Medical University Pleven
Faculty of Medicine
Department of Obstetrics and Gynaecology

D-r Eva Petrova Tsoneva

The role of ROMO1 in the pathogenesis of cervical carcinoma

Abstract of a Dissertation
For the award of educational and scientific degree

“Doctor”

Doctoral programme:Obstetrics and Gynaecology

Pleven 2026

Table of contents **Page**

The dissertation comprises 128 standard pages and is structured into the following sections: Introduction, Literature Review, Aim and Objectives, Materials and Methods, Results, Discussion, Conclusion, Conclusions, Contributions of the Dissertation, Publications, Scientific Forums Related to the Dissertation, Appendices, and References.

The work is illustrated with 20 figures and 4 tables. Only one appendix is included in the section “Appendices”. The bibliographic list contains 96 references.

In relation to the dissertation, three full-text publications and four scientific communications (posters) presented at European scientific forums have been produced.

The dissertation has been approved and submitted for public defense by an extended Department Council of the Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University – Pleven, and on the basis of Order of the Rector No. 1087/31.03.2026, before a scientific jury composed of:

Chairperson:

Corresponding Member Prof. Slavcho Tomov, MD, PhD,DSc

Members:

Corresponding Member Prof. Slavcho Tomov, MD, PhD,DSc

Prof. Yavor Kornovski, MD, PhD

Prof. Maria Angelova, MD, PhD

Assoc. Prof. Lachezar Tanchev, MD

Assoc. Prof. Ivan Ivanov, MD

Official Reviewers: Prof. Yavor Kornovski, MD, PhD

Assoc. Prof. Ivan Ivanov, MD

The materials related to the defense are published on the website of Medical University – Pleven: http://www.mu-pleven.bg/index.php/bg/

Abbreviations used in the text

AC – Adenocarcinoma

Akt – Protein kinase B

ASC – Adenosquamous carcinoma

CAF – Cancer-associated fibroblasts

CIN – Cervical intraepithelial neoplasia

CT – Computed tomography

Drp1 – Dynamin-related protein 1

EGFR – Epidermal growth factor receptor

E2F – Early region 2 binding factor

EMT – Epithelial–mesenchymal transition

FIGO – International Federation of Gynecology and Obstetrics

FR – Free radicals

GLUT1/4 – Glucose transporter 1/4

GSH – Glutathione

HIF-1α – Hypoxia-inducible factor 1 alpha

HLA – Human leukocyte antigen

HMA – Hexamethylene amiloride

HPV – Human papillomavirus

IHC – Immunohistochemistry

LSIL – Low-grade squamous intraepithelial lesion

LVSI – Lymphovascular space invasion

MDSC – Myeloid-derived suppressor cells

MFN1 – Mitofusin 1

MFN2 – Mitofusin 2

MRI – Magnetic resonance imaging

mTOR – Mechanistic target of rapamycin kinase

OMA2 – Oocyte maturation defective protein 2

OPA1 – Optic atrophy 1 protein

PD-1 – Programmed cell death protein 1

PD-L1/L2 – Programmed death ligand 1/2

PET – Positron emission tomography

PI3K – Phosphoinositide 3-kinase

PKM2 – Pyruvate kinase M2

pRb – Retinoblastoma protein

ROMO1 – Reactive oxygen species modulator 1

ROS – Reactive oxygen species

SCC – Squamous cell carcinoma

SCO2 – Synthesis of cytochrome c oxidase 2

SGLT – Sodium-glucose linked transporters

TAM – Tumor-associated macrophages

TGF-β – Transforming growth factor beta

TIGAR – TP53-induced glycolysis and apoptosis regulator

VEGF – Vascular endothelial growth factor

VHL – Von Hippel–Lindau tumor suppressor

Introduction

Cervical cancer remains one of the most significant malignant diseases affecting women worldwide, despite the availability of effective methods of primary and secondary prevention. The disease is closely associated with persistent infection with high-risk types of human papillomavirus (HPV), which represents a necessary prerequisite for the development of cervical intraepithelial neoplasia (CIN) and subsequent invasion. Despite the proven causal relationship between HPV and cervical carcinoma, progression to high-grade dysplasia and invasive disease is observed only in a proportion of infected women, suggesting the involvement of additional molecular and cellular mechanisms.

Current research shows that the viral oncoproteins E6 and E7 not only disrupt the cell cycle through inactivation of p53 and pRb, but also induce significant changes in cellular metabolism, mitochondrial function, and redox homeostasis. In this context, oxidative stress is regarded as an important mediator linking viral persistence, chronic inflammation, and genomic instability, all of which contribute to the progression from precancerous lesions to invasive carcinoma.

Despite the high sensitivity of current screening methods, including HPV testing, cytology, and colposcopy, their specificity for clinically significant lesions remains limited. This creates a need for additional biomarkers that can reflect active tumor transformation and support more precise risk stratification in HPV-positive patients.

In this context, growing attention is being directed toward mitochondria-related markers and regulators of redox balance. One potential candidate is ROMO1 (Reactive Oxygen Species Modulator 1), a protein of the inner mitochondrial membrane involved in the regulation of reactive oxygen species production and the

maintenance of mitochondrial function. Data from other malignancies suggest a relationship between increased ROMO1 expression and tumor progression, but its role in HPV-associated tumors remains insufficiently clarified.

