CANCER DIAGNOSIS & PROGNOSIS *6*: 322-334 (2026)
doi: 10.21873/cdp.10531

Immunohistochemical Expression of Endoplasmic Reticulum Stress Markers and their Association With Clinicopathological Characteristics and Survival Outcomes in Endometrial Cancer

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Abstract

Background/Aim: This study aimed to examine the immunohistochemical expression of Inositol-Requiring Enzyme 1 Alpha (IRE1) and Protein Kinase R-like ER Kinase (PERK) immunoreactivity scores (IRS) as emerging biomarkers on key clinicopathologic features and survival outcomes in endometrial cancer (EC).

Patients and Methods: Immunoreactive scores (IRS) for IRE1 and PERK were assessed in tumor samples from 73 EC survivors and compared with 20 benign endometrial controls. Associations between IRS values and clinicopathological variables were analyzed. Overall survival (OS) and disease-free survival (DFS) were evaluated using univariable and multivariable Cox proportional hazards models adjusted for age, FIGO stage, and tumor grade.

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Received December 9, 2025 | Revised December 30, 2025 | Accepted December 31, 2025

Results: IRE1-IRS and PERK-IRS were significantly higher in EC survivors compared to controls (p=0.015 and p<0.001, respectively) and PERK-IRS was higher in non-endometrioid than endometrioid tumors (p=0.017). EC survivors undergoing laparotomy exhibited higher PERK-IRS than those treated laparoscopically (p=0.009). Neither IRE1-IRS nor PERK-IRS was associated with OS or DFS, except for a negative association of high *vs*. low IRE1-IRS score and DFS in the unadjusted model (p=0.026) but not in the adjusted one.
Conclusion: IRE1 and PERK expression is up-regulated in endometrial cancer and correlates with selected clinicopathological features, particularly those linked to aggressive disease. However, neither marker demonstrated independent prognostic value for survival outcomes. Further studies are warranted to clarify the role of ER stress pathways in EC biology and their potential implications for risk stratification and targeted therapy.

Keywords: Endometrial cancer, ER stress, UPR, IRE1, PERK, survival.

Introduction

Endometrial cancer (EC) is the fourth most common cancer in women and the most prevalent malignancy affecting the female reproductive system, accounting for approximately 6% of all cancers in women (1). Tumoral abnormalities disrupt the microenvironment, leading to conditions such as ischemia, hypoxia, oxidative stress, nutrient imbalance, and DNA damage, which in turn disturb endoplasmic reticulum (ER) homeostasis (2). This results in the accumulation of misfolded or unfolded proteins, a condition known as ER stress (3). Prolonged ER stress influences tumorigenesis through both transcriptional and translational pathways (4). Additionally, ER stress modulates immune cell functions, weakening the anti-cancer immune response (5). ER-stressed tumor cells release factors that modulate the behavior of nearby leukocytes contributing to immune escape, resulting in further promoting tumor progression (6).

ER plays a crucial role in calcium homeostasis, protein folding, and transport in eukaryotic cells (7). Protein folding is highly sensitive to microenvironmental changes, and its disruption leads to the accumulation of misfolded proteins, triggering ER stress (8). The mammalian unfolded protein response (UPR) comprises three key ER membrane–resident sensors: protein kinase RNA (PKR)-like endoplasmic reticulum kinase (PERK), inositol-requiring protein-1 (IRE1), and activating transcription factor-6 (ATF6) (3, 9). These sensors detect protein folding abnormalities and activate the UPR to mitigate ER stress through various mechanisms (10). Recent studies have showed that UPR activation in EC influences cancer cell growth, invasion, survival in the tumor microenvironment, and resistance to chemotherapy (2, 11). The IRE1–XBP1 signaling axis is a central and well-characterized UPR pathway (12); it promotes cell survival by alleviating ER stress through enhancement of protein folding *via* XBP1s and by regulating UPR target genes alongside ATF6 (13, 14).

The PERK signaling, activated similarly to IRE1, phosphorylates eukaryotic translation initiation factor-2α (eIF2α) to reduce global protein synthesis and selectively increase ATF4 translation and affect both cell proliferation and apoptosis. PERK and IRE1 share structural similarities as transmembrane proteins and are both activated upon dissociation from the chaperone protein BiP/GRP78 (15). The dynamic regulation of IRE1 and PERK signaling events is critical in determining cellular fate (16). The PERK–eIF2α pathway has been reported to both facilitate and suppress malignant transformation in human cancers (17).

