The aim of the present study was to identify potential therapeutic targets that serve crucial roles in the progression of cervical cancer. Clinical data, RNA sequencing (RNAseq)-counts and micro (mi)RNA data regarding cervical squamous cell carcinoma were retrieved from The Cancer Genome Atlas, and analyses were performed using the University of California Santa Cruz database. RNAseq and miRNA data were stratified into 3 groups (according to the patients' age), and genes were re-annotated and preprocessed prior to Mfuzz time clustering analysis. Subsequently, enrichment analyses were performed in order to identify differentially expressed mRNAs (DEmRNAs) and a protein-protein interaction analysis network was constructed. miRNA-gene, miRNA-lncRNA, and long non-coding (lnc)RNA-mRNA pairs were collected and the lncRNA-miRNA-mRNA competing endogenous (ce)RNA network was established. Further enrichment analyses were performed in order to identify crucial mRNAs in the ceRNA network. Finally, survival and drug association analyses were performed. A total of 269 DEmRNAs (including alcohol dehydrogenase 7 (ADH7), vestigial-like family member 3 (VGLL3) and cytochrome P450, family 26, subfamily B, polypeptide 1 (CYP26B1)), 274 DElncRNAs (including LINC01133) and 16 DEmiRNAs (including miR-3065 and miR-330) were identified. There were 102 lncRNAs, 15 miRNAs, 15 mRNAs and 522 interaction pairs in the ceRNA network. In particular, ADH7 was regulated by miR-3065, and miR-3065 interacted with LINC01133 in the ceRNA network. Furthermore, ADH7 and CYP26B1 were enriched in the retinoic acid metabolic process and the retinol metabolism pathway. ADH7 and VGLL3 were significantly associated with the cervical cancer survival rate. ADH7, VGLL3, CYP26B1, miR-3065, miR-330, miR-499a and LINC01133 play pivotal roles in the progression of cervical cancer in different age groups.

Cervical cancer is the fourth most prevalent cancer in women worldwide, behind colon, breast and lung cancer, and represents a leading cause of tumor-associated mortality amongst women in developing countries (1). Notably, it is estimated that ~570,000 cervical cancer cases occurred worldwide in 2018 (2). Human papillomavirus (HPV) infection is a persistent infection that is associated with the tumorigenesis of cervical cancer; in particular, HPV type 16 (HPV16), which is present in >50% of patients (3). Previous studies have revealed that the distribution and prevalence of HPV genotypes differs depending on age cohort (4-6). Furthermore, HPV is a sexually transmitted infection and 75-80% of sexually active individuals are likely to be infected in their lifetime (7). Hence, the prevalence of both HPV and cervical cancer among women may be associated with age (8). For example, females aged 25-44 years have a demonstrated ability to clear or prevent HPV infection and, consequently, the infection rate of HPV in these women is lower than other age groups (9). However, over the past 10 years the prevalence of HPV has increased among gynecological outpatients aged between 20 and 24 years (8). This may be due to the long-term use of contraceptives, early coitus and an overall increase in stress levels (10,11). Therefore, research on differences in cervical cancer between various age groups may provide clinical guidance to help address the increasing prevalence of cervical cancer in young women.

There are currently several treatments for patients with cervical cancer, including radiotherapy, chemotherapy, interventional therapy and radical hysterectomy, and the overall survival rate of patients with cervical cancer has improved.
to a certain extent (12,13). However, lymphatic metastasis, postoperative recurrence and other toxic side effects, including secondary infection, radiation damage and malnutrition all have an adverse effect on therapeutic outcomes (14). Therefore, it is essential to investigate novel and effective prognostic indicators and therapeutic targets to improve cervical cancer therapy (15).