The present dissertation is focused on investigating ROMO1 expression as a potential biomarker in cervical neoplasia. Through immunohistochemical analysis of normal cervical tissue, precancerous lesions (CIN), and invasive carcinoma, the study aims to determine the expression profile of ROMO1, evaluate its associations with clinicopathological characteristics, and place this marker in the context of HPV-induced oxidative stress and mitochondrial dysfunction.

The obtained results contribute to a better understanding of the role of redox-mediated mechanisms in cervical carcinogenesis and provide a foundation for future studies aimed at validating ROMO1 as a diagnostic or prognostic biomarker.

III. AIM AND OBJECTIVES

Aim of the Study

Based on the literature data regarding the clinical and epidemiological significance of cervical cancer, the present study aimed to investigate the expression levels of ROMO1 in normal, precancerous, and cancerous tissues, to determine the relationship between ROMO1 expression levels and selected clinicopathological characteristics in patients with cervical cancer, and to identify the most appropriate statistically significant method for evaluating the expression of this biomarker.

Objectives

To achieve the main aim, the following objectives were defined:

2.1 To determine the levels of ROMO1 expression in normal, precancerous, and cancerous tissues.

2.2 To identify the most appropriate statistically significant method for assessing biomarker expression.

2.3 To determine whether a relationship exists between ROMO1 expression levels and the degree of dysplasia.

2.4 To compare the levels of biomarker expression with selected clinicopathological characteristics of the patients, including histological type, tumor size, tumor grade, lymphovascular invasion, N status, and FIGO stage.

IV. MATERIALS AND METHODS

1. Qualitative Method

A retrospective analysis of archival histological materials and clinical data was performed.

Patients

The study included 205 patients with morphologically confirmed invasive cervical carcinoma, classified into three histological subtypes: squamous cell carcinoma (SCC), adenocarcinoma (AC), and adenosquamous carcinoma (ASC). For comparative analysis, 41 cases of precancerous lesions (CIN) and 30 samples of normal cervical tissue were also included.

All cases were diagnosed at the Clinic of Oncogynecology, Medical University – Pleven, between 2015 and 2024. Since all available cases within this period were included, no prior sample size calculation was performed.

Demographic and clinicopathological data (age, FIGO stage, T stage, nodal status, histological type, tumor grade, and presence of lymphovascular space invasion – LVSI) were extracted from the hospital's electronic database.

The study was conducted as part of the research project:

"MeMoMi: Mechanisms of Modulation of the Immune Response in the Tumor and Its Microenvironment for Defining Prognostic Groups and Optimizing the Treatment Algorithm in Patients with Cervical Cancer" (Medical University – Pleven).

The project was approved by the Ethics Committee of Medical University – Pleven

(No. 656/29.06.2021). All patient data were analyzed in an aggregated and anonymized form.

CIN lesions were categorized as follows:

- **LSIL (CIN I)** – dysplasia involving up to one-third of the epithelial thickness

- **HSIL (CIN II/III)** – dysplasia involving more than one-third of the epithelial thickness

Invasive carcinomas were evaluated according to:

- histological subtype (SCC, AC, ASC)

- tumor differentiation grade (G1–G3)

- nodal status (N0/N1)

- presence of lymphovascular space invasion (LVSI)

Staging was performed according to the FIGO 2018 classification.

1.1. Patients characteristics

Patient characteristics are presented in Table 1.

Characteristic	**Number**	**%**
Healthy tissue	30	100
Precancerous lesion	41	100
LSIL	6	14.6
HSIL	35	85.4
Age		
>50	106	51.7
≤50	99	48.3
T stage		
T1b1	68	33.17
T1b2	82	40
T1b3	26	12.6
T2A	20	9.75
T2B	9	4.39
N stage		
N0	152	74.14

N1	53	25.85
FIGO stage		
FIGO I	138	67.3
FIGO II	14	6.82
FIGO III	53	25.85
Hystology		
AC	50	24.4
ASC	18	8.78
SCC	137	66.8
G		
G1	50	24.39
G2	101	49.26
G3	54	26.34
LVSI		
Да	47	22.92
Не	158	77.1
Total number	205	100

2. Morphological Method

2.1 Histological Examination

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 2–5 μm. After routine staining with hematoxylin and eosin (H&E), the slides were examined under a light microscope by a pathologist.

Tumors were classified and graded according to standard pathological criteria. Staging was performed according to the TNM classification (AJCC/UICC) and the FIGO 2018 system.

2.2 Immunohistochemical Analysis

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections (3 μm). An automated staining system (AutostainerLink 48, Dako, Denmark) was used.

After deparaffinization and antigen retrieval using citrate buffer (pH 6), the sections were incubated with a primary monoclonal antibody against ROMO1 (clone OTI2C12, Abcam) at a dilution of 1:150. Detection was performed using the EnVision FLEX polymer detection system (Dako) and visualized with a DAB chromogen.

All procedures were carried out according to the manufacturer’s protocols.

2.3 Evaluation of ROMO1 Expression

Tissue sections were evaluated microscopically at both low and high magnification. The following parameters were assessed:

- staining intensity
- percentage of positive tumor cells
- localization of expression

Due to the absence of a standardized scoring system for ROMO1, three semi-quantitative methods were applied:

- H-score
- Allred score
- Combined score

H-score

The H-score was calculated using the following formula:

H-score = (% of weakly stained cells × 1) + (% of moderately stained cells × 2) + (% of strongly stained cells × 3)

The resulting values ranged from 0 to 300.

