

Putative Prognostic Value of miR-15a and miR-221 in Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma

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Abstract

Background/Aim: Cervical cancer remains a major global health burden among women, with high morbidity and mortality driven by dysregulation of key cell cycle regulatory pathways. MicroRNAs (miRNAs) act as post-transcriptional regulators of genes controlling cell proliferation and differentiation. This study analyzed the expression and prognostic significance of six cell cycle-related miRNAs (hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222) in a cervical squamous cell carcinoma and endocervical adenocarcinoma cohort (TCGA-CESC) obtained from TCGA data.

Materials and Methods: Expression data from 306 CESC tumor tissues and two normal cervical tissue samples were retrieved from TCGA via the UALCAN portal. Differential expression of the selected miRNAs was assessed, and associations with clinical and pathological parameters – including tumor stage, histologic grade, lymph node involvement, and patient age – were evaluated. Kaplan–Meier and log-rank analyses were used to determine correlations between miRNA expression and overall survival (OS).

Results: Among the analyzed miRNAs, hsa-miR-15a, hsa-miR-106b, hsa-miR-221, and hsa-miR-222 were significantly up-regulated in CESC, whereas hsa-miR-195 was significantly down-regulated. Age-stratified analysis demonstrated higher expression of hsa-miR-15a, hsa-miR-106b, and hsa-miR-222 in younger and middle-aged patients, suggesting age-dependent modulation of cell cycle miRNAs. Across tumor stages and grades in CESC, hsa-miR-15a, hsa-miR-106b, hsa-miR-221, and hsa-miR-222 were significantly up-regulated in Stage II and in moderately differentiated tumors (Grades 2-3), whereas hsa-miR-195 remained consistently down-regulated across all stages and lower tumor grades. Kaplan–Meier survival analysis demonstrated that high expression of hsa-miR-15a ($p=0.023$) and hsa-miR-221 ($p=0.048$) significantly correlated with poorer overall survival, suggesting their potential prognostic values.

Conclusion: Dysregulated expression of cell-cycle-related miRNAs, particularly hsa-miR-15a and hsa-miR-221, may contribute to CESC progression and serve as potential prognostic biomarkers.

Keywords: Cervical cancer, CESC, microRNA, TCGA, UALCAN, hsa-miR-15a, hsa-miR-221, overall survival, biomarker.



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Introduction

Cervical cancer is a major global health concern and one of the leading causes of cancer-related morbidity and mortality in women (1). The two principal histological subtypes – cervical squamous cell carcinoma (CSCC) and endocervical adenocarcinoma – together account for the majority of cases, with squamous histology comprising approximately 75-90% of cases worldwide (2). CSCC typically develops through a stepwise progression from cervical intraepithelial neoplasia (CIN) to invasive carcinoma as a result of long-term infection with high-risk human papillomavirus (HPV) strains (3, 4). Nonetheless, its pathogenesis involves a multifactorial interplay of molecular, immunological, and clinical determinants that collectively influence tumor initiation, progression, and prognosis (5). These factors disrupt normal cell cycle regulation, promote uncontrolled proliferation, and facilitate invasion and metastasis (6-9).

MicroRNAs (miRNAs) are small non-coding RNAs, approximately 19-25 nucleotides in length, that control gene expression post-transcriptionally by binding to target mRNAs. They play critical roles in fundamental biological processes, including cell cycle progression, apoptosis, differentiation, and DNA damage response. Dysregulation of miRNAs has been implicated in the initiation, progression, and therapeutic response of various cancers, including cervical cancer (10, 11).

In particular, cell cycle/proliferation-related miRNAs such as hsa-miR-16, hsa-miR-15a, hsa-miR-195, hsa-miR-221, hsa-miR-222, hsa-miR-106b, and hsa-miR-93 have been shown to influence tumor growth by targeting key regulators of the G₁/S and G₂/M checkpoints, cyclins, and cyclin-dependent kinases (12-15). Altered expressions of these miRNAs can either promote oncogenesis or act as tumor suppressors, depending on their targets and cellular context (16).

The present study aims to examine the expression patterns and survival associations of six key cell cycle-related miRNAs in cervical squamous cell carcinoma and

endocervical adenocarcinoma obtained from The Cancer Genome Atlas (TCGA)-cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) database. The analysis was performed using University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN), a publicly accessible resource built on TCGA that enables comprehensive assessment of gene and microRNA expression in relation to clinical parameters (17, 18). Through UALCAN, we compared miRNA expression between tumor and normal tissues, assessed their associations with clinicopathological factors such as patient age, cancer stages, tumor grade, nodal metastasis status, overall survival, and analyzed their impact on patient survival. These findings may help identify potential prognostic biomarkers and therapeutic targets in cervical cancer.

Materials and Methods

Data source. The expression data of cell cycle/proliferation-related microRNAs (miRNAs) in TCGA-CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma) were obtained from UALCAN (<http://ualcan.path.uab.edu/>), which provides access to TCGA datasets *via* the Genomic Data Commons (GDC). Analyses were based on TCGA-CESC Level 3 miRNA-seq data normalized to log₂(TPM+1) values (17, 18). UALCAN enables analysis of tumor *versus* normal tissue expression, stratification by clinical features, and survival analysis. Analyses in this study were performed using the most recent publicly available version of UALCAN, accessed in September 2025.

Selection of miRNAs. Six miRNAs implicated in cell cycle regulation and tumor proliferation were selected for analysis: hsa-miR-15a, hsa-miR-195, hsa-miR-221, hsa-miR-222, hsa-miR-106b, and hsa-miR-93. These miRNAs were chosen based on published evidence demonstrating their involvement in regulation of cyclins, cyclin-dependent kinases (CDKs), and other key cell cycle checkpoints.

Table I. Expression pattern of cell cycle/proliferation-related miRNAs in CESC.

miRNA	Expression pattern in primary tumor compared to normal tissue	p-Value
hsa-miR-15a	Up-regulated	0.047
hsa-miR-93	Up-regulated	0.064
hsa-miR-106b	Up-regulated	<0.0001
hsa-miR-195	Down-regulated	0.00494
hsa-miR-221	Up-regulated	<0.0001
hsa-miR-222	Up-regulated	<0.0001

NS: Not significant; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma. *p*-Values calculated using Welch's *t*-test comparing tumor vs. normal cervical tissue.

Study population. TCGA-CESC cases with available miRNA expression data in UALCAN; TCGA normal cervical tissues served as controls. As implemented in UALCAN, TCGA-CESC aggregates cervical squamous cell carcinoma and endocervical adenocarcinoma; no histology-specific filtering was applied. Clinical parameters retrieved from UALCAN included: patient age, cancer stages, tumor grade, nodal metastasis status, overall survival.