MicroRNAs (miRNAs) are a group of endogenous short noncoding RNAs consisting of ~22 nucleotides that cause the degradation of mRNA by targeting the 3’ untranslated region (16,17). At present, several miRNAs have been demonstrated to influence the progression of cervical cancer. For instance, miR-152 expression is upregulated in the peripheral blood of patients with cervical intraepithelial neoplasia, serving as an early diagnostic and prognostic biomarker (18). Furthermore, miR-4429 sensitizes cervical cancer cells to irradiation via the targeting of RAD51 recombinate (19). Additionally, increasing evidence has demonstrated that the long non-coding (lnc)RNAs serve crucial roles in the migration, differentiation, invasion and proliferation of cells (20,21). Notably, several lncRNAs that regulate various cellular biological processes and influence the oncogenesis of cervical cancer have been identified (22). For example, the lncRNA CRNDE mediates the tumorigenesis of cervical cancer via inhibition of p53 upregulated modulator of apoptosis expression (PUMA) (23).

Recently, it has been demonstrated that certain lncRNAs and mRNA can co-operate with miRNAs, downregulating the expression of miRNAs by forming a competing endogenous (ce)RNA network (24). Hence, the present study hypothesized that lncRNAs may regulate mRNA expression via co-operation with miRNAs in cervical cancer. In the present study, the clinical data, RNA sequencing (RNA-seq)-counts and miRNA data in different age groups were integrated to further investigate the molecular mechanisms underlying cervical cancer development, resulting in the identification of several potential therapeutic targets of cervical cancer.

Materials and methods

Data source and preprocessing. The Cancer Genome Atlas (TCGA; https://cancergenome.nih.gov) is the largest database of cancer gene information, containing data regarding 29 common cancer types, 39 cancer subtypes and >14,500 cancer samples. The data describe various characteristics, including miRNA, RNA-seq, single nucleotide polymorphisms, copy number variations and methylation patterns, as well as detailed clinical information. The University of California Santa Cruz Genome Browser (UCSC; http://xena.ucsc.edu) (25) serves as a tool to view the recently assembled dataset (26). Subsequently, the data were pre-processed via matching, selecting and deleting data using the UCSC Xena platform. The RNAseq data were then divided into 3 groups according to the patients’ age: 1-40 years (84 samples), 41-60 years (157 samples) and >60 years (65 samples). Similarly, the miRNA data were assigned into 3 groups according to the patients’ age (as aforementioned), and the number of samples in each group was consistent with the RNAseq data.

TCGA genes re-annotation and data preprocessing. The HUGO Gene Nomenclature Committee (HGNC; http://www.genenames.org) is responsible for providing unique, standard and widespread symbols for human mRNA genes, protein-encoding genes and other genes. For each gene in the human genome, there is a unique ID and symbol listed in the HGNC database. Gene information of all symbols in the human genome were retrieved from the HGNC database for further data preprocessing. miRNAs and lncRNAs were distinguished from RNA-seq data for subsequent analysis.

The ENSG (European Neuroblastoma Study Group) number of genes in the RNAseq expression profiles from HGNC corresponded to the gene symbol (27,28). Genes that did not match were deleted. The clinical data were used for the subsequent survival analysis if they matched the RNA-seq expression profile. Analogously, the miRNAs were used for subsequent analysis if the samples were matched with RNA-seq expression profile samples and clinical samples.

RNA-seq Mfuzz time clustering analysis. mRNA, lncRNA and miRNA expression matrices were obtained from TCGA database, and the mean of the expression matrix was calculated according to each age group. mRNA data was eliminated if the standard deviation (SD) was <0.3; otherwise, lncRNA and miRNA were eliminated if their SD was <0.1. The Mfuzz package in R software (version 3.2.3) (https://www.r-project.org) was applied to perform soft clustering analysis of noise resistance to the degeneration of DEGs (29). In this process, the Fuzzy C-Means clustering method was adopted to conduct clustering analysis based on the variation of time and the change in expression levels. Finally, multiple clustering results were obtained and those with consistent expression changes were placed in the same group. DEMRNAs, DELncRNAs, and DEmiRNAs were identified in the group according to the difference in expression value (P<0.05 was used as the cut-off value).