Based on a pilot study involving 75 patients, the H-score demonstrated higher statistical sensitivity compared with the other scoring systems. Therefore, the final analysis of ROMO1 expression in the present study was performed using the H-score.

Evaluation in Normal Tissue and CIN

In normal tissue and CIN lesions, ROMO1 expression was evaluated qualitatively by assessing staining intensity and the distribution of positive cells across the epithelial layers, since no defined tumor area is present in these tissues.

3. Statistical Method

The collected and summarized data were analyzed using appropriate parametric and non-parametric statistical tests.

3.1 Statistical Analysis Applied in the Present Study

All statistical analyses were performed using the R environment (version 4.5.0, 2024; R Foundation for Statistical Computing, Vienna, Austria). Graphical presentation of the results was carried out using the ggplot2 (version 3.5.2) and ggpubr (version 0.6.1) packages for R. A p-value of <0.05 was accepted as the threshold for statistical significance.

The following methods were applied:

- **Descriptive analysis** – ROMO1 H-score and clinicopathological characteristics (T stage, N stage, histological subtype, and presence of lymphovascular space invasion – LVSI) were presented in tabular form using frequency distributions and contingency tables.

- **Comparative analysis** – the χ^2 (chi-square) test was used to assess the association between ROMO1 H-score and categorical variables (T stage, N

stage, histological subtype, and LVSI).

- **Analysis of quantitative variables** – age was compared between groups according to ROMO1 H-score levels using a two-sided t-test.

- **Graphical analysis** – visualization of the results was performed using graphs generated in the R environment.

V. Results

Objective 1. To determine the levels of ROMO1 expression in normal, precancerous, and cancerous tissues

Results of the immunohistochemical analysis

In normal cervical epithelium, ROMO1 showed high-intensity basal expression restricted exclusively to the lowest layer of the stratified squamous epithelium. None of the examined normal samples (0/30) demonstrated positivity in the suprabasal epithelial layers, indicating that in the healthy cervix ROMO1 is virtually not expressed outside the basal cells.

In contrast, all analyzed precancerous lesions (CIN, n = 41) showed positive ROMO1 expression in the layers of dysplastic cells. In LSIL (CIN I), positivity was mainly confined to the lower one-third of the epithelium; in HSIL (CIN II), it extended to approximately the lower two-thirds; and in HSIL (CIN III), it involved almost the entire thickness of the epithelial layer. Thus, the area of ROMO1-positive cells progressively increased with increasing severity of dysplasia.

Objective 2. To identify the most appropriate statistically significant method for evaluating biomarker expression

In a pilot study involving 75 patients, the H-score and Allred score were compared. Both methods demonstrated significantly higher ROMO1 levels in FIGO stage I

than in FIGO stages II and III. However, the H-score showed greater discriminatory power, reflected by stronger statistical associations between FIGO groups (e.g., FIGO I vs FIGO II: $p = 0.00012$; FIGO I vs FIGO III: $p = 0.0008$) compared with the Allred score.

An additional argument in favor of the H-score was the statistically significant difference it identified between patients with and without lymph node metastases ($p = 0.033$). Owing to its higher sensitivity and better reflection of tumor heterogeneity, the final evaluation of ROMO1 in the present study was performed exclusively using the H-score.

Objective 3. To determine whether there is an association between ROMO1 expression levels and the degree of dysplasia

Immunohistochemical analysis of squamous intraepithelial lesions demonstrated 100% ROMO1 expression in the suprabasal layers of abnormal cells in all examined CIN cases. The expression was diffuse, of moderate to strong intensity, and confined to the dysplastic areas.

In LSIL/CIN I, ROMO1 was expressed in cells involving approximately one-third of the epithelial thickness. In HSIL/CIN II, expression extended through one-third to two-thirds of the epithelial thickness, whereas in HSIL/CIN III it involved more than two-thirds, with intense staining distributed throughout almost the entire affected epithelial layer.

Objective 4. To compare biomarker expression levels with selected clinicopathological characteristics of the patients—tumor grade, histological type, FIGO stage, N status, T stage, and lymphovascular invasion

In invasive cervical carcinoma, ROMO1 expression was markedly heterogeneous, with cases showing negative, weak, and strong immunostaining. In most tumors, ROMO1 was positive to varying degrees, suggesting that oxidative imbalance is a common feature of cervical carcinoma.

No statistically significant association was found between ROMO1 expression and FIGO stage, tumor grade, presence of lymphovascular invasion, nodal status, or patient age ($p > 0.25$ for all variables). High ROMO1 expression was observed across all subgroups of these parameters, indicating that increased oxidative stress is a general characteristic of the disease, independent of traditional prognostic factors (Figures 1 and 2).

However, two statistically significant associations were identified (Figure 3). First, ROMO1 expression varied according to tumor histological type ($p = 0.02$), with squamous cell carcinoma (SCC) showing the highest expression, followed by adenosquamous carcinoma (ASC), whereas adenocarcinoma (AC) showed the lowest expression.