Expression analysis. Expression levels of the selected miRNAs were analyzed in UALCAN using TCGA miRNA-seq data [$\log_2(\text{TPM}+1)$]. For each normal/tumor subgroup, UALCAN displays box-and-whisker plots showing the interquartile range (IQR) – minimum, 1st quartile, median, 3rd quartile, and maximum – after outlier filtering *via* a descriptive PERL module. Welch's *t*-test was used by the portal to estimate the significance of differences in expression between normal and primary tumors and among tumor subgroups defined by clinicopathological features. Unless otherwise indicated, $p < 0.05$ was considered statistically significant. All box-plot figures were generated directly in UALCAN (TCGA-CESC).

Survival analysis. Overall survival was evaluated in UALCAN by dichotomizing primary tumor samples into High expression ($\geq 3^{\text{rd}}$ quartile) and Low/Medium expression ($< 3^{\text{rd}}$ quartile). Kaplan–Meier curves were generated, and log-rank tests were used to derive *p*-values (R packages survival and survminer were used,

implemented by the portal, R Foundation, Vienna, Austria). Survival plots were generated by UALCAN.

Results

Differential expression of cell cycle–related miRNAs in CESC.

The expression of six cell cycle/proliferation-associated miRNAs (hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222) were evaluated in CESC tumor tissues (n=306) compared to normal cervical tissues (n=2) using TCGA datasets *via* UALCAN.

As shown in Table I, hsa-miR-15a, hsa-miR-106b, hsa-miR-221, and hsa-miR-222 were significantly up-regulated in tumor tissues relative to normal tissues. hsa-miR-93 displayed moderate up-regulation ($p=0.064$), while hsa-miR-106b, hsa-miR-221, and hsa-miR-222 exhibited highly significant overexpression ($p < 0.0001$ for all). In contrast, the tumor suppressor miRNA hsa-miR-195 was significantly down-regulated in tumors compared to normal tissue ($p=0.0049$) (Figure 1).

Age-stratified variation in cell cycle miRNA expression.

To examine potential age-related patterns in miRNA dysregulation, the six key cell cycle/proliferation-associated miRNAs (hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222) were analyzed across four patient age groups (21-40, 41-60, 61-80, and 81-100 years) in CESC tumor tissues.

hsa-miR-15a was significantly up-regulated in younger and middle-aged patients, with elevated expression in the

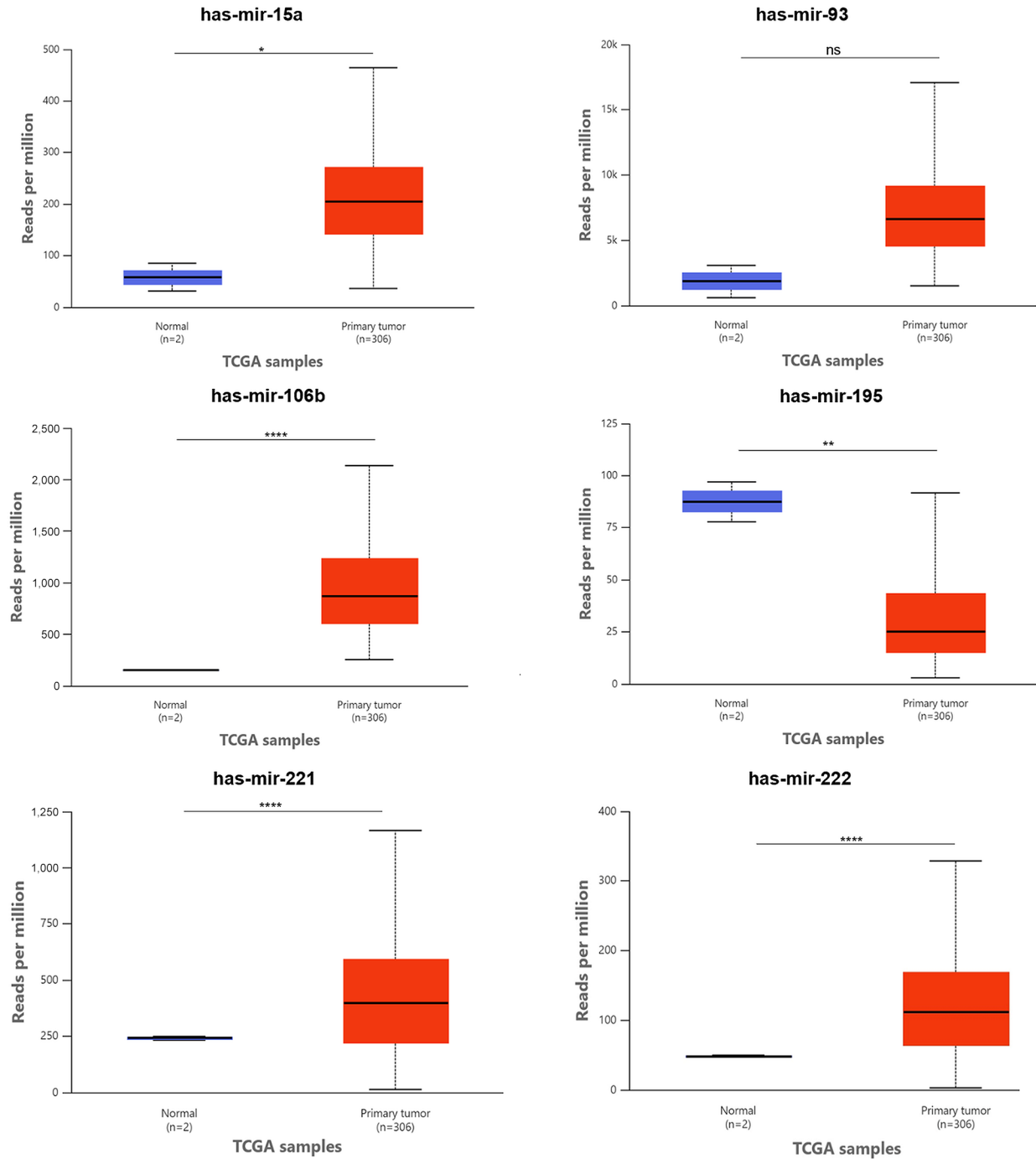


Figure 1. Differential expression levels of six selected cell cycle/proliferation miRNAs in normal versus primary tumor tissues in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC). Box plots display the expression levels (reads per million) of six selected miRNAs – hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222 – in The Cancer Genome Atlas (TCGA)-CESC primary tumor samples (n=306) compared with normal cervical tissues (n=2) obtained from TCGA via the UALCAN portal. hsa-miR-15a, hsa-miR-106b, hsa-miR-221, and hsa-miR-222 were significantly up-regulated, whereas hsa-miR-195 was significantly down-regulated in tumor tissues; hsa-miR-93 showed no significant change. Statistical analysis was performed using Welch's t-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. ns: Not significant.

