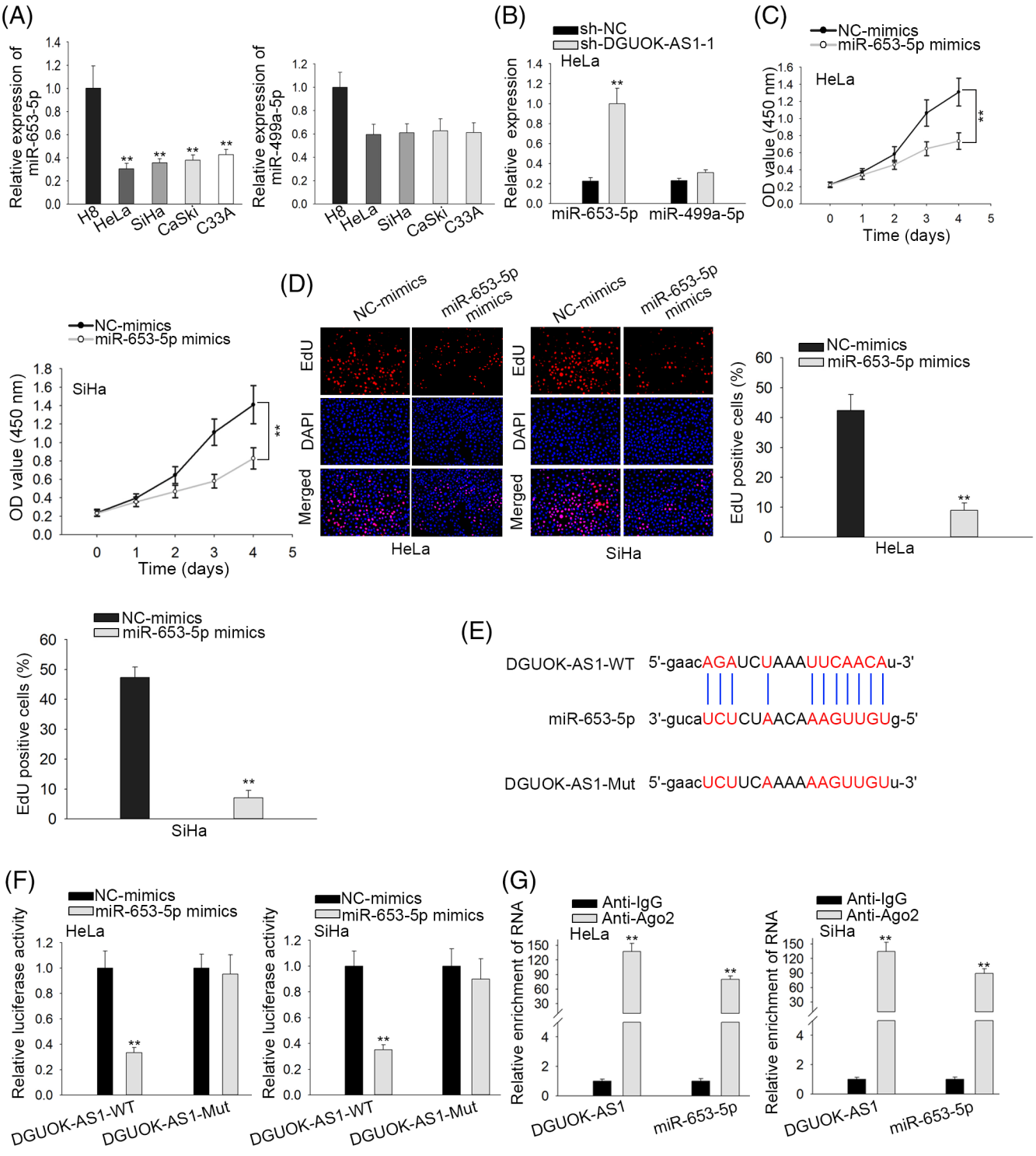

FIGURE 2 DGUOK-AS1 works as a natural sponge of miR-653-5p in cervical cancer (CC). A, Real-time quantitative polymerase chain reaction (RT-qPCR) unmasked the expression of miR-653-5p and miR-499a-5p in CC cell lines and the normal cell line. B, RT-qPCR indicated the expression of miR-653-5p and miR-499a-5p in HeLa cells treated with sh-DGUOK-AS1 or sh-NC. C,D, CCK-8 and EdU assays evaluated the growth capacity of HeLa and SiHa cells after transfection with miR-653-5p mimics. E, The putative binding site between miR-653-5p and DGUOK-AS1. F, Dual-luciferase reporter assay measured the luciferase activity of DGUOK-AS1-WT and DGUOK-AS1-Mut. G, RNA immunoprecipitation (RIP) assay tested the enrichment of DGUOK-AS1 and miR-653-5p in anti-Ago2 group. Anti-IgG served as negative control. Experiments were performed at least three times. $^{**}P < .01$

