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Evaluation of PAM Signaling Pathway Genetic Alterations and Their Clinical-Prognostic Relevance in Cervical Cancer

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Abstract

Purpose. This study aimed to evaluate the association between genetic alterations in the PI3K/AKT/mTOR (PAM) signaling pathway and overall survival (OS) in patients with cervical cancer.

Materials and methods. A total of 116 HPV-negative female patients diagnosed with cervical cancer at the National Oncology Center (2017–2020) were included in this retrospective cohort study. DNA was extracted from FFPE tumor samples using Qiagen kits. Mutational analyses of PIK3CA, AKT1, PTEN, and mTOR genes were performed using real-time PCR (EntroGen) and RT-qPCR. Survival analyses were conducted using the Kaplan–Meier method and log-rank test.

Results. Patients without genetic alterations (wild-type) showed a median OS of 49.6 months, while those harboring at least one mutation had a markedly reduced median OS of 8.8 months ($p<0.001$). A stepwise decline in survival was observed with an increasing number of genetic alterations, and cases with ≥2 mutations exhibited extremely poor prognosis.

Conclusion. The number of genetic alterations within the PI3K/AKT/mTOR signaling pathway serves as an independent prognostic factor in cervical cancer. Higher molecular alteration burden correlates with more aggressive disease progression and reduced survival. These findings highlight the clinical value of incorporating molecular profiling into personalized treatment strategies for cervical cancer patients.

Keywords: cervical cancer, PI3K/AKT/mTOR, genetic alteration, prognostic biomarker, survival

НА ПЕРВУЮ | НА СЛЕДУЮЩУЮ | НА ПРЕДЫДУЩУЮ | К СОДЕРЖАНИЮ

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Оценка генетических изменений сигнального пути PAM и их клинико-прогностической значимости при раке шейки матки

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Резюме

Цель. Настоящее исследование было направлено на оценку связи между генетическими изменениями в сигнальном пути PI3K/AKT/mTOR (PAM) и общей выживаемостью (ОВ) у пациенток с раком шейки матки.

Материалы и методы. В ретроспективное когортное исследование были включены 116 пациенток с отрицательным статусом ВПЧ, у которых диагностирован рак шейки матки в Национальном онкологическом центре (2017–2020 гг.). ДНК извлекали из образцов опухолевой ткани, фиксированных в формалине и заключенных в парафин (FFPE), с использованием наборов Qiagen. Мутационный анализ генов PIK3CA, AKT1, PTEN и mTOR проводили методом полимеразной цепной реакции в реальном времени (EntroGen) и количественной ПЦР в реальном времени (RT-qPCR). Анализ выживаемости выполняли с использованием метода Каплана – Мейера и логрангового теста (log-rank test).

Результаты. У пациенток без генетических изменений (дикий тип) медиана общей выживаемости составила 49,6 месяца, тогда как у пациенток, имевших хотя бы одну мутацию, медиана ОВ была значительно снижена и составила 8,8 месяца ($p<0,001$). Наблюдалось ступенчатое снижение выживаемости по мере увеличения количества генетических изменений, а случаи с ≥2 мутациями характеризовались крайне неблагоприятным прогнозом.

Заключение. Количество генетических изменений в сигнальном пути PI3K/AKT/mTOR является независимым прогностическим фактором при раке шейки матки. Более высокая нагрузка молекулярных изменений коррелирует с более агрессивным течением заболевания и снижением выживаемости. Полученные данные подчеркивают клиническую значимость включения молекулярного профилирования в стратегии персонализированного лечения пациенток с раком шейки матки.

Ключевые слова: рак шейки матки, PI3K/AKT/mTOR, генетические изменения, прогностический биомаркер, выживаемость

■ INTRODUCTION

Cervical cancer (CC) remains one of the most common malignant tumors among women and continues to constitute a significant public health burden, particularly in low- and middle-income countries due to its high incidence and mortality rates.

The pathogenesis of the disease is a multistep process characterized not only by persistent high-risk HPV infection but also by the involvement of genetic and epigenetic alterations occurring across multiple signaling pathways. One such pathway, the PI3K/AKT/mTOR (PAM) pathway, is considered one of the most frequently activated signaling networks in human cancers and plays a key regulatory role in cell growth, differentiation, metabolism, and apoptosis [1].