Table II. Expression of cell cycle-related miRNAs by age group in CESC.

miRNA	21-40 years	41-60 years	61-80 years	81-100 years
miR-15a	Up ($p=0.03$)	Up ($p=0.04$)	NS ($p>0.05$)	NS ($p>0.05$)
miR-93	Up ($p=0.05$)	Up ($p=0.04$)	NS ($p>0.05$)	Up ($p=0.01$)
miR-106b	Up ($p<0.0001$)	Up ($p<0.0001$)	Up ($p<0.0001$)	Up ($p=0.06$)
miR-195	Down ($p=0.02$)	Down ($p=0.0007$)	Down ($p=0.02$)	Down ($p=0.02$)
miR-221	NS ($p>0.05$)	Up ($p<0.0001$)	Up ($p=0.0025$)	NS ($p>0.05$)
miR-222	Up ($p<0.0001$)	Up ($p<0.0001$)	Up ($p<0.0001$)	NS ($p=0.18$)

NS: Not significant; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma. p -Values calculated using Welch's t -test comparing tumor vs. normal cervical tissue.

21-40 ($p=0.03$) and 41-60-year groups ($p=0.04$), whereas no significant differences were observed in the 61-80 and 81-100-year groups ($p>0.05$). Similarly, hsa-miR-106b was significantly overexpressed in nearly all age groups, including 21-40 ($p<0.0001$), 41-60 ($p<0.0001$), 61-80 ($p<0.0001$), and 81-100 ($p=0.06$), indicating consistent up-regulation across the patient age spectrum.

Furthermore, hsa-miR-222 was shown to be significantly elevated in patients aged 21-40 ($p<0.0001$), 41-60 ($p<0.0001$), and 61-80 ($p<0.0001$), while the oldest group (81-100 years) did not show significant changes ($p=0.18$), suggesting a gradual decline in expression with advancing age. hsa-miR-93 showed elevated expression in 21-40 ($p=0.05$), 41-60 ($p=0.04$), and 81-100-year groups ($p=0.01$), indicating partial age-dependent modulation.

hsa-miR-221 was significantly up-regulated in middle-aged patients, including 41-60 ($p<0.0001$) and 61-80-year groups ($p=0.0025$), while younger and older age groups did not show significant changes. In contrast, the tumor suppressor miRNA hsa-miR-195 was consistently down-regulated across all age groups, with significant reductions observed in 21-40 ($p=0.02$), 41-60 ($p=0.0007$), 61-80 ($p=0.02$), and 81-100-year groups ($p=0.02$) (Table II, Figure 2).

These findings indicate that oncogenic miRNAs are predominantly up-regulated in early- to middle-aged patients, whereas tumor suppressor miRNAs exhibit consistent down-regulation across the age spectrum, highlighting age-dependent dysregulation of cell cycle pathways in CESC tissues.

miRNA expression patterns across cancer stages. To investigate potential variations in miRNA expression during tumor progression, the six key cell cycle/proliferation-related miRNAs (hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222) were examined across cancer stages I-IV in CESC tumor tissues using TCGA dataset. hsa-miR-15a was significantly up-regulated in Stage I ($p=0.039$), Stage II ($p=0.041$), and Stage IV ($p=0.029$) relative to normal tissue, whereas Stage III ($p>0.05$) did not show a statistically significant change. In contrast, hsa-miR-195 was consistently down-regulated across all stages, with Stage I ($p=0.0124$), Stage II ($p<0.0001$), Stage III ($p=0.020$), and Stage IV ($p=0.007$), reflecting progressive loss of tumor-suppressor activity.

Oncogenic miRNAs hsa-miR-106b, hsa-miR-222, and hsa-miR-221 were up-regulated in early- to mid-stages. hsa-miR-106b was significantly elevated across all stages ($p<0.0001$). hsa-miR-222 showed significant overexpression in Stages I-III ($p<0.0001$, 3.04×10^{-7} , 2.45×10^{-4}), with a significant decrease in Stage IV ($p=0.0146$). hsa-miR-221 was significantly up-regulated in Stage II ($p<0.0001$) and Stage III ($p=0.007$), while Stages I and IV were not significant. hsa-miR-93 exhibited a trend toward up-regulation across stages, but differences were not statistically significant ($p>0.05$) (Table III and Figure 3).

Association between tumor grade and cell cycle miRNA expression. To examine how cell cycle/proliferation-related miRNAs vary with tumor differentiation, the selected six key miRNAs were evaluated across tumor