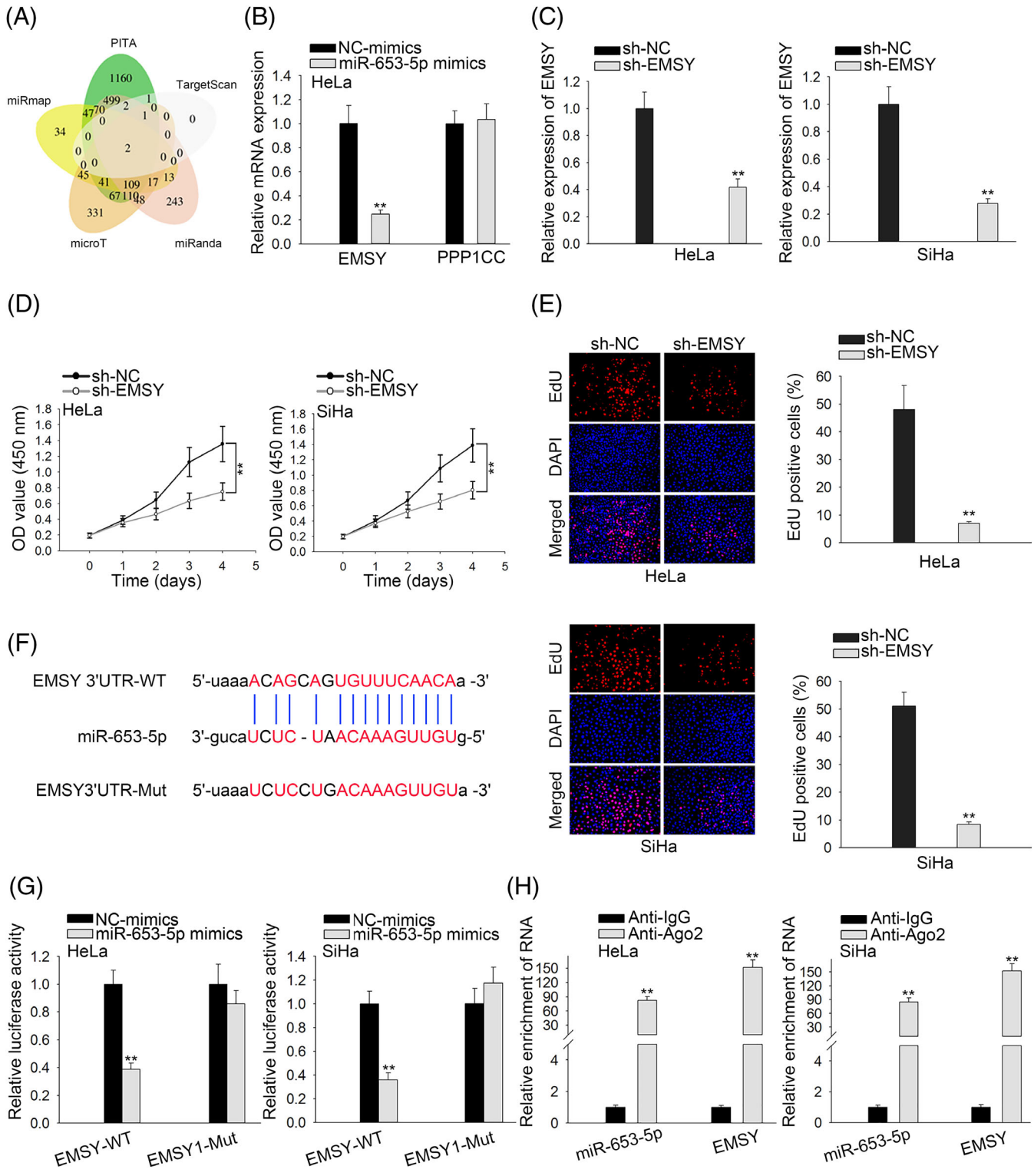

FIGURE 3 DGUOK-AS1 sponges miR-653-5p to elevate EMSY in cervical cancer (CC). A, The intersection of five bioinformatics tools (PITA, miRanda, miRmap, microT, and TargetScan) exhibited the mRNAs that might bind with miR-653-5p. B, Real-time quantitative polymerase chain reaction (RT-qPCR) measured the expression of EMSY and PPP1CC in HeLa and SiHa cells proceeded with miR-653-3p mimics or NC mimics. C, Interference efficiency of EMSY was confirmed by RT-qPCR. D,E, CCK-8 and EdU assays assessed the growth ability of HeLa and SiHa cells after EMSY depletion. F, The putative binding sites between miR-653-5p and EMSY. G, Dual-luciferase reporter assay tested the luciferase activity of EMSY-WT or EMSY-Mut. H, RNA immunoprecipitation (RIP) assay tested the enrichment of miR-653-5p and EMSY in anti-Ago2 group. Anti-IgG served as negative control. Experiments were performed at least three times. *$P < .01$

real-time quantitative PCR (RT-qPCR) with GAPDH as the cytoplasmic normalizer and U2 as the nuclear normalizer.

2.8 | Dual-luciferase reporter assay

DGUOK-AS1 or 3′-UTR of EMSY and the mutant forms of them were subcloned into pEZX-MT06 vectors containing firefly luciferase gene (Genecopoeia, Guangzhou, China). A pRL-TK vector that contained Renilla luciferase gene was used as the internal control. The constructed products were cotransfected with miR-653-5p mimics and NC mimics for 24 hours. Luciferase activities were calculated by employing a Dual-Luciferase Reporter Assay System (Promega).

2.9 | RNA immunoprecipitation

RNA immunoprecipitation (RIP) assay was performed by utilizing RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA) following the manufacturer's instructions. Antibodies of Ago2 and IgG were used to carry out RNA immunoprecipitation. And the enrichment of DGUOK-AS1, miR-653-5p, and EMSY was evaluated by RT-PCR. Reverse Transcription with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher) and cDNA RT-PCR with GoTaq qPCR Master Mix (Promega) were also used here.

2.10 | Comet assay

DNA damage was measured by comet assay. In brief, transfected CC cancer cells were reaped and suspended with PBS. Then cells together with low melting point agarose at the ratio of 1:200 were preserved on a slide, which had been coated with 1% regular agarose. The slide solidified at 4°C was fixed with a cold lysis buffer at 4°C. After 50 minutes, the slides were dried and dipped in electrophoresis solution. Twenty-five minutes later, electrophoresis was conducted at 300 mA and 25 V, followed by dyeing using ethidium bromide. The slide was neutralized, washed, and detected with the help of a fluorescent microscope (Olympus, Tokyo, Japan).