Second, a significant inverse association was found between ROMO1 expression and tumor size/local invasion (pT stage; $p = 0.035$). Higher ROMO1 levels were more frequently observed in earlier local stages, whereas expression decreased in more advanced tumors. For example, 52% of pT1b2 tumors showed high ROMO1 expression, whereas this was observed in only 11% of pT2a tumors.

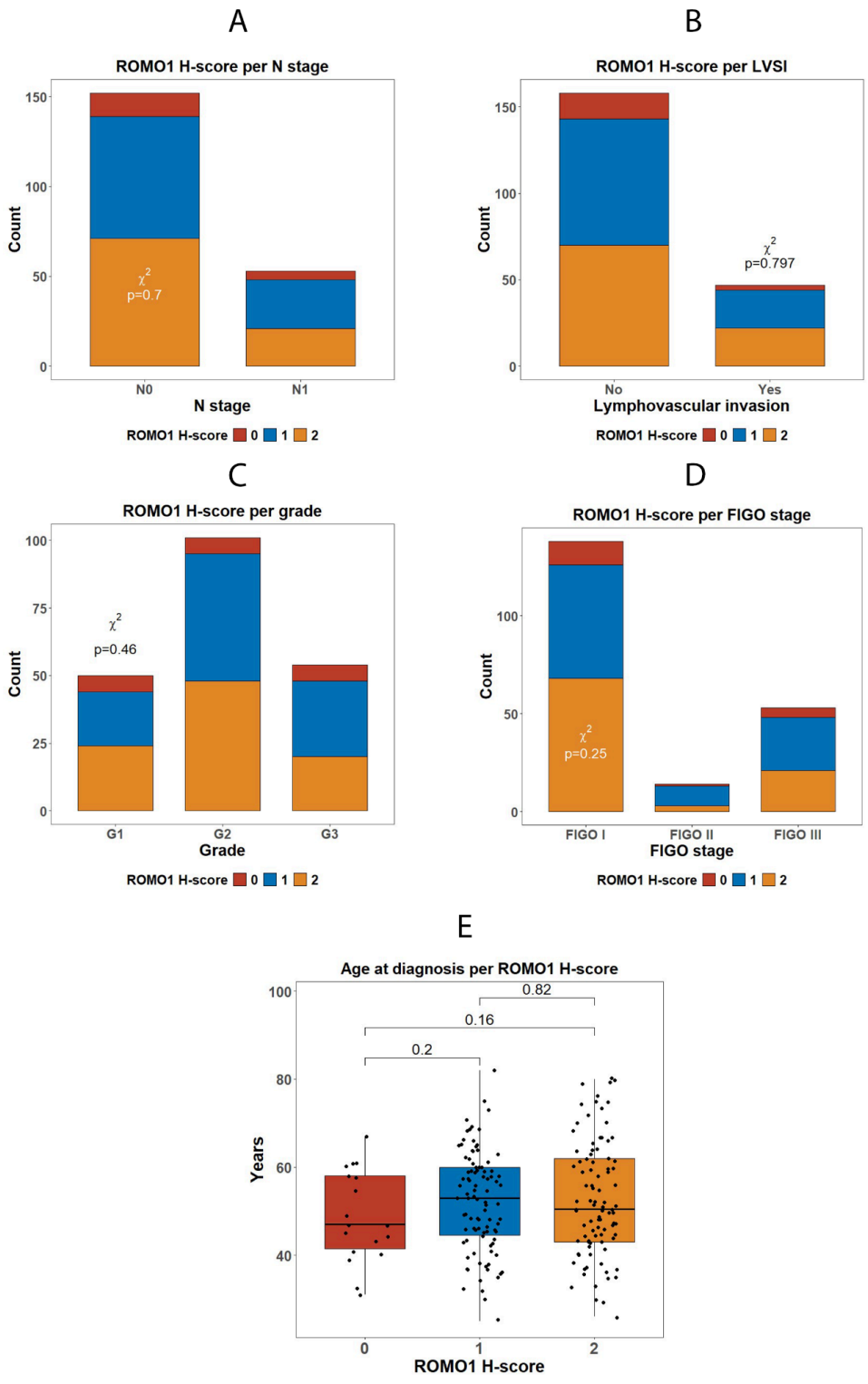

Figure 1. Association between ROMO1 expression and clinicopathological characteristics and patient age in invasive cervical carcinoma.