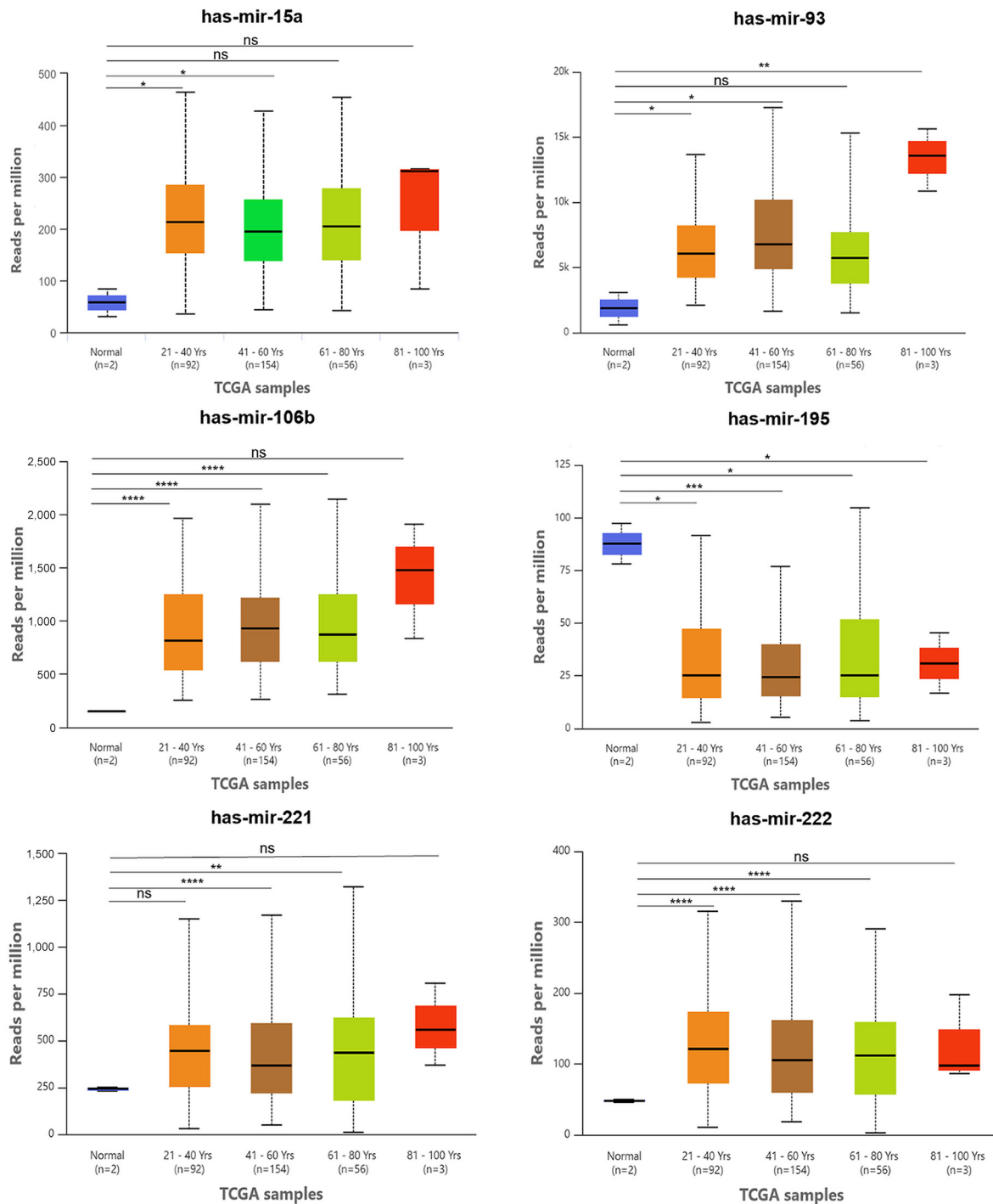


Figure 2. Expression of cell cycle/proliferation-associated miRNAs in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) across patient age groups. Box-and-whisker plots [log₂(TPM+1)] show expression of hsa-miR-15a, hsa-miR-195, hsa-miR-222, hsa-miR-106b, hsa-miR-93, and hsa-miR-221 in normal cervical tissue (n=2) and across patient age groups: 21-40 (n=92), 41-60 (n=154), 61-80 (n=56), and 81-100 years (n=3), using TCGA-CESC data accessed via UALCAN. Boxes depict the interquartile range (IQR) with median; whiskers indicate minimum and maximum after outlier filtering as implemented by the portal. p-Values as reported by UALCAN (Welch's t-test); *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ns: Not significant.

Table III. *miRNA levels across tumor stages in CESC.*

miRNA	Stage I	Stage II	Stage III	Stage IV
hsa-miR-15a	Up ($p=0.039$)	Up ($p=0.041$)	NS ($p>0.05$)	Up ($p=0.029$)
hsa-miR-93	NS ($p>0.05$)	NS ($p>0.05$)	NS ($p>0.05$)	NS ($p>0.05$)
hsa-miR-106b	Up ($p<0.001$)	Up ($p<0.001$)	Up ($p<0.001$)	Up ($p<0.001$)
hsa-miR-195	Down ($p=0.012$)	Down ($p<0.001$)	Down ($p=0.020$)	Down ($p=0.007$)
hsa-miR-221	NS ($p>0.05$)	Up ($p<0.001$)	Up ($p=0.007$)	NS ($p>0.05$)
hsa-miR-222	Up ($p<0.001$)	Up ($p<0.001$)	Up ($p<0.001$)	Down ($p=0.015$)

NS: Not significant; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma. p -Values calculated using Welch's t -test comparing tumor vs. normal cervical tissue.

grades 1-4 in CESC using the TCGA dataset. hsa-miR-15a was significantly up-regulated in Grade 2 ($p=0.046$) and Grade 3 ($p=0.048$) relative to normal tissues, whereas Grade 1 ($p>0.05$) and Grade 4 ($p>0.05$) did not show statistically significant changes. Similarly, hsa-miR-195 was down-regulated in Grades 1-3 (Grade 1, $p=0.019$; Grade 2, $p<0.0001$; Grade 3, $p=0.012$), while Grade 4 ($p>0.05$) showed no significant difference.

Oncogenic miRNAs hsa-miR-106b, hsa-miR-221, and hsa-miR-222 were up-regulated in Grades 1-3, with highly significant changes for hsa-miR-106b and hsa-miR-222 ($p<0.0001$) and for hsa-miR-221 in Grades 2 and 3 (Grade 2, $p<0.0001$; Grade 3, $p=0.007$). Grade 4 did not reach statistical significance for most oncogenic miRNAs. hsa-miR-93 showed a non-significant trend across all grades ($p>0.05$) (Table IV and Figure 4).

Expression of cell cycle/proliferation miRNAs across nodal metastasis status in CESC. To examine whether cell cycle/proliferation-related miRNAs vary with nodal metastasis, the selected six key miRNAs were examined across tumors without nodal metastasis (N0) and those with nodal metastasis (N1) using TCGA datasets. hsa-miR-15a and hsa-miR-93 showed non-significant trends toward higher expression in both N0 ($p=0.052$ and 0.079 , respectively) and N1 ($p=0.065$ and 0.088 , respectively) compared to normal tissue. hsa-miR-106b and hsa-miR-222 were significantly up-regulated in both N0 and N1 tumors ($p<0.0001$), indicating consistent oncogenic overexpression regardless of nodal involvement.

hsa-miR-195 was significantly down-regulated in both N0 ($p<0.001$) and N1 ($p=0.0113$) groups. In contrast, hsa-miR-221 was significantly up-regulated in N0 tumors ($p<0.0001$) but not in N1 tumors ($p=0.223$), suggesting variable expression depending on nodal involvement (Table V and Figure 5). No statistically significant difference was observed between N0 and N1 groups for any of the analyzed miRNAs (all $p>0.05$).

Association of miRNA expression with overall survival in CESC patients. To evaluate the prognostic significance of candidate miRNAs in CESC tumor tissues, Kaplan–Meier survival analyses were performed for hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222. Patients were stratified into high and low/medium expression groups, and overall survival (OS) was compared. High expression of hsa-miR-15a was significantly associated with reduced OS ($p=0.023$), indicating a potential oncogenic role in patient prognosis. Similarly, elevated hsa-miR-221 expression correlated with poorer survival ($p=0.048$). In contrast, no significant differences in OS were observed for hsa-miR-93 ($p=0.13$), hsa-miR-106b ($p=0.66$), hsa-miR-195 ($p=0.59$), or hsa-miR-222 ($p=0.053$), although trends toward reduced survival were noted in some high-expression groups (Table VI and Figure 6).