Enrichment analyses. The clusterProfiler (version 3.2.11; http://www.biocductor.org/packages/release/bioc/html/clusterProfiler.html) package of R software was used to conduct functional Gene Ontology (http://geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (30) functional enrichment analyses for the DEMRNAs. P<0.05 was considered to indicate a statistically significant difference.

Protein-protein interaction (PPI) analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; version 10.0; http://www.string-db.org/) database was used to search and predict a PPI network. The database can be used for >2,000 species, containing 9.6 million different proteins and 13.8 million types of protein interactions. Notably, the PPI network included direct physical interactions as well as indirect functional correlations (31). According to the RNA-seq Mfuzz time clustering analysis, mRNAs that were significantly

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associated with the age of patients with cervical cancer were selected and used to analyze the interaction between the mRNAs identified in the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://www.string-db.org). mRNA data with a standard deviation value <0.3 was eliminated. P<0.05 was considered to indicate a statistically significant difference.

**Co-expression analysis of DEmRNAs and DElncRNAs.** mRNA and lncRNA expression matrices were obtained via RNA-seq Mfuzz time clustering analysis and co-expression was calculated using the Pearson correlation coefficient method (6). Using DEmRNAs and DElncRNAs data, the co-expression association was calculated and the mRNA-lncRNA co-expressed pairs were obtained. P<0.05 and an absolute correlation coefficient value |r| >0.4 were considered to indicate a significant correlation. An r-value >0 was considered to indicate of a positive correlation, whereas r <0 was considered as a representative of a negative correlation. The correlation strength was highest when the r-li-value was closest to 1.

**Prediction of lncRNA and mRNA targeted by miRNA.** miRWalk (32,33), RNA22 (34), miRanda (http://miranda.org.uk), Targetscan (http://www.targetscan.org/vert_72), miRDB (http://www.mirdb.org) and miRMap tools (http://mirmap.ezlab.org) were applied to predict mRNAs targeting miRNA. The miRWalk, miRanda, Targetscan and RNAhybrid tools were also used for predicting lncRNAs targeting miRNA. The target mRNAs and lncRNAs were arbitrarily selected if they were predicted by >6 and ≥2 retrieval tools among the aforementioned tools, respectively. The parameter settings in the miRWalk tool were selected as miRNA and official symbol. Species was set as human and the database retrieval association was set as ‘OR’ across all databases.

**ceRNA network construction and mRNA enrichment analysis.** The miRNA-mRNA, miRNA-lncRNA and co-expressed lncRNA-mRNA pairs were identified as aforementioned. Subsequently, lncRNAs and target genes that were associated with the same miRNA were selected, and the lncRNA-miRNA-mRNA ceRNA network was constructed using Cytoscape software (version 3.4.0) (35,36). Thereafter, functional analyses for crucial mRNAs located in the ceRNA network were conducted as aforementioned. P<0.05 was considered to indicate a statistically significant difference.

**Survival analysis.** The optimal cut-off of key genes was obtained according to the expression value, survival time and survival state based on Survminer package (version 0.4.2) (https://www.rdocumentation.org/packages/survminer/versions/0.4.2) of R software. Genes were considered to be upregulated if the expression value was greater than the optimal cut-off value; conversely, genes lower than the cut-off value were considered to be downregulated. Subsequently, the survival analysis and survival test were performed utilizing survival in the R package (version 2.4.2-6). The significance cut-off was set as P<0.05. mRNAs with a significant correlation between survival analysis and prognosis were selected and the significant results of survival analysis were plotted on the Kaplan-Meier curve using SPSS software (version 16.0).

**Drug association analysis.** The prediction of targeted drugs was performed using the Drug-Gene Interaction (DGIdb) online database (http://www.dgidxdb.org/search_interactions) based on the pre-identified mRNAs located in the ceRNA network and using the following parameter settings, FDA approved and antineoplastic. The interaction network of drug-target genes was constructed using Cytoscape software.