2.11 | Statistical analysis

SPSS version 22.0 (SPSS, IL, USA) was employed for statistical analysis, and data were expressed as mean ± standard deviation (SD) after experiments at least in triplicate. And differences between groups were obtained using Student's *t* test or ANOVA. The survival rate of CC patients was analysed by applying Kaplan-Meier method and calculated utilizing the log-rank test. Results with *P* value under .05 were considered to be statistically significant.

3 | RESULTS

3.1 | Cytoplasmic DGUOK-AS1 promotes proliferation of CC cells

Database from TCGA demonstrated that DGUOK-AS1 was notably overexpressed in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) tissues in comparison with the noncancerous tissues (Figure 1A). Besides, RT-qPCR detected that the expression of DGUOK-AS1 was obviously upregulated in CC tumour tissues (n = 50) than adjacent nontumour tissues (n = 50) (Figure S1A). Meanwhile, Kaplan-Meier curve was depicted and demonstrated that CC patients with more expression of DGUOK-AS1 possessed lower survival rate (Figure S1B). In Table 1, the correlation between clinical features of CC patients and the expression of DGUOK-AS1 was exhibited. High expression of DGUOK-AS1 was closely associated with tumour size (>4 cm) and FIGO stage (III-IV). Further, in order to monitor the expression of DGUOK-AS1 in CC cell lines and normal cervical epithelial cell line, RT-qPCR was conducted again. As shown in Figure 1B, DGUOK-AS1 was significantly increased in the CC cell lines. Subsequently, we silenced DGUOK-AS1 with sh-DGUOK-AS1-1/2/3, among which sh-DGUOK-AS1-1 and sh-DGUOK-AS1-2 presented higher knockdown efficiency so that they were used in the follow-up assays (Figure 1C). To investigate cell proliferation, CCK-8 and EdU assays were successively performed. As shown in Figure 1D, cell proliferation was markedly impeded in HeLa and SiHa cells after DGUOK-AS1 depletion. Figure 1E also exhibited consistent results. Afterwards, we localized DGUOK-AS1 and found it predominantly located in the cytoplasm (Figure 1F). Taking these together, DGUOK-AS1 contributes to proliferation of CC cells.