Discussion

CESC represent the predominant histological subtypes of cervical cancer and are primarily driven by persistent

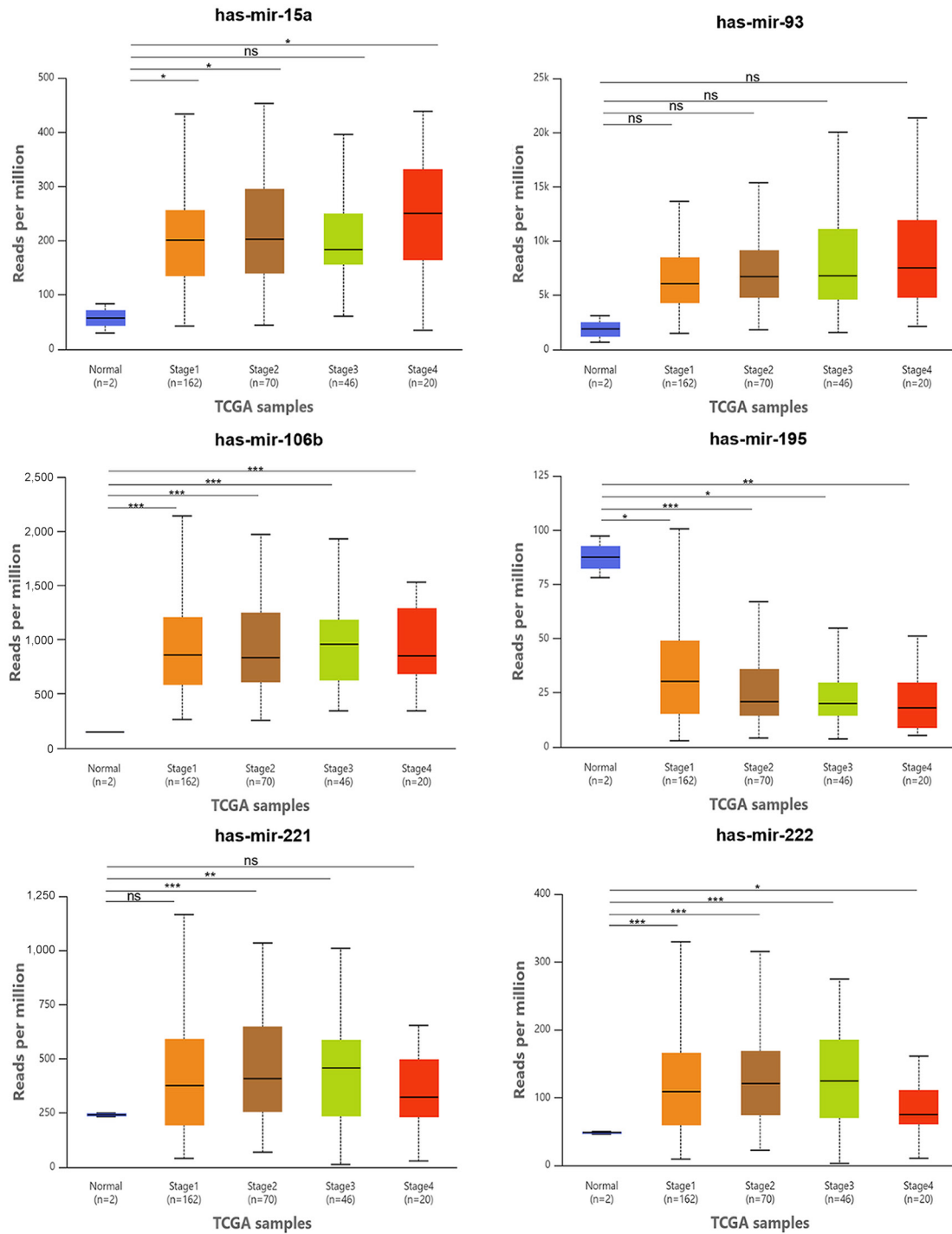


Figure 3. Stage-wise expression of six cell cycle/proliferation-related miRNAs in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (TCGA/UALCAN). Box-and-whisker plots [$\log_2(\text{TPM}+1)$] show expression of hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222 in normal tissue (n=2) and CESC stages I (n=162), II (n=70), III (n=46), and IV (n=20) using TCGA-CESC data accessed via UALCAN. Boxes indicate IQR with median; whiskers denote minimum and maximum after outlier filtering as implemented by the portal. p-Values as reported by UALCAN (Welch's t-test); * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. ns: Not significant.

Table IV. *miRNA expression by tumor grade in CESC.*

miRNA	Grade 1	Grade 2	Grade 3	Grade 4
hsa-miR-15a	NS ($p>0.05$)	Up ($p=0.046$)	Up ($p=0.048$)	NS ($p>0.05$)
hsa-miR-93	NS ($p>0.05$)	NS ($p>0.05$)	NS ($p>0.05$)	NS ($p>0.05$)
hsa-miR-106b	Up ($p<0.0001$)	Up ($p<0.0001$)	Up ($p<0.0001$)	NS ($p>0.05$)
hsa-miR-195	Down ($p=0.019$)	Down ($p<0.0001$)	Down ($p=0.012$)	NS ($p>0.05$)
hsa-miR-221	NS ($p>0.05$)	Up ($p<0.0001$)	Up ($p=0.007$)	NS ($p>0.05$)
hsa-miR-222	Up ($p<0.0001$)	Up ($p<0.0001$)	Up ($p<0.0001$)	NS ($p>0.05$)

NS: Not significant; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma. p -Values calculated using Welch's t -test comparing tumor vs. normal cervical tissue.

infection with HR-HPV strains (1). Recent screening studies further highlight the substantial burden of HR-HPV infections, including the frequent coexistence of multiple HR-HPV types in clinical practice, underscoring the central etiologic role of HPV in cervical carcinogenesis (19). Moreover, recent whole-genome sequencing of HPV-positive cervical and head-and-neck squamous cell carcinomas delineates recurrent genomic alterations and pathways that may intersect with miRNA-mediated regulation (20). Increasing evidence suggests that miRNAs play central roles in its pathogenesis by regulating genes involved in the cell cycle, apoptosis, and DNA repair (10, 11). Prior studies have demonstrated that aberrant expression of several cell cycle-associated miRNAs contribute to malignant transformation and progression in cervical and other epithelial cancers (21). This study builds upon that foundation by evaluating the expression and prognostic implications of six key miRNAs—hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222—in a large CESC cohort from TCGA, which comprises Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma, stratified by clinicopathological features and age.

Among these, hsa-miR-221 and hsa-miR-222 have been previously identified as oncomiRs in cervical and breast cancers, where they promote proliferation by targeting CDKN1B (p27Kip1) and PTEN, leading to deregulation of cell cycle checkpoints (22, 23). Our findings support and extend these reports, showing consistent up-regulation of hsa-miR-221 and hsa-miR-222 in early-to-mid stage and moderately differentiated CESC

tumors. Notably, hsa-miR-221 was also significantly associated with worse overall survival, consistent with previous studies indicating its prognostic significance in gynecological cancers (24).

hsa-miR-15a has been classically considered a tumor suppressor in hematological malignancies due to its targeting of BCL2, yet several recent studies have reported its paradoxical oncogenic activity in solid tumors, including cervical cancer (25). Our results align with this emerging evidence showing up-regulation of hsa-miR-15a in CESC tumor tissues and a significant association with poor prognosis. These findings suggest a context-dependent role for miR-15a, possibly influenced by the local tumor microenvironment or differential target gene expression.