Recent molecular studies have demonstrated that mutations in PIK3CA, AKT1, and PTEN-genes encoding components of the PAM pathway – along with alterations in their expression levels are strongly associated with tumor initiation, progression, and therapeutic resistance in both HPV-positive and HPV-negative cervical carcinomas [2, 3]. Notably, the frequent co-occurrence of PIK3CA hotspot mutations and PTEN loss leads to persistent pathway activation and contributes to a more aggressive tumor phenotype [1, 3].

Clinical investigations have shown that PIK3CA mutation status is associated with poorer overall survival (OS) in many patients and may significantly increase mortality risk in stage IB–II CC patients undergoing radical chemoradiotherapy [4]. These findings have been partially confirmed in larger cohorts and meta-analyses, which report that both overall and disease-free survival, are frequently reduced in individuals harboring PIK3CA mutations [5]. More recent studies emphasize that evaluating PIK3CA mutational status together with copy number variation (CNV) may provide additional prognostic information regarding post-chemoradiotherapy outcomes [6].

Moreover, studies conducted in the Azerbaijani population have indicated that genetic alterations in key PAM pathway genes (PIK3CA, AKT1, mTOR, PTEN) may influence tumor biological behavior and survival outcomes, particularly among HPV-negative CC patients [7]. These results highlight that treatment strategy and multimodal approaches may be critical for improving overall survival in patients exhibiting active and stable PAM pathway signaling [7].

Collectively, current evidence suggests that the number and type of genetic alterations within the PAM signaling pathway may serve as prognostic and potentially predictive biomarkers in cervical cancer management [1, 3, 4, 6]. The present study aims to evaluate the association between PAM-related genetic alterations and overall survival in cervical cancer patients, providing substantial theoretical and practical significance for identifying high-risk groups and planning personalized therapeutic strategies [5, 7].

■ MATERIALS AND METHODS

This study was a retrospective cohort investigation based on clinical and molecular-genetic analyses and was conducted at the Departments of Oncogynecology and Molecular Oncology Laboratory (MOL) of the National Oncology Center (NOC) of the Ministry of Health of the Republic of Azerbaijan during the years 2017–2020. The study was approved by the Ethics Committee of the National Oncology Center (Protocol No. D/8-1074). The participants were women who presented to the NOC Department for the Diagnosis and Treatment of Precancerous Diseases and were diagnosed with cervical cancer. Molecular-genetic analyses were performed at the Molecular Oncology Laboratory within the NOC. A total of 116 HPV-negative patients were included in the study.

DNA Extraction

Genetic material (DNA) was isolated from tumor samples embedded in paraffin blocks (FFPE) using Qiagen kits in accordance with the manufacturer's protocol [8].

Genotyping of the PIK3CA Gene

Genotyping analysis of the PIK3CA gene was performed using the PIK3CA kit (cat. PI3K-RT48, EntroGen Inc., CA, USA) following the manufacturer's guidelines. The analysis was carried out on genomic DNA extracted from FFPE tumor samples using real-time PCR (qPCR). This kit detects 11 somatic mutations in the PIK3CA gene (C420R; Exon 9: E542K, E545A, E545D, E545G, E545K, Q546E, Q546R; Exon 20: H1047L, H1047R, H1047Y) in a 6-reaction/test format and requires signal reading through FAM, ROX, and Cy5 channels. Internal and external controls were provided by the manufacturer. Measurements were performed on a real-time PCR instrument capable of reading FAM/ROX/Cy5 channels, and the interpretation of results as well as quality criteria (successful internal control and presence of mutation-specific signal) were evaluated according to the manufacturer' algorithm. It should be noted that genotyping was performed using commercially available qPCR-based hotspot mutation kits, which detect a predefined panel of 11 common PIK3CA mutations. Due to the absence of next-generation sequencing (NGS) infrastructure in our setting, rare or non-hotspot variants outside the targeted regions could not be assessed. Consequently, the mutation profile reported in this study reflects only the spectrum of alterations detectable by the applied assays.