3.2 | DGUOK-AS1 works as a natural sponge of miR-653-5p in CC

The potential molecular mechanism of DGUOK-AS1 in CC was further investigated. It was predicted by starBase v2.0 (http://starbase.sysu.edu.cn/index.php) that two miRNAs (miR-653-5p and miR-499a-5p) have binding potential with DGUOK-AS1. The expression patterns of miR-653-5p and miR-499a-5p were addressed. It was worth noting that miR-653-5p was remarkably decreased in four CC cell lines compared with the normal cell line while miR-499a-5p showed less notable difference (Figure 2A). Besides, the impact of DGUOK-AS1 on the expression levels of miR-653-5p and miR-499a-5p was evaluated. As demonstrated by Figure 2B, miR-653-5p was obviously increased after DGUOK-AS1 depletion, whereas no observable change happened to the expression level of miR-499a-5p. Proliferative capacity of HeLa and SiHa cells was apparently crippled after they were treated with miR-653-5p mimics (Figure 2C,D). The presumptive binding sites between DGUOK-AS1 and miR-653-5p were listed in Figure 2E, and the effectiveness of sites was confirmed by dual-luciferase reporter assay (Figure 2F). RIP assay was also carried out and measured the prominent enrichment of DGUOK-AS1 and miR-

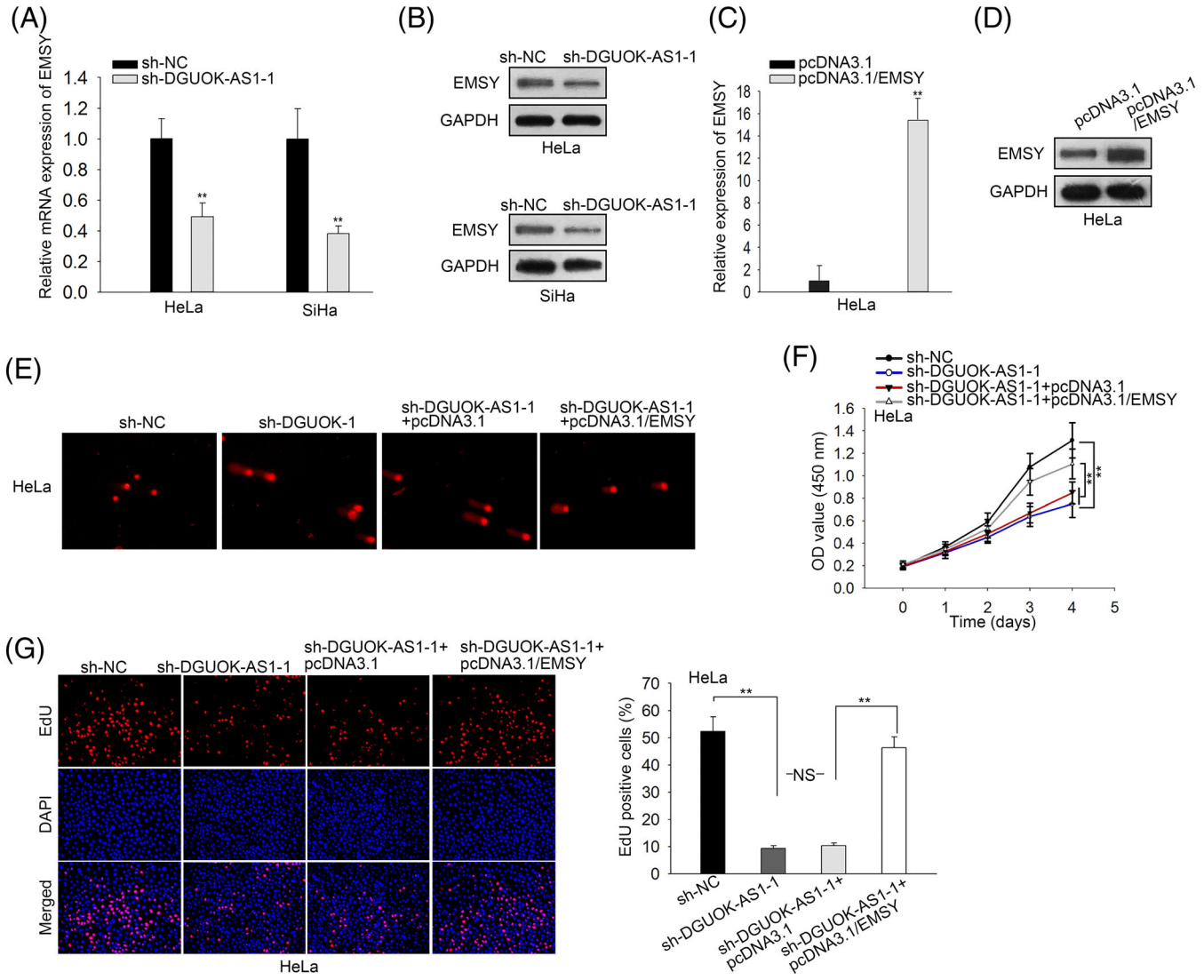

FIGURE 4 EMSY is required for DGUOK-AS1 to induce cell proliferation and repress DNA damage in cervical cancer (CC). A,B, Real-time quantitative polymerase chain reaction (RT-qPCR) and western blot assays detected the expression of EMSY at mRNA and protein levels in HeLa and SiHa cells treated with sh-DGUOK-AS1-1 or sh-NC. C,D, RT-qPCR and western blot assays confirmed overexpression efficiency of pcDNA3.1/EMSY at mRNA and protein levels in HeLa cells. E, Comet assay was performed to measure DNA damage. F,G, CCK-8 and EdU assays evaluated cell viability. NS meant no significance. $^{**}P < .01$