While our study found up-regulation of hsa-miR-106b-5p and hsa-miR-93-5p in CESC, prior studies reported their down-regulation and tumor-suppressive roles, with overexpression suppressing proliferation and migration by targeting oncogenes like FGF4 and being regulated by circRNAs (26, 27). These discrepancies may reflect tumor heterogeneity or cohort-specific epigenetic factors.

Conversely, in colorectal cancer, both miRNAs are commonly up-regulated and promote tumor progression *via* PTEN, p21, CDK6, and the PI3K/Akt pathway (28, 29). However, in some CRC studies, miR-106b-5p also showed tumor-suppressive effects by inhibiting metastasis through CTSA and FAT4 (30, 31), highlighting their context-dependent dual roles. Our study confirms their up-regulation in CESC, particularly in younger patients and early-stage tumors. While hsa-miR-106b was up-

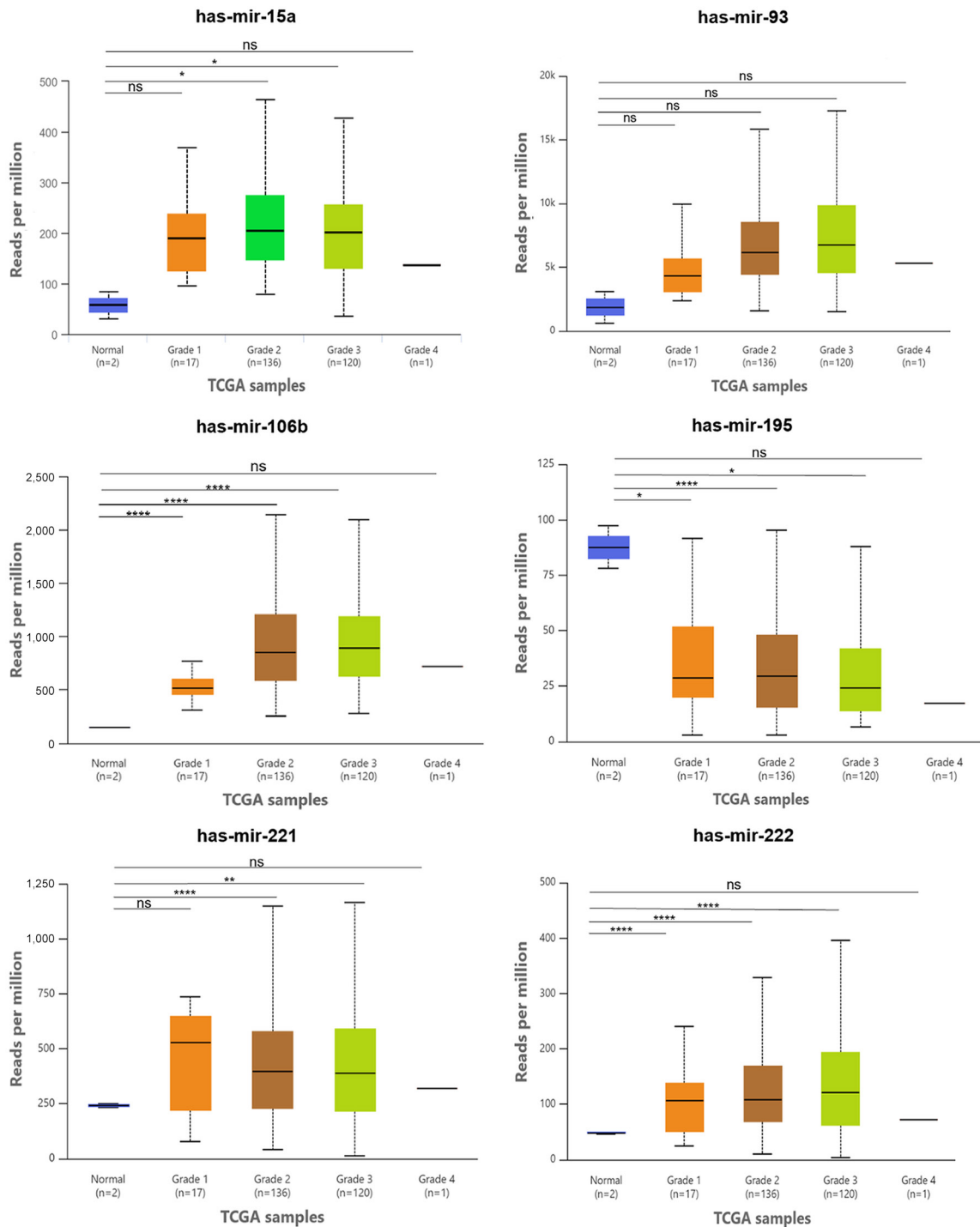


Figure 4. Expression of six cell cycle/proliferation miRNAs across tumor grades (1-4) in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (TCGA/UALCAN). Box-and-whisker plots $[\log_2(\text{TPM}+1)]$ show expression of hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222 in normal tissue (n=2) and tumor grades G1 (n=17), G2 (n=136), G3 (n=120), and G4 (n=1) using TCGA-CESC data accessed via UALCAN. Boxes indicate IQR with median; whiskers denote minimum and maximum after outlier filtering as implemented by the portal. p-Values as reported by UALCAN (Welch's t-test); * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. ns: Not significant.

Table V. Nodal metastasis and miRNA expression in CESC.

miRNA	N0 (No Metastasis)	N1 (Metastasis)
hsa-miR-15a	NS ($p=0.052$)	NS ($p=0.065$)
hsa-miR-93	NS ($p=0.079$)	NS ($p=0.088$)
hsa-miR-106b	Up ($p<0.0001$)	Up ($p<0.0001$)
hsa-miR-195	Down ($p<0.001$)	Down ($p=0.0113$)
hsa-miR-221	Up ($p<0.0001$)	NS ($p=0.223$)
hsa-miR-222	Up ($p<0.0001$)	Up ($p<0.0001$)

NS: Not significant; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma. p -Values calculated using Welch's t -test comparing tumor vs. normal cervical tissue.

regulated across all clinical subgroups, it did not correlate with overall survival, suggesting that its role may be more relevant to tumor initiation rather than progression.

In contrast, we observed consistent down-regulation of hsa-miR-195 across all age groups, tumor stages, grades, and nodal statuses in CESC tumor tissues, supporting its well-recognized tumor suppressive role. This aligns with previous studies linking low miR-195 expression to advanced disease, metastasis, and poor prognosis in cervical cancer (32, 33). miR-195 is known to inhibit cancer progression by targeting key oncogenes involved in cell cycle regulation and invasion (34, 35). Our findings further highlight its potential as a prognostic biomarker and therapeutic target in CESC.