Gene Expression Analysis (PTEN, AKT1, mTOR)

Quantitative assessment of gene expression was performed using a one-step RT-qPCR protocol. Primers were synthesized by DİT (Turkey). Reactions were prepared in accordance with the manufacturer's instructions, and –RT, NTC, and appropriate positive control samples were included in each run. Measurements were conducted on the CFX96 Real-Time PCR platform (Bio-Rad, USA). Primer/probe sets for target and reference genes were based on manufacturer-validated or literature-validated designs [9]. Ct values were determined using [software name, version], and relative expression levels were calculated using the ΔΔCt method normalized to [selected reference gene(s)]. The absence of amplification signals in –RT and NTC reactions was considered a quality criterion. Expression analysis of PTEN, AKT1 and mTOR was performed for exploratory purposes; however, these findings were not included in the final statistical model, as the primary scope of the study was centered on genomic alterations.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA). Data distribution was evaluated using the Shapiro – Wilk test. Parametric tests (e.g., Student's t-test, ANOVA) were used for normally distributed data, while the Mann–Whitney U and Kruskal – Wallis tests were applied for non-normally distributed data. Categorical variables were compared using the χ^2 (chi-square) or Fisher's exact test. Survival analyses were performed using the Kaplan – Meier method, and differences between groups were assessed with the log-rank test. Multivariate analysis was conducted using the Cox proportional hazards model. Results were presented as mean ± standard deviation (SD) or median (min–max), and statistical significance was defined as $p<0.05$.

■ RESULTS AND DISCUSSION

A total of 116 HPV-negative female patients diagnosed with cervical cancer were included in the study. Molecular analyses were performed on the major components of the PAM signaling pathway, and samples without detectable genetic alterations were classified as wild type (WT).

The distribution of PAM pathway mutations across age groups was assessed using the chi-square (χ^2) test. Although the mutation rate appeared numerically higher in the 50–59-year age group (78.4%) compared with the younger (≤39 years: 55.6%) and older groups (40–49 years: 64.7%; ≥60 years: 66.7%), this difference did not reach statistical significance. The chi-square analysis demonstrated no significant association between age category and the presence of mutations (χ^2=3.31, df=3, p=0.346). These results indicate that PAM pathway genetic alterations are relatively evenly distributed across age groups and that mutation occurrence is independent of patient age in this cohort.

Table 1
Distribution of mutations across age groups and most frequent co-mutations

Age group (years)	Total patients (n)	Mutation-positive (n)	Mutation rate (%)	Most frequent co-mutations
≤39	18	10	55.6%	PTEN loss; PIK3CA E545K
40–49	34	22	64.7%	PIK3CA E545K; PIK3CA H1047R
50–59	37	29	78.4%	PIK3CA H1047R; AKT1 E17K
≥60	27	18	66.7%	PIK3CA H1047R; mTOR alterations
Total	116	79	68.1%	–

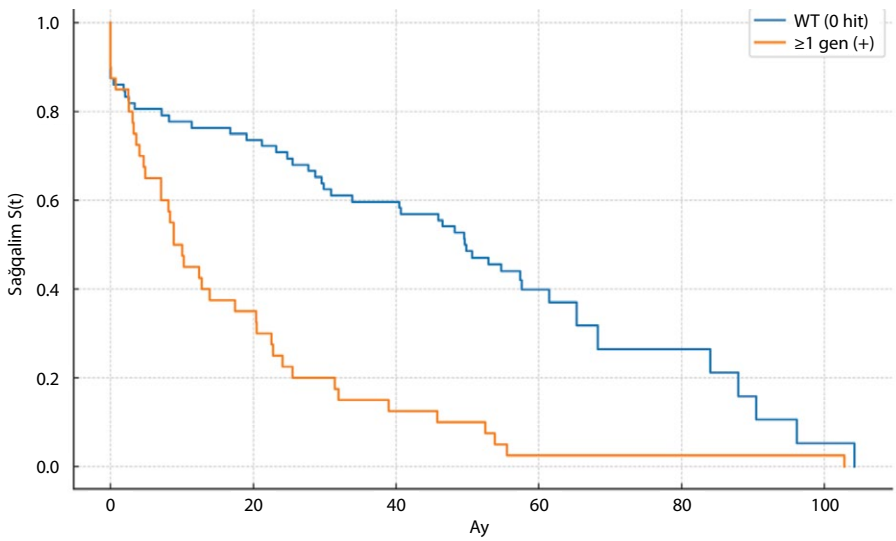

Fig. 1. Kaplan – Meier survival curves showing overall survival (OS) for patients in the WT (0 hit) group and those with at least one gene mutation (≥1 gene (+))

In Figure 1, the overall survival (OS) of patients in the WT group is compared with the OS of patients in whom at least one mutation was identified. Overall survival is presented in months.