653-5p in anti-Ago2 group (Figure 2G). All the data above suggested that DGUOK-AS1 works as a natural sponge of miR-653-5p in CC and miR-653-5p suppresses CC cell proliferation.

3.3 | DGUOK-AS1 sponges miR-653-5p to elevate EMSY in CC

Prediction of miR-653-5p target genes was carried out using starBase v2.0 (http://starbase.sysu.edu.cn/index.php). We firstly obtained 2193 mRNAs containing binding sites with miR-653-5p (Table S1). Then based on these restrictions (CLIP-Data ≥ 1; Degradome-Data ≥ 0; pan-Cancer ≥ 0; programNum ≥ 1; program: microT, miRanda, miRmap, PITA, TargetScan; target: all), EMSY and PPP1CC were screened out (Figure 3A). After transfection of miR-653-5p mimics, the expression of EMSY strikingly declined, while no significant change of PPP1CC expression was captured (Figure 3B). Therefore, EMSY was selected in the following research. We then evaluated the influence of EMSY silence on CC cell proliferation. HeLa and SiHa cells transfected with sh-EMSY were subjected to CCK-8 and EdU assays. The knockdown efficiency of EMSY was testified by RT-qPCR (Figure 3C). As elucidated by Figure 3D,E, cell proliferation ability was much attenuated after EMSY silence. Luciferase reporter assay was later performed on the basis of the putative binding sites exhibited in Figure 3F. The results manifested that the relative luciferase activity of EMSY-WT was inhibited by elevated miR-653-5p while that of EMSY-Mut was not affected (Figure 3G). RIP assay further verified the direct interaction between miR-653-5p and EMSY (Figure 3H). In summary, DGUOK-AS1 sequesters miR-653-5p to elevate EMSY, which promotes cell proliferation in CC.

3.4 | EMSY/miR-653-5p is required for DGUOK-AS1 to induce cell proliferation and repress DNA damage of in CC