Recent studies highlight the dual role of ER stress in cancer, as it can either promote cell survival or trigger apoptosis depending on the balance between the ER protein folding load and capacity (18, 19). Moderate ER stress enhances cancer cell survival and confers resistance to chemotherapy, whereas severe ER stress induces apoptosis (20). Understanding the mechanisms

governing ER stress and UPR signaling in EC provides valuable insights into potential therapeutic strategies targeting these pathways.

The aim of this study was to investigate the immunohistochemical expression of IRE1 and PERK in EC and analyze their associations with clinicopathological parameters and EC survival. As there is a lack of similar studies in the literature, our study aimed to provide important insights into tumor progression, prognostic factors, and potential therapeutic targets, thereby advancing our understanding of EC.

Patients and Methods

Study design, inclusion, and exclusion criteria. This study is a retrospective chart review of electronic medical records of 73 women who underwent hysterectomy with or without lymphadenectomy as part of their treatment for histologically confirmed EC at the 2nd Department of Obstetrics and Gynecology of Aristotle University of Thessaloniki, General Hospital Ippokratio, Thessaloniki, between 2019 and 2024. The median age was 64 years (range=45-83 years). Furthermore, 20 female patients with benign endometrial conditions served as negative controls for the IRE-IRS and PERK-IRS expression.

Female patients were considered eligible for inclusion in the study if they had undergone surgery for EC within the aforementioned time frame and had available formalin-fixed, paraffin-embedded (FFPE) tumor samples suitable for immunohistochemical analysis. Exclusion criteria included insufficient tumor material for analysis or neoadjuvant chemotherapy or radiotherapy of EC survivors prior to surgery, which could potentially alter protein expression. The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of General Hospital of Thessaloniki, Thessaloniki, Greece (Approval No. 105/2025, 17/01/2025). Written informed consent was waived due to the retrospective nature of the study.

Clinicopathologic data collection. Demographic and clinicopathologic characteristics were retrieved from electronic medical records. These included age, tumor histologic subtype, tumor grade, International Federation of Gynecology and Obstetrics (FIGO) stage, presence of lymphovascular space invasion (LVSI), and details of any administered adjuvant therapy (radiotherapy, chemotherapy, or hormonal therapy). In addition, the surgical reports were reviewed to obtain information regarding the type of surgery performed and the extent of lymph node dissection.

Tissue processing and immunohistochemistry. This study retrospectively analyzed the expression of IRE1 and PERK in EC tissues using immunohistochemistry (IHC). Following surgical resection, endometrial tissue specimens were fixed in 10% neutral-buffered formalin, dehydrated, and embedded in paraffin to ensure long-term preservation. Serial sections, each 5 μm thick, were prepared from the FFPE blocks and mounted on positively charged slides. For routine histopathological evaluation, sections were stained with hematoxylin and eosin (H&E). The slides were initially incubated at 70°C for 30 min to ensure complete adherence of the tissue, followed by overnight cooling in an oven, where the temperature was gradually reduced from 60°C to 23°C. This process facilitated the removal of excess paraffin, ensuring optimal antigen exposure. IHC staining was performed using the automated BOND-MAX system (Leica Biosystems, Deer Park, IL, USA). After deparaffinization, heat-induced antigen retrieval was carried out using BOND Epitope Retrieval Solution 2 (EDTA-based, pH 9.0, Cat. No. AR9640; Leica Biosystems) at 98°C for 20 min. Endogenous peroxidase activity was blocked with BOND Peroxide Block (Cat. No. DS9800; Leica Biosystems) for 10 min to prevent non-specific staining. Appropriate tissue (pancreas) was used as positive controls, while omission of primary antibodies was used as a negative control.

For the detection of IRE1 and PERK expression, tissue sections were incubated with specific primary antibodies: rabbit polyclonal IRE1 (E-AB-93217; Elabscience, Houston, TX, USA) and mouse monoclonal PERK (B-5:

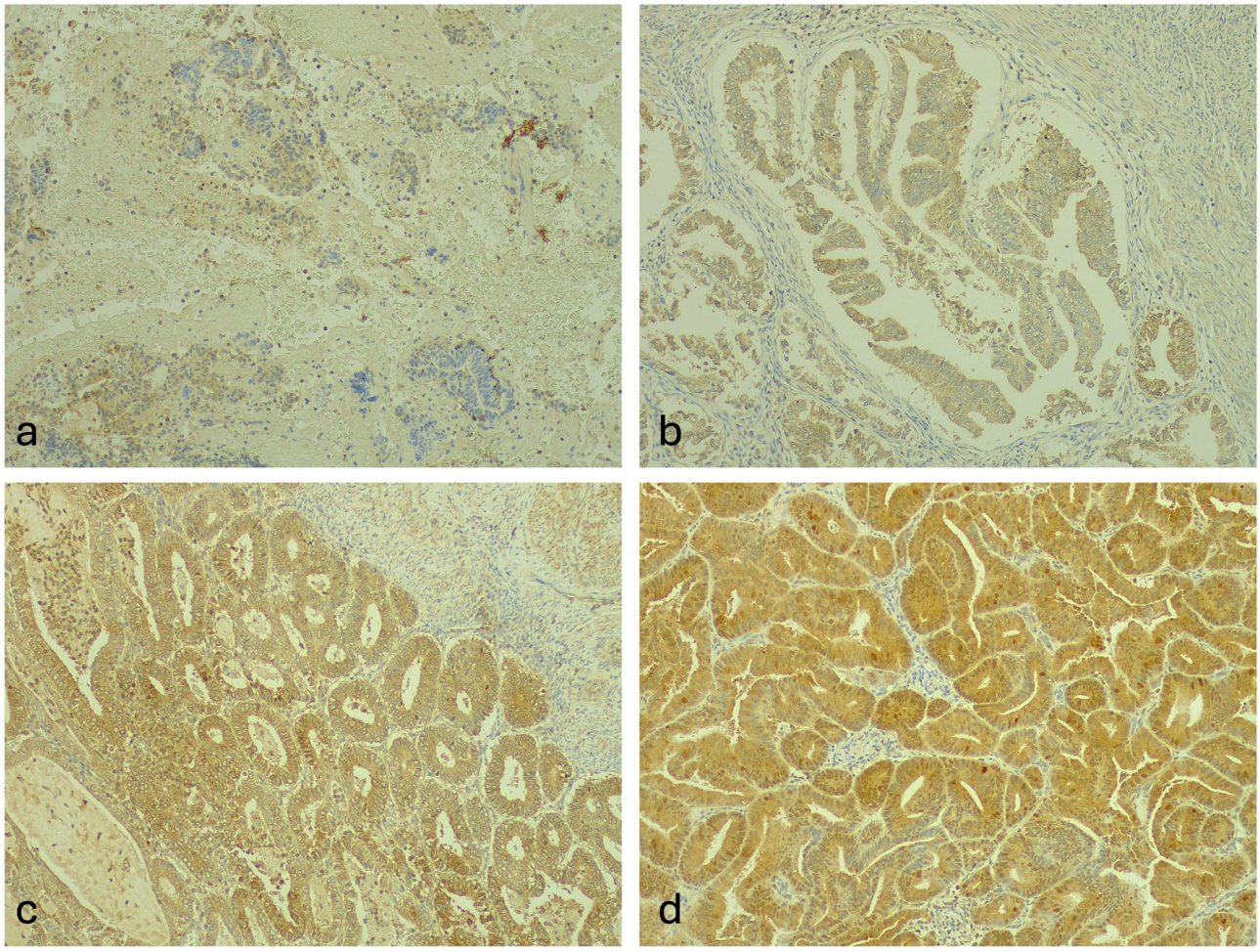

Figure 1. *Inositol-requiring enzyme 1 (IRE1) immunohistochemical stain: (a) Magnification 100× intensity score 0 (no color reaction), (b) Magnification 100× intensity score 1 (low intensity of color reaction), (c) Magnification 100× intensity score 2 (average intensity of color reaction), and (d) Magnification 100× intensity score 3 (intense color reaction).*

sc-377400; Santa Cruz Biotechnology, Dallas, TX, USA), both at a dilution of 1:50. Following primary antibody incubation, a polymer-based detection system was applied for 10 min, followed by visualization using BOND Polymer Refine Detection DAB Chromogen (Cat. No. DS9800; Leica Biosystems) for another 10 min. Counterstaining was performed using Mayer's hematoxylin, after which the slides were sequentially dehydrated in graded alcohol solutions and cleared in xylene. The stained sections were then examined under a Nikon Eclipse E200 microscope to assess the expression of IRE1 and PERK. Immunostaining intensity and distribution were evaluated based on a semi-quantitative scoring system, considering both the staining intensity and the percentage of positive tumor cells (Figure 1, Figure 2).