According to the analysis, the median overall survival was 49.6 months in the WT group, whereas it was only 8.8 months in the ≥1 gene (+) group. The difference between the groups was highly statistically significant (log-rank test: χ^2=20.24; p=7×10^{-6}). As clearly illustrated by the curve, patients carrying at least one genetic alteration in the PI3K/AKT/mTOR pathway exhibited a markedly reduced survival. The vast majority of these patients died within the first 12 months. In contrast, survival was considerably longer in the WT group, with a substantial proportion of patients living 36–60 months or more. These findings indicate that the presence of genetic alterations in the PI3K/AKT/mTOR signaling pathway is a strong indicator of poor prognosis in cervical cancer patients. Multigene alterations are associated with a more aggressive clinical course, underscoring the potential value of these genetic changes both as prognostic markers and as therapeutic targets in clinical practice.

In Figure 2, the overall survival (OS) durations of patients with mutations detected in individual genes of the PAM signaling pathway are presented in comparison with the wild-type (WT) group.

This analysis was conducted to evaluate the impact of the number of genetic alterations (mutations) in the PAM signaling pathway on the overall survival (OS) of patients. According to the results, the median overall survival in patients without any genetic alterations (WT, 0 alterations) was approximately 49.6 months. In patients with a single genetic alteration, this value decreased to 17.4 months (p=0.004). In the group with two genetic alterations, median survival dropped to only 8.3 months (p=0.0001). Notably, patients harboring

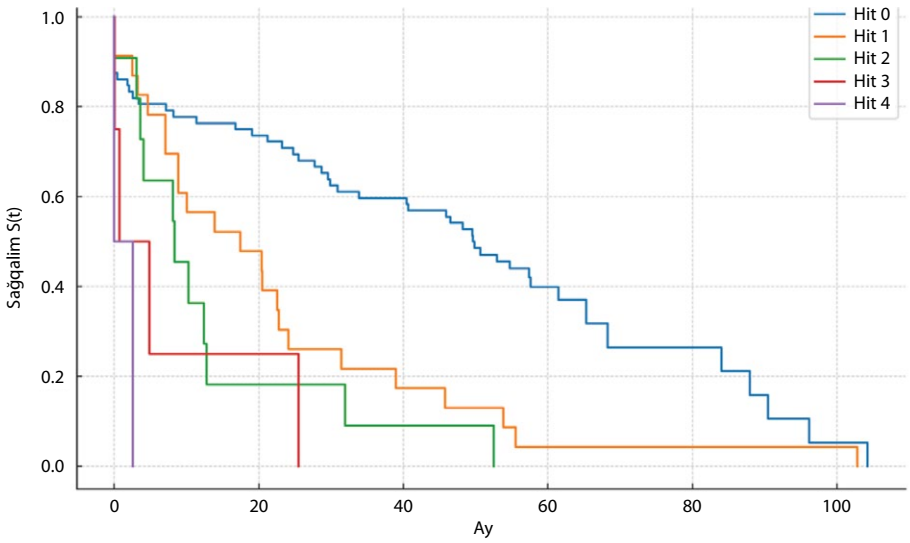

Fig. 2. Kaplan – Meier survival curves illustrating overall survival (OS) of patients stratified by the number of genetic alterations, ranging from 0 to 4

Table 2
Recommended follow-up schedule by molecular risk

Risk Group	0–24 months	24–60 months	>60 months
Low risk (WT)	Clinical assessment every 6 months; pelvic US/MRI as clinically indicated	Annual clinical follow-up	Annual surveillance
Intermediate risk (1 mutation / isolated PIK3CA+)	Clinical assessment every 4–6 months with optional imaging	Clinical evaluation every 6–12 months	Annual surveillance
High risk (≥2 mutations / non-PIK3CA+)	Clinical assessment every 3 months; pelvic MRI routinely; PET/CT if indicated	Clinical evaluation every 6 months	Annual surveillance
Very high risk (≥3 mutations and/or AKT1+PTEN co-alterations)	Clinical evaluation every 3 months + imaging; early restaging recommended	Clinical evaluation every 6 months	Annual surveillance

three genetic alterations had a median survival of 0.7 months (p=0.001), and in the group with four alterations, median survival was essentially 0.0 months (p=0.003).

As illustrated by the curves, survival among wild-type (WT) patients was longer and more stable. However, each additional genetic alteration (an added mutation in the PI3K/AKT/mTOR signaling pathway) led to a monotonic worsening of overall survival, resulting in a pronounced downward shift in the survival curves. These findings indicate that as the molecular damage burden increases, the clinical course of the disease becomes more aggressive and overall survival is significantly reduced.