**Statistical analysis.** Statistical analyses were performed using SPSS software (version 16.0). The Pearson correlation coefficient method was used to analyze co-expression between DEmRNAs and DElncRNAs. Data are presented as the mean ± standard deviation. The independent-samples student t-test was used to compare data between two groups and the χ² test was used to compare the count data groups. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Mfuzz time clustering analysis of cervical cancer progression in different age groups.** The design ideas and analysis process are detailed in Fig. S1. The Mfuzz analysis results for cervical cancer are presented in Table I. The results show that there were 269 DEmRNAs; 30 upregulated [including alcohol dehydrogenase 7 (ADH7)] and 239 downregulated [including vestigial-like family member 3 (VGLL3) and cytochrome P450, family 26, subfamily B, polypeptide 1 (CYP26B1)]. A total of 274 DElncRNAs were identified; 131 upregulated...
(e.g. LINC01133) and 143 downregulated, and 16 DEmiRNAs were discovered; 8 upregulated (including miR-499a) and 8 downregulated (including miR-3065 and -330) (Table SI). In particular, ADH7 expression was upregulated in patients >60 years, while VGLL3 and CYP26B1 expression were downregulated in the older age groups (Table SII).

Table II. Hub nodes in the protein-protein interaction network.

| Gene symbol | Degree | Betweenness  | Closeness  |
|-------------|--------|-------------|------------|
| CSF2        | 14.0   | 4759.71880  | 0.052211303|
| KLK3        | 11.0   | 4843.13800  | 0.051845074|
| WNT3A       | 11.0   | 4234.45750  | 0.051328503|
| HIST1H2BD   | 10.0   | 187.71416   | 0.050535080|
| HIST1H2BJ   | 10.0   | 187.71416   | 0.050535080|
| HIST1H2BO   | 10.0   | 187.71416   | 0.050535080|
| HIST1H2BM   | 10.0   | 187.71416   | 0.050535080|
| FOXJ1       | 9.0    | 3495.36670  | 0.049985297|
| CDKN2A      | 9.0    | 4913.64550  | 0.052372150|
| HOXB1       | 9.0    | 1055.40840  | 0.050073640|

Figure 1. PPI network for DEmRNAs. A PPI network was constructed for the pre-identified DEmRNAs using the Search Tool for the Retrieval of Interacting Genes/Proteins database. Red and green circles represent the upregulated and downregulated mRNA, respectively. There are 292 interaction pairs and 173 nodes in the PPI network. DEmRNAs, differentially expressed mRNAs; PPI, protein-protein interaction.

PPI network with age of DEmRNAs in cervical cancer. A total of 292 interaction pairs and 173 nodes identified in the PPI network (Fig. 1). The hub nodes are presented in Table II.

Enrichment analyses with age change of DEmRNAs in cervical cancer. A total of 57 functional terms were obtained.
for the DEmRNAs (Fig. 2A). For upregulated DEmRNAs, the top 10 enriched functions are listed in Table III, such as ‘cofactor binding’, ‘modified amino acid binding’ (e.g., ADH7, etc.) and ‘oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as an acceptor’. Similarly, the top 10 enriched functions for downregulated DEmRNAs included ‘extracellular matrix structural constituent’, ‘receptor ligand activity’ and ‘growth factor receptor binding’. DEmRNAs were enriched in 17 KEGG pathways (Fig. 2B). GO, Gene Ontology; BP, biological processes; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEmRNAs, differentially expressed mRNAs.
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(Table IV). Downregulated DEGs were enriched in 13 KEGG pathways, (Table IV).

cRNA network construction. In total, 632 mRNA-lncRNA interactions were identified according to the aforementioned method, including 169 mRNAs and 106 lncRNAs (Table SIII). A total of 1,841 pairs of miRNA-mRNA interactions (consisting of 1,518 mRNAs and 13 miRNAs) were also identified, followed by a total of 15 overlapped mRNAs and 7 corresponding miRNAs derived from the miRNA-mRNA and mRNA-lncRNA interactions. In addition, 90,026 pairs of miRNA-lncRNA interactions were extracted, including 15,069 lncRNAs and 16 miRNAs. Similarly, 102 overlapped lncRNAs and 15 corresponding miRNAs were extracted from those miRNA-lncRNA and lncRNA-mRNA interactions. Finally, the lncRNA-miRNA-mRNA ceRNA network was established utilizing those 102 lncRNAs, 15 miRNAs, 15 mRNAs and 522 interaction pairs (Fig. 3).