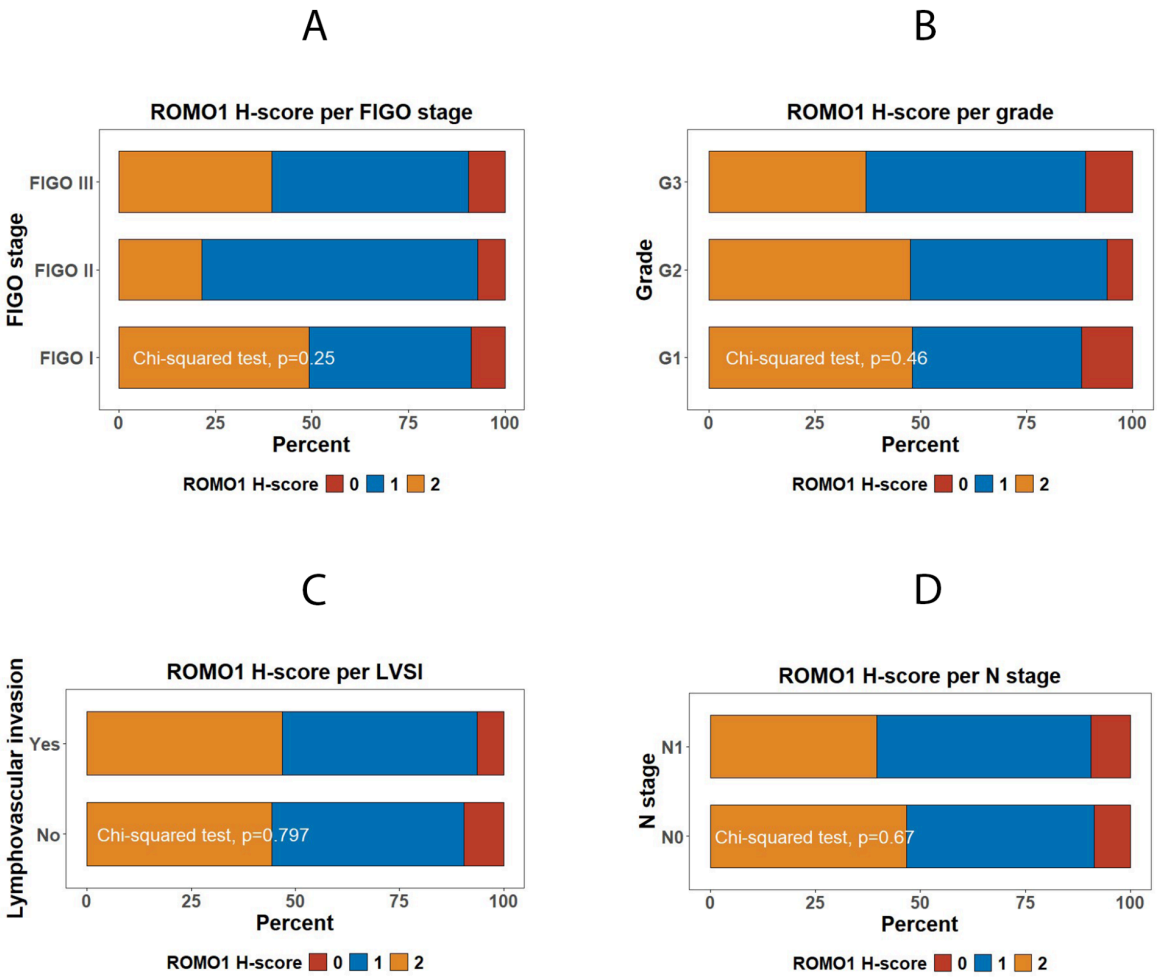

Figure 2. Association between ROMO1 expression and clinicopathological characteristics in invasive cervical carcinoma.