After DGUOK-AS1 silence, the expression of EMSY was remarkably reduced, not only at the mRNA level but also at the protein level (Figure 4A,B). CC cells treated with pcDNA3.1/EMSY held significantly increased level of EMSY, as verified by RT-qPCR and western blot (Figure 4C,D). Intriguingly, EMSY has been reported to exert functions related to DNA repair.[11,12] So we reasoned that DGUOK-AS1 and EMSY might affect DNA damage and repair in CC. Comet assay was carried out, and it was demonstrated that DNA damage was enhanced when DGUOK-AS1 was knocked down, whereas increased EMSY abolished this trend (Figure 4E). In addition, impaired proliferation ability of CC cells caused by DGUOK-AS1 depletion was revived when EMSY was overexpressed (Figure 4F,G). With regard to the correlation between DGUOK-AS1 and miR-653-5p in regulating CC cell proliferation, rescuing experiments were also performed. The knockdown efficiency of miR-653-5p inhibitor was confirmed (Figure S2A). In a similar way, comet assay verified that miR-653-5p inhibitor alleviated DGUOK-AS1 depletion-mediated DNA damage (Figure S2B). Additionally, the sh-DGUOK-AS1-suppressed CC cell proliferation was encouraged after knocking down miR-653-5p (Figure S2C,D). Given all the results above, it can be concluded that EMSY/miR-653-5p is required for DGUOK-AS1 to induce proliferation and repress DNA repair of CC cells.

4 | DISCUSSION

As one of the most predominant causes of deaths in female around the globe, CC has attracted increasing attention.[13,14] The underlying mechanisms of CC initiation and progression are sophisticated, involving large quantities of dysregulated oncogenes and antioncogenes.

LncRNAs used to be regarded as dark matter in genome or transcriptional noise. However, a great many of lncRNAs have been accepted as key regulators in tumourigenesis and progression of CC. For instance, lncRNA C5orf66-AS1 promotes the proliferation of CC cells.[15] LncRNA NCK1-AS1 promotes cell proliferation and stimulates cell cycle progression in CC.[16] Lnc-CC3 increases metastasis in CC.[17] Nonetheless, there are many lncRNAs remaining poorly understood, and unveiling new CC-related lncRNAs may be beneficial to find new treatment strategies for CC. DGUOK-AS1 was an upregulated lncRNA in CESC tissues according to TCGA public database. However, there were few researches about the function of lncRNA DGUOK-AS1 in CC. In this research, we found the aberrant expression of DGUOK-AS1 in CC tissues and cell lines. In addition, DGUOK-AS1 depletion strikingly obstructed cell proliferation in CC.

It has been widely recognized that cytoplasmic lncRNAs probably regulate mRNAs via posttranscriptionally sequestering miRNAs and thus exert crucial function in cancers.[18] For example, HOTAIR sponges miR-206 to induce Bcl-w signalling and thereby facilitates the proliferation of breast cancer cells.[19] LncRNA HULC promotes EMT to facilitate tumourigenesis and metastasis of hepatocellular carcinoma through regulating the expression of miR-200a-3p/ZEB1.[20] Upregulation of lncRNA XIST benefits CC progression via modulating miR-140-5p and ORC1.[21] MiR-653-5p has been verified as tumour inhibitor in nonsmall cell lung cancer and neuroblastoma.[22,23] In our study, we found decreased expression of miR-653-5p in CC cell lines, and further, the inhibiting effect of miR-653-5p on CC cell proliferation was elucidated. Besides, the miR-653-5p was sponged by DGUOK-AS1 in CC.

EMSY is a putative target gene of miR-653-5p, and its oncogene role in cancers has been reported.[24-26] In the current study, we demonstrated that EMSY was negatively regulated by miR-653-5p and EMSY silence led to attenuation of CC cell growth. And interestingly, it drew our attention that EMSY is closely associated with DNA repair.[11,12] It is known that abnormity of DNA repair could lead to tumourigenesis. Accordingly, we elucidated that DGUOK-AS1 knockdown aggravated DNA damage but EMSY overexpression or miR-653-5p suppression reversed this trend and relieved DNA damage. At last, we performed a battery of rescue assays and found that DGUOK-AS1 facilitates growth of CC cells via increasing expression of EMSY or decreasing that of miR-653-5p.

To conclude, it is initially manifested that DGUOK-AS1 contributed to CC cell proliferation via acting as a ceRNA of miR-653-5p to deregulate EMSY in this research, which may shed new light on discovery of therapeutic strategies for CC patients.

ACKNOWLEDGEMENTS

We thank for all supports for this research.

CONFLICT OF INTEREST

The authors declare that no competing interests are involved in this study.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Wu N, Song H, Ren Y, Tao S, Li S. DGUOK-AS1 promotes cell proliferation in cervical cancer via acting as a ceRNA of miR-653-5p. *Cell Biochem Funct*. 2020;38:870–879. https://doi.org/10.1002/cbf.3506