Enrichment analysis with age change of crucial mRNAs in cervical cancer. Enrichment analysis for crucial mRNA located in the ceRNA network was implemented; the results identified 20 functional terms (Fig. 4A) and 5 KEGG pathways (Fig. 4B). Here, the top 10 enriched functions of DEMRNAs are listed in Table VA, such as the ‘retinoic acid metabolic process’ (e.g., ADH7, CYP26B1, etc.), ‘regulation of stem cell population maintenance’ and ‘endodermal cell differentiation’. In addition, the top 10 enriched KEGG pathways for DEMRNAs are listed in Table VB, including ‘retinol metabolism’, ‘glycosaminoglycan degradation’ and maturity onset diabetes of the young.

Crucial mRNA survival analysis and drug association analysis. In total, the survival data of 265 samples were obtained and the results are presented in Tables VI and SIV. In particular, the crucial mRNAs ADH7 (Fig. 5A) and VELL3 (Fig. 5B) were significantly associated with cervical cancer survival rate (P<0.05). The prediction of targeted drugs was performed for ADH7 and VGLL3. While no associated drugs were revealed to be associated with VGLL3, two drugs (CHEMBL1161866 and ethanol) were associated with ADH7. This allows for development of intervention targets for ADH7.

Table III. GO enrichment analysis for differentially expressed mRNAs.

A, Upregulated

| Term                      | Description                                                                 | P-value     | Key genes                  |
|---------------------------|------------------------------------------------------------------------------|-------------|----------------------------|
| GO:0048037                | Cofactor binding                                                            | 4.77x10^-03 | CYP4Z1, DDO                |
| GO:0072341                | Modified amino acid binding                                                  | 5.07x10^-03 | CPNE6, GSTM2               |
| GO:0016616               | Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor | 1.28x10^-02 | ADH7, PHGDH                |
| GO:0140161               | Monocarboxylate:sodium symporter activity                                  | 1.42x10^-02 | SLC6A12                    |
| GO:00333130              | Acetylcholine receptor binding                                              | 1.56x10^-02 | NRXN1                      |
| GO:0043295               | Glutathione binding                                                          | 1.56x10^-02 | GSTM2                      |
| GO:0016614               | Oxidoreductase activity, acting on CH-OH group of donors                    | 1.63x10^-02 | ADH7, PHGDH                |
| GO:1900750               | Oligopeptide binding                                                         | 1.70x10^-02 | GSTM2                      |
| GO:0015355               | Secondary active monocarboxylate transmembrane transporter activity         | 1.84x10^-02 | SLC6A12                    |
| GO:0019841                | Retinol binding                                                              | 2.12x10^-02 | ADH7                       |

B, Downregulated

| Term                      | Description                                                                 | P-value     | Key genes                  |
|---------------------------|------------------------------------------------------------------------------|-------------|----------------------------|
| GO:0005201                | Extracellular matrix structural constituent                                | 2.56x10^-06 | MUC4, FBLN2                |
| GO:0048018                | Receptor ligand activity                                                     | 2.83x10^-05 | IL18, SA1                  |
| GO:0070851                | Growth factor receptor binding                                               | 1.39x10^-04 | CSF2, FG5                  |
| GO:0045504                | Dynein heavy chain binding                                                   | 4.77x10^-04 | DNA2, DNA11                |
| GO:0005125                | Cytokine activity                                                           | 9.35x10^-04 | IL18, WNT7A                |
| GO:0008509                | Anion transmembrane transporter activity                                     | 1.86x10^-03 | ABCB11, AQP6               |
| GO:0015081                | Sodium ion transmembrane transporter activity                               | 2.44x10^-03 | ABCB11, NALCN              |
| GO:0045503                | Dynein light chain binding                                                   | 2.44x10^-03 | DNA2, DNA11                |
| GO:0008083                | Growth factor activity                                                       | 2.61x10^-03 | CLCF1, CSF2                |
| GO:0022804                | Active transmembrane transporter activity                                    | 3.41x10^-03 | ATP2C2, SLC13A3            |
Discussion