Evaluation and classification of immunostaining. To ensure objective and reproducible assessment, immunostaining results were independently evaluated by two experienced pathologists who were blinded to the clinical outcomes of the participants. The evaluation criteria were based on both the staining intensity and the proportion of positive tumor cells. Staining intensity was classified using a four-tiered scale: 0 (no staining), 1 (weak), 2 (moderate), and 3

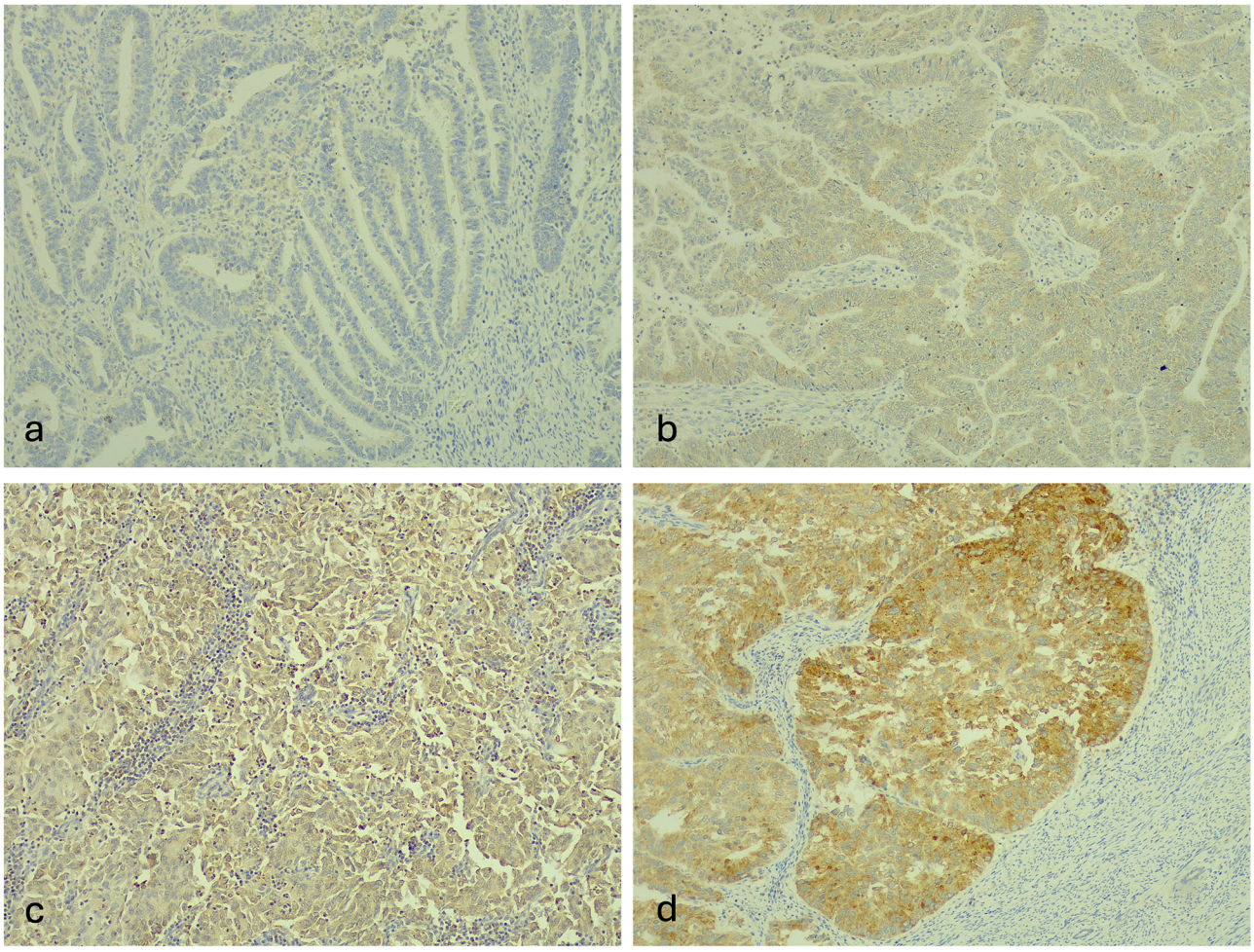

Figure 2. *Protein kinase RNA-like endoplasmic reticulum kinase (PERK) immunohistochemical stain: (a) Magnification 100× intensity score 0 (no color reaction), (b) 1 Magnification 100× intensity score 1 (low intensity of color reaction), (c) Magnification 100× intensity score 2 (average intensity of color reaction), and (d) Magnification 100× intensity score 3 (intense color reaction).*

(strong). The percentage of immunoreactive tumor cells was also categorized into four groups: 1 (0% positive cells), 2 (1-10%), 3 (11-50%), and 4 (51-100%). For each case, the semi-quantitative immunoreactive score (IRS) was derived by multiplying the staining intensity score by the percentage of positive cells, resulting in a final IRS ranging from 0 to 12 (with possible values for the scores being 0, 1, 2, 3, 4, 6, 8, 9, and 12). Any discrepancies between the two pathologists were resolved by consensus after a joint re-evaluation of the slides. High-resolution digital images were captured using a Nikon DS-Fi3 camera attached to the microscope. Standardized magnifications (×100, ×200, and ×400) were used to assess staining intensity and cellular distribution. Images were processed and analyzed using NIS-Elements software (Nikon, Tokyo, Japan) to ensure consistent exposure, contrast, and color balance. Representative images of IRE1 and PERK expression were selected to illustrate different staining patterns.