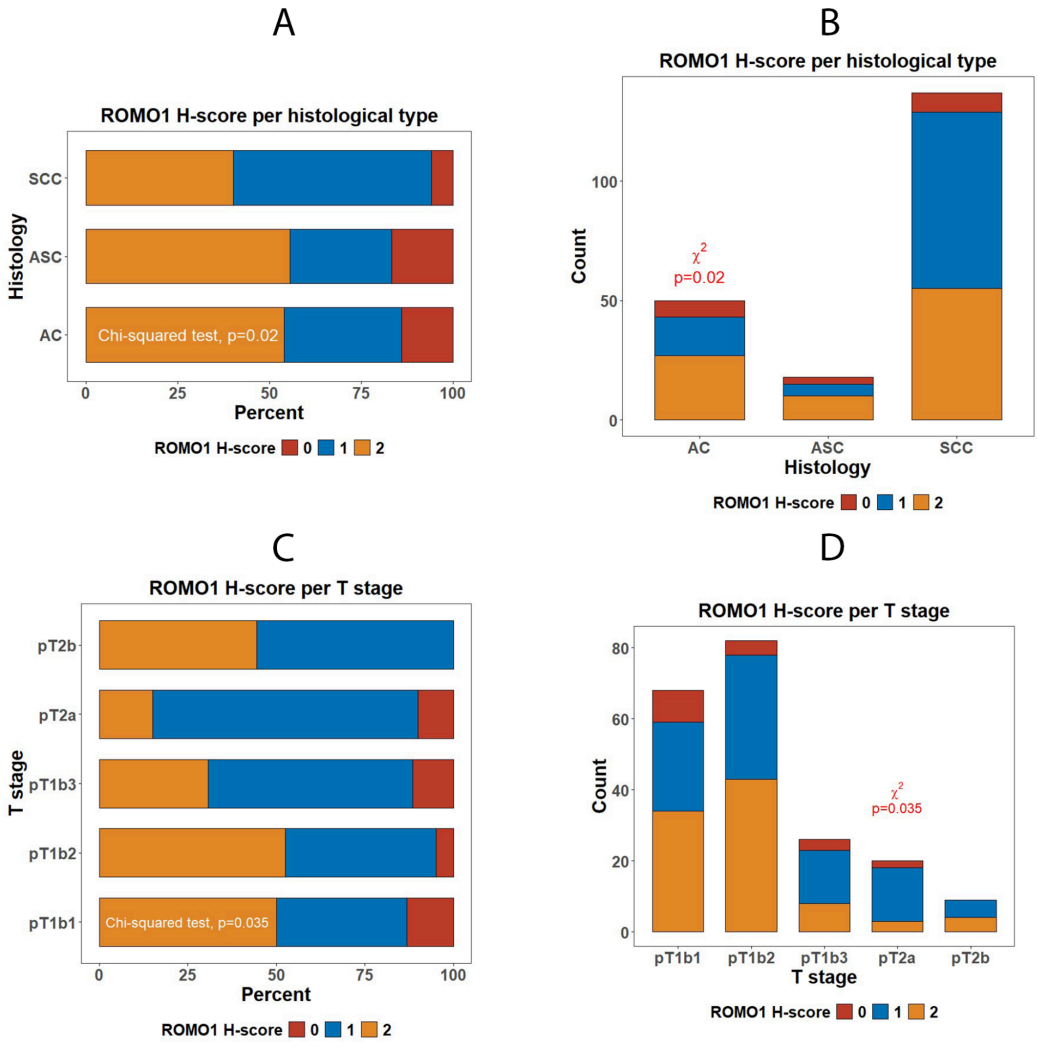

Figure 3. Distribution of ROMO1 expression according to histological subtype and primary tumor stage in invasive cervical carcinoma.

VI. Discussion

The present study is, to our knowledge, the first to investigate the immunohistochemical expression of ROMO1 in histologically verified samples of normal cervical tissue, precancerous lesions, and invasive cervical carcinoma. The obtained results outline a clear expression profile of this marker during cervical neoplasia.

In normal cervical epithelium, ROMO1 expression is practically restricted to the basal layer, whereas all precancerous lesions show diffuse positivity in dysplastic cells. This finding indicates that a pronounced redox imbalance is activated already in the early stages of HPV-induced transformation, reflected by increased ROMO1 expression.

In invasive carcinoma, ROMO1 expression demonstrates marked heterogeneity. No statistically significant association was observed with FIGO stage, tumor grade, lymphovascular invasion, nodal status, or patient age. However, two significant associations were identified—according to histological subtype and pT stage. Squamous cell carcinoma showed higher ROMO1 expression compared with adenocarcinoma and adenosquamous carcinoma, which is likely related to its stronger association with HPV and its characteristic evolution through CIN. In addition, higher ROMO1 expression was more frequently observed in earlier local stages, while it decreased with increasing tumor invasion.

This pattern differs from that observed in several HPV-negative malignancies, such as lung, hepatocellular, and colorectal cancers, where increased ROMO1 expression is associated with advanced disease and poorer prognosis. The findings of the present study suggest that in cervical carcinoma ROMO1 exhibits a distinct, context-dependent behavior.

Based on these observations, a biphasic model of ROMO1 behavior in HPV-associated cervical carcinogenesis can be proposed. In the early stages of HPV infection, the viral oncoproteins E6 and E7 induce oxidative stress, mitochondrial dysfunction, and metabolic reprogramming. In this context, early induction of ROMO1 may represent an adaptive response that supports cell survival under conditions of increased oxidative burden. With disease progression, the accumulation of genetic and epigenetic alterations, HPV genome integration, and worsening mitochondrial dysfunction may lead to a reconfiguration of redox regulation and a subsequent reduction in ROMO1 expression.

This observation is consistent with current views of ROMO1 as a molecule with a dual function—acting both as a regulator of reactive oxygen species and as a mitochondrial cation channel involved in maintaining mitochondrial homeostasis. Therefore, ROMO1 expression does not necessarily follow a linear increase during tumor progression, but rather reflects the dynamic state of cellular redox balance.

From a clinical perspective, ROMO1 may have potential as an additional diagnostic marker, since it is virtually absent in normal epithelium but clearly expressed in dysplastic and neoplastic cells. This could support the morphological evaluation of difficult or borderline cases. From a therapeutic standpoint, the marker and its associated redox pathways open avenues for future research aimed at modulating mitochondrial function and oxidative stress.

The present study also has several limitations. The immunohistochemical method used provides a semi-quantitative assessment, and no universally accepted standard for ROMO1 evaluation currently exists. The study is single-center, and the lack of long-term follow-up data does not allow definitive conclusions regarding the prognostic value of the marker. In addition, viral parameters such as E6/E7 activity and HPV integration status were not directly analyzed. Nevertheless, the findings offer a new perspective on HPV-induced carcinogenesis and identify ROMO1 as an important component of its biological mechanisms.

VII. Conclusion

The present dissertation summarizes and integrates the obtained results regarding the role of ROMO1 in the context of HPV-associated cervical carcinogenesis, with particular emphasis on the relationship between oxidative stress, mitochondrial dysfunction, and the progression from precancerous lesions to invasive carcinoma.

The main conclusion is that ROMO1 represents a dynamic biomarker along the spectrum "normal epithelium → CIN → invasive carcinoma." In normal cervical tissue, ROMO1 expression is minimal or limited, whereas in precancerous lesions (LSIL/HSIL; CIN I–III) the intensity of marker expression increases with the progression of the CIN stage. This finding supports the concept that oxidative stress is an early and essential component of HPV-induced transformation.

In invasive carcinoma, ROMO1 expression demonstrates marked heterogeneity, with a tendency toward higher levels in earlier local stages, while advanced local invasion is associated with a decline in high expression levels. This pattern is particularly noteworthy because it differs from observations in several non-HPV-associated tumors, where elevated ROMO1 expression is more commonly associated with advanced disease and poor prognosis.