Cervical cancer represents a leading cause of tumor-associated mortality among women, seriously threatening the health of females (2). It has been revealed that the prevalence of HPV and cervical cancer among women may be associated with their age (8). Thus, it is essential to investigate the molecular mechanism underlying cervical cancer in different ages. In total, 14 DEmiRs, 11 DElncRNAs and 16 DEmiRNAs were identified based on the different age groups in the present study. Additionally, 102 lncRNAs, 15 miRNAs and 15 mRNAs were confirmed in the ceRNA network. In particular, ADH7 was regulated by miR-3065, and miR-3065 was associated with LINC01133 in the ceRNA network. Furthermore, DEmRNAs located in the ceRNA network were involved in 5 KEGG pathways, such as 'retinol metabolism' (ADH7 and CYP26B1). Analogously, DEmRNAs were enriched in several functions, such as the ‘steroid hormone metabolic process’, ‘stem cell population maintenance’ and ‘retinoic acid metabolic process’ (ADH7 and CYP26B1). Furthermore, ADH7 and VGLL3 were significantly associated with cervical cancer survival rate.

It has been revealed that retinoids have the ability to regulate cellular differentiation and proliferation, as well as epithelial-to-mesenchymal transition via specific nuclear retinoic-acid receptors (37,38). Retinoids are considered as inhibitors of cervical cancer progression due to their interaction with the HPV proteins E6 and E7 (38). Furthermore, Gariglio et al (39) demonstrated that retinoid deficiency is associated with cervical squamous metaplasia. In a recent study, lecithin retinol acyltransferase and retinol dehydrogenase 12, which are associated with retinol metabolism, have been identified to suppress the apoptosis, migration and invasiveness of cervical cancer cells (40). These data from the present study indicated that genes correlated with retinol metabolism may influence cervical cancer progression. Whereas CYP26B1 was downregulated in cervical cancer samples, ADH7 was upregulated. Notably, in the current study, both CYP26B1 and ADH7 were revealed to influence the retinol metabolism pathway and the retinoic acid metabolic process. ADH7 is as a member of the alcohol dehydrogenase family and, thus, may be involved in the synthesis of retinoic acid, which is a hormone crucial for cellular differentiation (41). It has been reported that the upregulation of CYP26B1 may result in the progression of cervical malignancies (42). Following survival analysis, ADH7 was revealed to be significantly associated with the cervical cancer survival rate. Therefore, it was speculated that ADH7 and CYP26B1 participate in cervical cancer progression via the regulation of the retinol metabolism pathway and the retinoic

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**Table IV. KEGG pathway enrichment analysis for differentially expressed mRNAs.**

| Term                  | Description                        | P-value       | Key genes         |
|-----------------------|------------------------------------|---------------|-------------------|
| hsa00982              | Drug metabolism-cytochrome P450     | 5.15x10^-3    | ADH7, GSTM2       |
| hsa00980              | Metabolism of xenobiotics by cytochrome P450 | 5.72x10^-3    | ADH7, GSTM2       |
| hsa05204              | Chemical carcinogenesis             | 6.63x10^-3    | ADH7, GSTM2       |
| hsa04727              | GABAergic synapse                   | 7.77x10^-3    | GABRB2, SLC6A12   |