Our age-stratified analysis revealed higher expression of hsa-miR-15a, hsa-miR-106b, and hsa-miR-222 in younger and middle-aged CESC patients, which may suggest biological influences such as hormonal signaling. miR-222 has been shown to be hormonally regulated in prostate and breast cancer promoting androgen independence in prostate cancer (36). However, its hormonal regulation in cervical cancer remains unexplored. For miR-15a and miR-106b, studies suggest roles in hormone-related cancers like endometrial and breast cancer, but without direct hormonal regulation demonstrated (37, 38). While our findings suggest age-related variation in miRNA expression, the underlying hormonal or epigenetic mechanisms remain to be clarified in CESC and warrant further investigation.

Table VI. Kaplan–Meier analysis of miRNA expression and patient survival in CESC.

miRNA	High expression vs. Low/Medium	p -Value
hsa-miR-15a	Poorer OS	0.023
hsa-miR-93	Trend toward poorer OS	0.13
hsa-miR-106b	No significant difference	0.66
hsa-miR-195	No significant difference	0.59
hsa-miR-221	Poorer OS	0.048
hsa-miR-222	Trend toward poorer OS	0.053

OS, Overall survival; NS, not significant; CESC; cervical squamous cell carcinoma and endocervical adenocarcinoma. p -Values calculated using log-rank test comparing tumor vs. normal cervical tissues. Significant p -values are shown in bold.

Interestingly, no significant expression differences were observed between nodal metastasis groups for most miRNAs, suggesting that their primary role may lie in early tumorigenesis rather than metastatic progression. This aligns with previous studies showing that while some miRNAs are associated with lymph node metastasis in specific cancers, such links are not universal and often context-dependent (39, 40). Our findings support the notion that lymphatic dissemination in cervical cancer may be governed by pathways beyond miRNA regulation.

Importantly, our survival analysis identified hsa-miR-15a and hsa-miR-221 as potential markers of poor prognosis in cervical cancer. This aligns with prior studies reporting elevated miR-15a expression associated with unfavorable outcomes in SCC, although it is not consistently validated as an independent prognostic factor (41, 42). Similarly, miR-221 has been linked to lymphatic spread and tumor aggressiveness, including its presence in tumor-derived exosomes (43). However, its role as an independent prognostic marker in cervical cancer remains unconfirmed. These results suggest that while both miRNAs are associated with disease progression, their prognostic utility may be context-dependent and require further validation through multivariate models.

Despite these findings, several limitations should be acknowledged. The TCGA-CESC cohort comprises more than 80% squamous cell carcinoma and a smaller proportion of endocervical adenocarcinoma cases; therefore, the absence

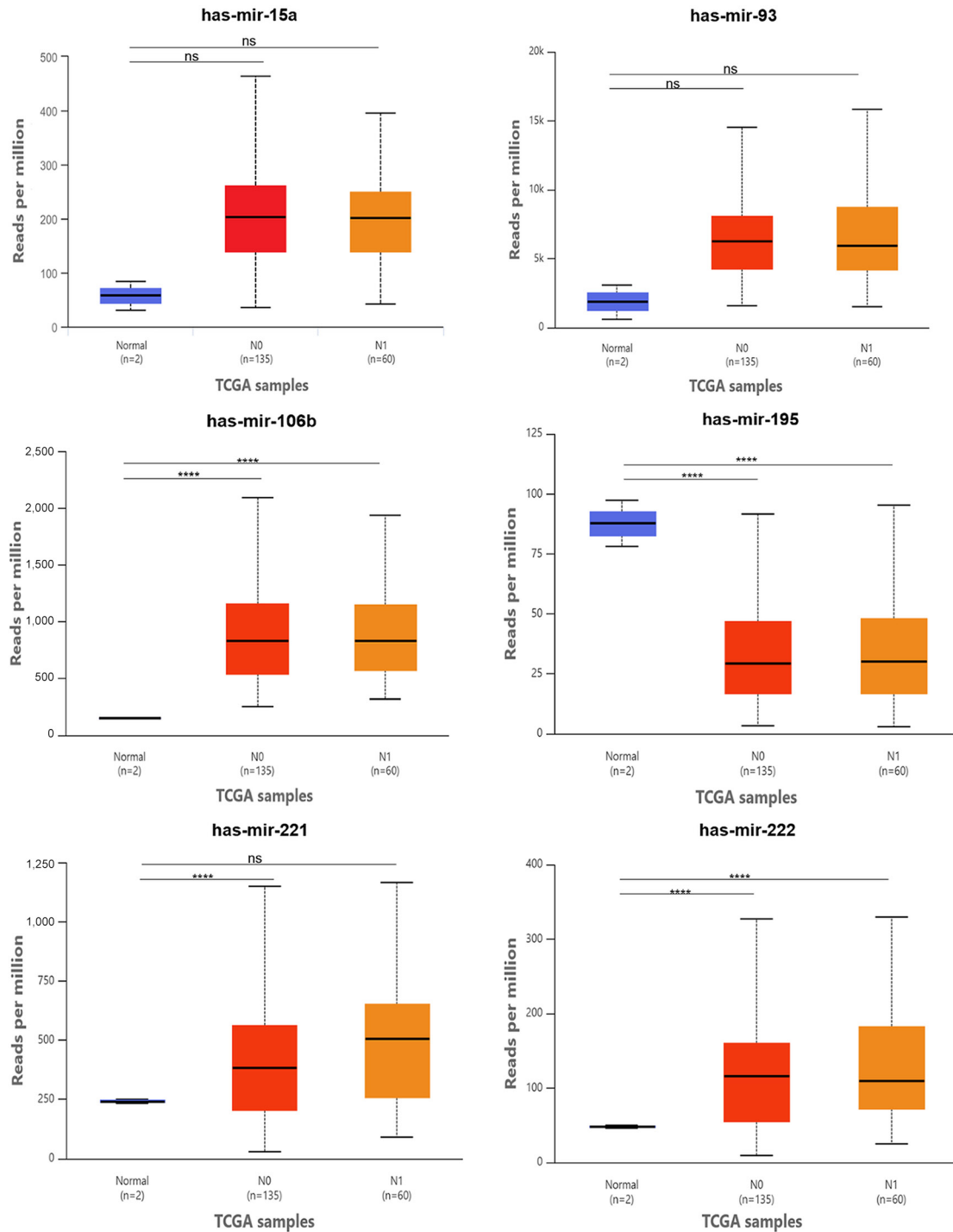


Figure 5. Expression of six cell cycle/proliferation miRNAs in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) based on nodal metastasis status (N0 vs. N1) from TCGA/UALCAN. Box-and-whisker plots [$\log_2(\text{TPM}+1)$] show expression of has-miR-15a, has-miR-93, has-miR-106b, has-miR-195, has-miR-221, and has-miR-222 in normal tissue (n=2) and CESC tumors stratified by N0 (n=135) and N1 (n=60) using TCGA-CESC data accessed via UALCAN. Boxes indicate IQR with median; whiskers denote minimum and maximum after outlier filtering as implemented by the portal. p-Values as reported by UALCAN (Welch's t-test); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. ns: Not significant.