From a methodological perspective, this work establishes the H-score as the most appropriate semi-quantitative method for evaluating ROMO1 expression in tissue, as it combines staining intensity and the proportion of positive cells, thereby providing greater sensitivity for detecting clinically relevant differences. Based on

this validation step, the final analyses were performed exclusively using the H-score, ensuring consistency and robustness of the statistical evaluation.

From a scientific standpoint, the present study proposes a hypothetical integrative model, in which ROMO1 participates in an early adaptive response to HPV-induced oxidative stress, while during tumor evolution a reprogramming of redox regulation occurs, leading to downregulation of ROMO1 expression, which may facilitate cellular damage and sustain tumor progression. This concept opens important avenues for future research, particularly prospective studies evaluating the prognostic value of ROMO1 in longitudinal patient cohorts.

In conclusion, the results support the hypothesis that ROMO1 is an important component of HPV-associated cervical carcinogenesis, and that its expression carries significant biological and potential diagnostic relevance. This work provides a foundation for the future validation of ROMO1 as a biomarker in larger prospective cohorts and for further elucidation of the molecular mechanisms underlying its distinctive biphasic behavior in cervical carcinoma.

VIII. Conclusions

1. ROMO1 demonstrates a distinctly different expression profile across the spectrum of normal cervical tissue → SIL/CIN → invasive carcinoma, supporting its involvement in HPV-associated redox dysregulation and early tumor transformation.

2. Based on methodological comparison, the H-score was established as the most appropriate semi-quantitative method for evaluating ROMO1, as it integrates staining intensity and the proportion of positive cells and shows greater sensitivity for detecting clinically relevant differences. For this reason, the final analyses and interpretations of ROMO1 expression in the present dissertation are based exclusively on the H-score, without the inclusion of parallel scoring systems (Allred/Combined), in order to ensure methodological consistency and comparability of the results.

3. The ROMO1 H-score did not show statistically significant associations with several clinicopathological parameters, including tumor grading, lymphovascular invasion, nodal status, and FIGO stage within the studied cohort.

4. In invasive cervical carcinoma, ROMO1 expression is heterogeneous, with cases demonstrating negative, low, and high levels of immunostaining,

reflecting varying degrees of activation or compensation of oxidative stress among tumors.

5. A significant association between ROMO1 expression and histological subtype was observed, with squamous cell carcinoma (SCC) demonstrating more frequent and higher expression compared with adenocarcinoma (AC) and adenosquamous carcinoma (ASC).

6. Significant variability in ROMO1 expression according to pT stage (tumor size) was also identified, with higher levels more frequently observed in earlier local stages and decreasing with more advanced disease, supporting a model of early activation followed by reduction of ROMO1 expression during the progression of local invasion.

7. These findings provide a foundation for future studies aimed at:(i) linking ROMO1 expression with viral parameters (E6/E7 activity and HPV integration status),(ii) directly measuring ROS levels and mitochondrial function in relation to ROMO1, and(iii) evaluating the prognostic value of ROMO1 in prospective cohorts with long-term follow-up.

IX. Contribution of the dissertation

Contributions of scientific- practical and/or original character

1. The expression profile of ROMO1 across the spectrum of normal cervical tissue → CIN → invasive carcinoma has been described and verified, providing a practical framework for the interpretation of ROMO1 in routine immunohistochemical (IHC) diagnostics.

2. The H-score has been methodologically validated as the optimal semi-quantitative method for the evaluation of ROMO1, compared with alternative scoring systems, and a consistent approach for final assessment based solely on H-score has been introduced.

3. Statistically significant associations between ROMO1 expression and histological subtype as well as T stage in invasive cervical carcinoma (SCC vs AC/ASC; pT stage) have been identified, contributing to more precise clinicopathological characterization of tumors.

Contributions of scientific- practical character

1. An integrative model for the role of ROMO1 in HPV-associated cervical carcinogenesis has been proposed, linking virus-induced oxidative

stress, mitochondrial dysfunction, and the dynamics of ROMO1 expression.

2. A “biphasic” model of ROMO1 behavior has been formulated and substantiated-characterized by strong expression in precancerous lesions and early invasion (adaptive redox response) followed by a tendency toward reduced expression during progression to more advanced local invasion. This model appears specific to HPV-associated neoplasms and differs from patterns observed in many HPV-negative tumors.

3. The concept has been established that ROMO1 reflects an early redox adaptation rather than a classical marker of advanced disease, which explains the absence of stable associations with FIGO stage, tumor grading, lymphovascular invasion (LVI), nodal status, and patient age within the studied cohort.

4. Testable hypotheses and priority directions for future research have been defined, including investigation of the relationship between ROMO1 and viral parameters (E6/E7 activity and integration status), functional correlations with ROS production and mitochondrial function, and prospective evaluation of its prognostic value.

X. Publications, participation in scientific forums, courses and projects related to the dissertation

1) Publications of scientific results in full-text articles and/or reports related to the dissertation work:

1. Tsoneva E., Dimitrova P. D., Metodiev M., Shivarov V., Vasileva-Slaveva M., Yordanov A. "The effects of ROMO1 on cervical cancer progression", Pathol Res Pract, Vol. 248, 2023:154561 (ISSN 0344-0338), Online (ISSN 1618-0984) – DOI: 10.1016/j.prp.2023.154561.