**B. Downregulated**

| Term                  | Description                        | P-value       | Key genes         |
|-----------------------|------------------------------------|---------------|-------------------|
| hsa04510              | Focal adhesion                      | 4.94x10^-5    | VAV2, ACTN1       |
| hsa05322              | Systemic lupus erythematosus        | 5.25x10^-5    | HIST1H2AE, HIST1H2BO |
| hsa05146              | Amebiasis                           | 2.19x10^-4    | CSF2, PRKCG       |
| hsa04670              | Leukocyte transendothelial migration| 5.99x10^-4    | VAV2, ACTN1       |
| hsa05034              | Alcoholism                          | 2.27x10^-3    | HIST1H2AE, HIST1H2BO |
| hsa04930              | Type II diabetes mellitus           | 2.87x10^-3    | MAFA, IRS1        |
| hsa04950              | Maturity onset diabetes of the young| 4.44x10^-3    | MAFA, GCK         |
| hsa05203              | Viral carcinogenesis                | 4.74x10^-3    | CDKN2A, IRF7      |
| hsa04060              | Cytokine-cytokine receptor interaction| 4.68x10^-3   | IL18, CLCF1       |
| hsa00601              | Glycosphingolipid biosynthesis-lacto and neolacto series | 4.95x10^-3 | FUT3, B3GNT3 |
| hsa04974              | Protein digestion and absorption    | 6.13x10^-3    | MME, COL4A6       |
| hsa05323              | Rheumatoid arthritis                | 6.42x10^-3    | IL18, CSF2        |
| hsa04640              | Hematopoietic cell lineage          | 8.37x10^-3    | CSF2, CSF3        |

GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
acid metabolic process. In particular, ADH7 expression levels gradually increased with an increase in patient age, while CYP26B1 expression gradually declined with a decrease in patient age, indicating that ADH7 and CYP26B1 are correlated with the differential progression of cervical cancer between different age groups.

It has been revealed that miR-499a influences the tumorigenesis of several different types of cancer, including breast cancer (43) and hepatocellular carcinoma (44). In addition, miR-499a contributes to the migration and proliferation of hepatitis B virus-associated hepatocellular carcinoma (HCC) cells (45). Recently, miR-499a-5p has been observed to promote migration, proliferation and epithelial-to-mesenchymal transition in metastatic lung cancer cell lines, via modulation of the mTOR pathway (46); however, the functions of miR-499 in cervical cancer development have not yet been fully elucidated. In the present study, miR-299a expression was upregulated in cervical cancer samples and was revealed to downregulate CYP26B1 expression. Thus, miR-499a may be associated with the tumorigenesis of cervical cancer via regulation of CYP26B1.

A previous study has suggested that downregulation of lncRNA WT1 inhibited cervical carcinoma cell migration and invasiveness via the regulation of miR-330-5p, indicating that miR-330-5p may influence cervical cancer progression (47). Although, to the best of our knowledge, there is currently no evidence that vestigial-like family member 3 (VGLL3) is associated with cervical cancer progression, the present study

Figure 3. ceRNA regulatory network comprising miRNAs, mRNAs and lncRNAs. miRNA-gene, miRNA-lncRNA and co-expressed lncRNA-mRNA pairs were identified and the lncRNA-miRNA-mRNA ceRNA network was constructed. Thereafter, functional analyses for crucial mRNAs located in the ceRNA network was performed. Red and green colors represent upregulated and downregulated genes, respectively. Triangles, circles and diamonds represent miRNAs, mRNAs and lncRNAs, respectively. The red line and the grey line refer to the pairs of lncRNA-mRNA interactions and miRNA-mRNA interactions, respectively. ceRNA, competing endogenous RNA; miRNA, microRNA; lncRNA, long non-coding RNA.
Figure 4. Enrichment analysis for crucial mRNA located in the ceRNA network was implemented. The R package clusterProfiler was applied to perform functional GO and KEGG pathway enrichment analyses for the DEmRNAs. The horizontal axis refers to the count of mRNAs, and the vertical axis represents the detailed terms of (A) GO-BP function or (B) KEGG pathway. The size of the dot represents the percentage of the total number of enriched mRNAs. The color gradient correlates to significance, whereby red indicates a higher significance. ceRNA, competing endogenous RNA; GO, Gene Ontology; BP, biological processes; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEmRNAs, differentially expressed mRNAs.
indicated that VGLL3 is regulated by miR-330 and was significantly correlated with cervical cancer survival rate. Therefore, it is speculated that VGLL3 may influence cervical cancer carcinogenesis via the regulation of miR-330 expression.