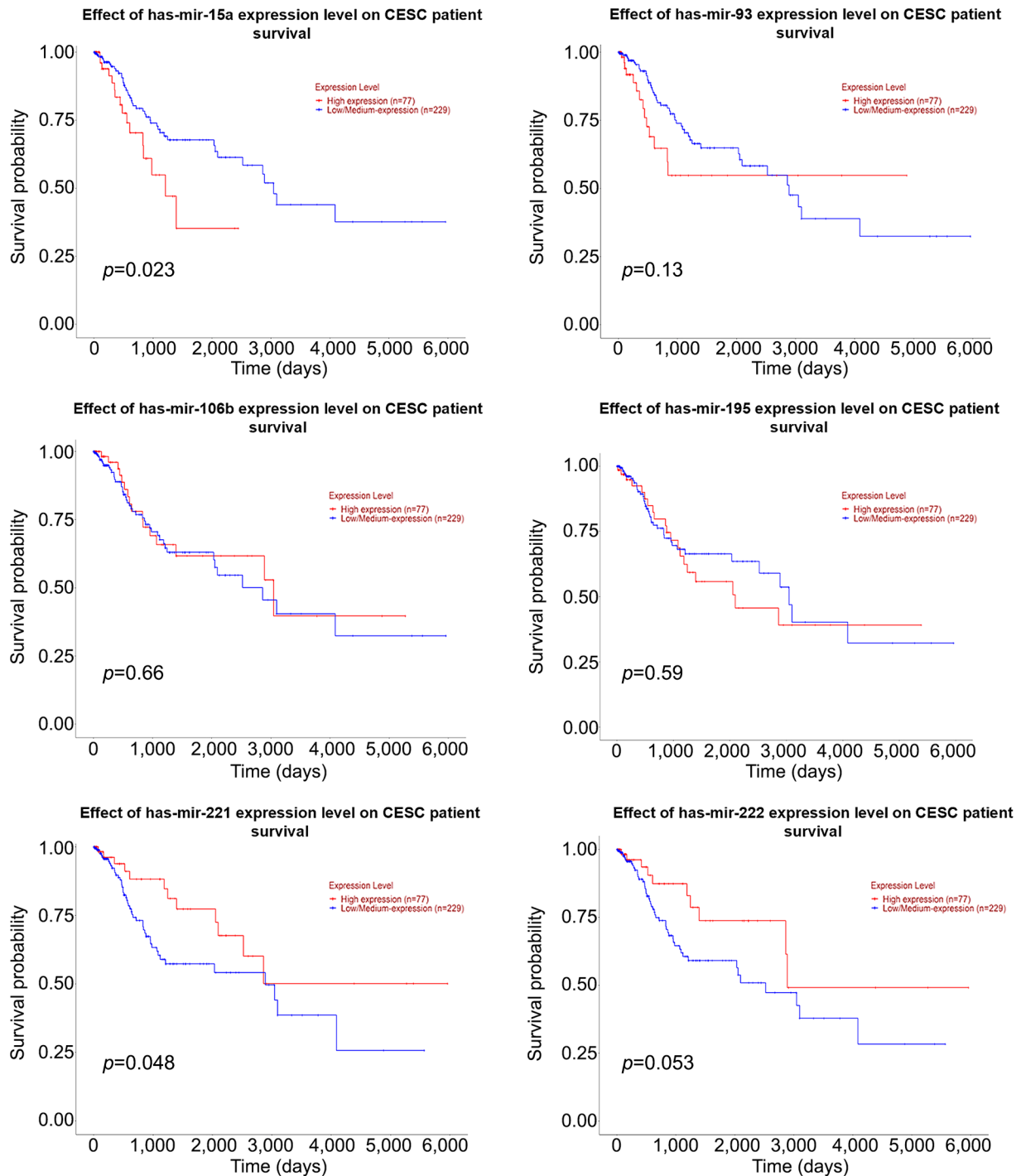


Figure 6. Kaplan–Meier survival analysis of cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patients stratified by miRNA expression levels. Survival curves depict overall survival in patients with high (red) versus low/medium (blue) expression of hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-195, hsa-miR-221, and hsa-miR-222. The number of patients in each group is indicated in the legend. Statistical significance was determined using the log-rank test, with p-Values shown on each panel. High expression of hsa-miR-15a ($p=0.023$) and hsa-miR-221 ($p=0.048$) was associated with reduced survival, whereas other miRNAs showed non-significant trends ($p>0.05$).

of histology-specific stratification may limit the generalizability of the results. Moreover, the analysis relied on TCGA-CESC data containing only two normal cervical tissue samples, which constrains the robustness of tumor–normal differential expression comparisons. In addition, information on HPV subtype, a key determinant of cervical carcinogenesis, was unavailable for stratified analysis, potentially obscuring the influence of viral heterogeneity on miRNA regulation. Finally, although statistically significant associations were identified, this study lacked experimental validation to confirm the mechanistic roles of the candidate miRNAs. To strengthen the translational potential of these findings, future research should focus on experimental validation of miRNA targets, investigate their role in HPV-positive *versus* HPV-negative tumors, and explore their potential as therapeutic targets through miRNA mimics or antagomiRs. Integration with immune profiling and proteomic data may also uncover novel miRNA-mediated regulatory axes that influence immune evasion or therapy resistance.

Conclusion

This study reinforces and extends existing knowledge on the role of cell cycle–regulatory miRNAs in CESC. The up-regulation of hsa-miR-15a, hsa-miR-93, hsa-miR-106b, hsa-miR-221, and hsa-miR-222, alongside consistent down-regulation of the tumor suppressor hsa-miR-195, underlines the dysregulation of miRNA networks governing cell proliferation and differentiation in CESC. Notably, the prognostic significance of hsa-miR-15a and hsa-miR-221 suggests their potential utility as biomarkers for risk stratification. By contextualizing these findings within prior literature and identifying novel age- and stage-specific patterns, this study contributes to a more nuanced understanding of miRNA biology in CESC and highlights opportunities for miRNA-targeted diagnostics and therapies.

Conflicts of Interest

The Authors report no conflicts of interest related to the content of this study.

Authors' Contributions

The Author was solely responsible for the study conception, data analysis, interpretation, manuscript writing, and final approval.

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Artificial Intelligence (AI) Disclosure

During the preparation of this manuscript, a large language model (ChatGPT, OpenAI) was used solely for language editing and stylistic improvements in select paragraphs. No sections involving the generation, analysis, or interpretation of research data were produced by generative AI. All scientific content was created and verified by the author. Furthermore, no figures or visual data were generated or modified using generative AI or machine learning–based image enhancement tools.

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