As evident from the curves, survival in wild-type (WT) patients is longer and more stable. However, each additional genetic alteration (an added mutation within the PI3K/AKT/mTOR signaling pathway) leads to a monotonic deterioration in overall survival, resulting in a pronounced decline in the survival curves. These findings demonstrate that as the molecular damage burden increases, the clinical course of the disease becomes more aggressive and overall survival decreases significantly.

At the same time, it was determined that multigene alterations (≥2 mutations) within the PI3K/AKT/mTOR pathway exert a profoundly negative prognostic effect. A cumulative impact of genetic damage is observed in patients, whereby each additional mutation markedly increases the risk of mortality. The reported results are consistent with other studies conducted in this field and align with the observations of previous authors [10, 11].

Thus, determining the number of genetic alterations (mutations) holds substantial clinical value both for accurately assessing disease prognosis and for guiding the future personalization of molecular targeted therapy strategies.

Limitations

Although mRNA expression analysis for PTEN, AKT1 and mTOR was performed as part of the methodological workflow, these results were intentionally excluded from the final manuscript. The primary aim of the study was to evaluate the prognostic significance of genomic alterations, and integrating transcriptional expression data would have required complex comparative modeling with PIK3CA mutational status, an area already extensively characterized in previous publications. To maintain analytical focus, avoid redundancy,

and ensure methodological consistency, the present work reports only the genomic-level findings. Future studies will explore the combined prognostic impact of gene expression profiles and mutational alterations in a more comprehensive analytical framework.

■ CONCLUSION

In cervical cancer patients, the number of genetic alterations (mutations) within the PI3K/AKT/mTOR signaling pathway was identified as an independent prognostic indicator of overall survival. Each additional mutation is associated with a significant reduction in survival duration, and cases exhibiting two or more genetic alterations ("multigene damage") are strongly associated with an extremely poor prognosis.

■ REFERENCES

1. Glaviano A., Diana P., Bosco B., et al. (2023). PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Molecular Cancer,* 2023;22:138. https://doi.org/10.1186/s12943-023-01827-6
2. Bossler F., Hoppe-Seyler K., Hoppe-Seyler F. PI3K/AKT/mTOR signaling regulates the virus/host cell crosstalk in HPV-positive cervical cancer cells. *International Journal of Molecular Sciences,* 2019;20(9):2188. https://doi.org/10.3390/ijms20092188
3. Bahrami A., Hasanzadeh M., Hassanian S.M., et al. The potential value of the PI3K/Akt/mTOR signaling pathway for assessing prognosis in cervical cancer and as a target for therapy. *Journal of Cellular Biochemistry,* 2017;118(12):4163–4169. https://doi.org/10.1002/jcb.26067
4. McIntyre J.B., Wu J.S., Craighead P.S., et al. PIK3CA mutational status and overall survival in patients with cervical cancer treated with radical chemoradiotherapy. *Gynecologic Oncology,* 2013;128(3):409–414. https://doi.org/10.1016/j.ygyno.2012.12.019
5. Pergialiotis V., Nikolaou C., Haidopoulos D., et al. PIK3CA mutations and their impact on survival outcomes of patients with cervical cancer: A systematic review. *Acta Cytologica,* 2020;64(6):547–555. https://doi.org/10.1159/000509095
6. Martell K., McIntyre J.B., Kornaga E.N., et al. PIK3CA mutation and CNV status and post-chemoradiotherapy survival in patients with cervical cancer. *Gynecologic Oncology,* 2020;158(3):776–784. https://doi.org/10.1016/j.ygyno.2020.06.506
7. Mardanova K.S., Rahimzade S.E., Aliyev J.A., et al. Cervical cancer with the active and stable PI3K/MTOR/AKT pathway in Azerbaijan patients. *Journal of Cervical Cancer,* 2024;1(2):1–9. https://doi.org/10.14302/issn.2997-2108.jcc-23-4838
8. www.qiagen.com/shop QIAamp DNA FFPE Tissue Kit
9. Livak K.J., Schmittgen T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−ΔΔC(T)) Method. *Methods.* 2001;25(4):402–408. doi: 10.1006/meth.2001.1262
10. Wu W., Zhao S., Zhang X. Effects of AKT1 E17K hotspot mutation: A pan-cancer perspective with relevance to cervical cancer. *Oncology Letters.* 2020;20(6):300–308. https://doi.org/10.3892/ol.2020.12165
11. Di Fiore R., Anneo A., Tesoriere G. The role of FBXW7 in gynecologic malignancies and links to PI3K signaling. *Cancers (Basel).* 2023;15(3):700. https://doi.org/10.3390/cancers15030700