Table VI. Survival analysis for differentially expressed mRNAs.

| Genes     | P-value     | High median | Low median |
|-----------|-------------|-------------|------------|
| ADH7      | 2.44x10^{-02}a | 136.2000000 | N/A        |
| AHNAK2    | 5.20x10^{-01}  | 101.5333333 | N/A        |
| CRIM1     | 5.15x10^{-01}  | N/A         | 101.5333333 |
| CYP26B1   | 7.59x10^{-01}  | 103.2333333 | 101.5333333 |
| DNALI1    | 3.05x10^{-01}  | 136.2000000 | 101.5333333 |
| GABRB2    | 8.53x10^{-01}  | 103.2333333 | 136.2000000 |
| GDA       | 6.01x10^{-01}  | 103.2333333 | N/A        |
| HMGA2     | 8.94x10^{-01}  | 136.2000000 | 101.5333333 |
| HNF1B     | 6.79x10^{-01}  | 103.2333333 | 136.2000000 |
| HPSE      | 5.82x10^{-02}  | N/A         | 103.2333333 |
| KCNS1     | 2.00x10^{-01}  | N/A         | 96.26666667 |
| PCDHGB7   | 5.13x10^{-01}  | 136.2000000 | 101.5333333 |
| RASGRF1   | 6.53x10^{-01}  | NA          | 96.26666667 |
| VGLL3     | 2.87x10^{-02}a | 84.00000000 | N/A        |
| ZNF662    | 7.24x10^{-01}  | 103.2333333 | 136.2      |

N/A, not applicable. The data was missing within the database or incomputable.
Notably, VGLL3 expression gradually declined with decreasing patient age, suggesting that VGLL3 may be associated with the progression of cervical cancer in different age groups.

Numerous studies have indicated that the lncRNA LINC01133 serves as a regulator of various cancer types; for example, LINC01133 was revealed to suppress the invasiveness, migration and proliferation of nasopharyngeal carcinoma cells, via interaction with Y box binding protein 1 (48). Furthermore, Zheng et al (49) demonstrated that LINC01133 contributes to the progression of HCC via activation of the PI3K/AKT pathway. It has also been identified that LINC01133 was upregulated in lung squamous cell cancer cells and was associated with poorer overall survival outcomes (50). Finally, LINC01133 was determined to be associated with the survival time of patients with cervical squamous cell carcinoma (26). In the present study, LINC01133 was upregulated in cervical cancer samples and was demonstrated to interact with miR-3065-5p. Currently, the role of miR-3065 in cervical cancer progression is yet to be elucidated. However, a previous study indicated that miR-3065-5p expression is associated with the tumorigenesis of clear cell renal cell carcinomas (51). Notably, in the present study miR-3065 expression was down-regulated in cervical cancer samples and was revealed to regulate ADH7 expression. Hence, LINC01133 may serve as a ceRNA, via interaction with miR-3065, to upregulate ADH7 expression, resulting in the progression of cervical cancer.

Overall, ADH7, VGLL3, CYP26B1, miR-3065, miR-330, miR-499a and LINC01133 may influence the progression of cervical cancer in different age groups. These genes may therefore represent novel targets for the diagnosis or treatment of patients with cervical cancer. There limitations of the present study include insufficient sample size, lack of clinical practice and lack of research on the mechanism of intermolecular interactions. For future studies, the predicted results should be validated using experimental data, and the molecular mechanism of cervical cancer carcinogenesis requires further clarification.

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Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions
SD, XH, JZ, BX, LX, DG and WZ contributed to the design of the present study. SD, XH and WZ drafted the initial manuscript. JZ, BX, LX and DG interpreted the data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.
Competing interests
The authors declare that they have no competing interests.

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