2. Tsoneva E., Damyanova P., V. Metodiev M., Shivarov V., Vasileva-Slaveva M., Gorcheva Z., Ivanova Y., Kornovski Y., Kostov S., Slavchev S., Nikolova M., Yordanov A., Watrowski R. "ROMO1 as a Diagnostic Biomarker in Cervical Neoplasia: Evidence from Normal, Pre-Invasive, and Invasive Lesions", Diagnostics (Basel), Vol. 16, No. 1, 2025:24 (EISSN 2075-4418), DOI: 10.3390/diagnostics16010024.

3. Tsoneva E., Yordanov A. "HPV Oncoproteins and Mitochondrial Reprogramming: The Central Role of ROMO1 in Oxidative Stress and Metabolic Shifts", Cells, Vol. 14, No. 20, 2025:1629 (EISSN 2073-4409), DOI: 10.3390/cells14201629.

2) **Participation in scientific forums related to the dissertation work**

1. 07.03.-10.03.2024 European society of gynaecological oncology

(ESGO), Barcelona, Spain

-Tsoneva E., Yordanov A., Kostov S., Ivanova Y., Kornovski Y., Slavchev S., Hasan I., Vasileva-Slaveva M., Shivarov V., Dimitrova P. D., Karakadieva K. “Exploring the prognostic relevance of ROMO1 expression and its association with histological type and lymph node status in cervical cancer”, poster abstract in Int J Gynecol Cancer, 2024; S1048-891X(24)06505-8. Постер

- Tsoneva E., Yordanov A., Kostov S., Ivanova Y., Kornovski Y., Slavchev S., Hasan I., Vasileva-Slaveva M., Shivarov V., Dimitrova P. D. “Infiltration by $FOXP3^{+}$ Tregs in cervical cancer is dependent on histological subtype”, poster presentation at ESGO 2024 – European Society of Gynaecological Oncology Congress, Barcelona, Spain, 2024. Published as a poster abstract in International Journal of Gynecological Cancer, 2024; S1048-891X(24)07392-4. Постер

2. 26.02-28.02.2026 European society of gynaecological oncology (ESGO), Copenhagen, Denmark

-Tsoneva E., Yordanov A., Kostov S., Vasileva-Slaveva M., Shivarov V., Metodiev M., Damyanova P. “ROMO1 expression defines an oxidative stress-driven window in HPV-induced cervical carcinogenesis”, poster abstract accepted for presentation at ESGO 2026 – European Society of Gynaecological Oncology Congress, 2026. To be published in International Journal of Gynecological Cancer. Постер

3. 18.03.-21.03.2026 EUROGIN, Vienna, Austria

-Tsoneva E., Yordanov A., Kostov S.“Early up-regulation of ROMO1 in cervical intraepithelial neoplasia: evidence of HPV-driven oxidative stress in precancerous lesions”, poster presentation, Cervical Neoplasia section, EUROGIN

Congress, 18–21 March 2026.

3) **Participation on scientific projects**

1. MeMoMi: Mechanisms of Modulation of the Immune Response in the Tumor and Its Microenvironment for the Definition of Prognostic Groups and Optimization of the Treatment Algorithm in Patients with Cervical Cancer.КП-06-ПН-43/28

XI. Appendices

COPYRIGHT PERMISSION FORM

Title(s) of the Image(s): Terese Winslow LLC owns the copyright to the following image(s):

Title(s) of illustration(s): ***Cervical Changes, Stages IA1 and IA2 Cervical Cancer, Stages IB1 and IB2, IB3 Cervical Cancer, Stage IV Cervical Cancer***

Description of the Work: Terese Winslow LLC hereby grants permission to reproduce the above image(s) for use in the work specified:

Dissertation title: Connection of ROMO1 with cervical cancerogenesis

University: Medical University Pleven, Bulgaria

Digital Object Identifier (DOI) and Journal ISSN, if available: n/a

License Granted: Terese Winslow LLC hereby grants limited, non-exclusive worldwide print and electronic rights only for use in the Work specified. Terese Winslow LLC grants such rights "AS IS" without representation or warranty of any kind and shall have no liability in connection with such license.

Restrictions: Reproduction for use in any other work, derivative works, or by any third party by manual or electronic methods is prohibited. Ownership of original artwork, copyright, and all rights not specifically transferred herein remain the exclusive property of Terese Winslow LLC. Additional license(s) are required for ancillary usage(s).

Credit must be placed adjacent to the image(s) in the following format:

© (copyright year as seen on the illustration) Terese Winslow LLC, U.S. Govt. has certain rights

Permission granted to:
Name: Eva Tsoneva
Mailing address: Sveta Troitsa, Sofia, Bulgaria
Email address: dretsoneva@gmail.com

Phone number: +359889631779

Signature _____ ***Eva Tsoneva*** _____ ***Date*** ***24.01.2026***
Name

Signature _____ Date January 26, 2026
Terese Winslow, CMI, Member

Terese Winslow LLC, Medical Illustration
714 South Fairfax Street, Alexandria, Virginia 22314
(703) 836-9121
terese@teresewinslow.com
www.teresewinslow.com

1