Statistical analysis. The statistical analyses included 1) the comparison of ER stress markers of EC survivors with controls, 2) the examination of associations between ER stress markers and clinicopathological characteristics, and 3) two survival analyses, one for the overall and one

for disease-free survival, aiming to assess the potential prognostic value of the IRE1 and PERK expression.

The linear correlation between IRE1-IRS and PERK-IRS expression scores was assessed using Pearson's correlation coefficient. Continuous variables are summarized as medians with interquartile ranges (IQR), while categorical variables are presented as frequencies and percentages. The normality of continuous variables was assessed using the Shapiro–Wilk test. Given the non-normal distribution of the data, non-parametric methods were applied for group comparisons. The Mann–Whitney *U*-test was used to compare continuous variables between two groups, while the Kruskal–Wallis test was employed for comparisons involving more than two independent groups. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, depending on the expected cell counts in contingency tables.

For the survival analyses, the time scale used was months since the hysterectomy. Overall survival (OS) concerned all-cause mortality. Disease-free survival (DFS) event was defined as tumor recurrence, tumor progression (to a higher stage/grade including metastasis), or death due to cancer. Cox proportional hazards (PH) regression models were employed to obtain hazard ratios (HR) and 95% confidence intervals (CI). The PH assumption was tested with the Schoenfeld residuals. For the ER stress marker scores, a continuous and a binary (0-9, 12) approach was taken. The results are presented as an unadjusted model and an adjusted model that includes age (in years, as a continuous variable), FIGO stage as a four-category (I, II, III, IV) factor variable, and tumor grade as a three-category (1, 2, 3) factor variable. Interaction between the two markers was also examined. A p-value<0.05 was considered statistically significant. The statistical analyses were performed using Stata version 16 (StataCorp LLC, College Station, TX, USA) and R package "*survival*".

Results

Levels of expression in EC survivors and controls. IRE1-IRS was elevated among the 73 EC survivors compared to the 20 controls (median 8, IQR=4-9 *vs*. median 4, IQR=3-8; p=0.015) and similarly, PERK-IRS was higher in EC survivors than in controls (median 8, IQR= 4-8 *vs*. median 3, IQR=1-4; p<0.001), indicating a significant up-regulation of both markers in cancerous tissues.

Clinicopathologic characteristics of EC survivors. Among the 73 EC survivors, a significant positive correlation (r=0.35, p=0.002) was observed between IRE1-IRS and PERK-IRS. Regarding the clinicopathologic characteristics, 80.8% had an endometrioid histology while 19.2% had a non-endometrioid histology. The surgical approach was nearly equally divided between laparoscopic (49.3%) and laparotomy (50.7%) methods, and common comorbidities included hypertension (43.8%) and diabetes mellitus (19.2%).

IRE1-IRS did not differ significantly between endometrioid and non-endometrioid histologies (p=0.585). In contrast, PERK-IRS was significantly higher in women with non-endometrioid histology compared with those having endometrioid histology [median, 8 (8-12) *vs*. 6 (4-8); p=0.017]. There was no significant association between hypertension and IRE1-IRS (p=0.161) or PERK-IRS (p=0.601), nor between diabetes mellitus and IRE1-IRS (p=0.268) or PERK-IRS (p=0.852). IRE1-IRS did not differ between women undergoing laparoscopic *versus* laparotomy procedures (p=0.068), but women undergoing laparotomy exhibited significantly higher PERK-IRS compared with those treated laparoscopically [median, 8 (4-9) *vs*. 6 (4-8); p=0.009). Women who underwent omentectomy compared to those who did not, had significantly higher PERK-IRS [median, 8 (8-12) *vs*. 6 (4-8); p<0.001] but not IRE1-IRS (p=0.214).

Having a sentinel lymph node procedure was not associated with IRE1-IRS (p=0.943); however, women who had undergone sentinel lymph node biopsy had significantly lower PERK-IRS compared to those that did not (p=0.012). No significant differences in either IRE1-IRS or PERK-IRS were observed with positive sentinel lymph nodes or the performance of pelvic or paraaortic lymphadenectomy. Presence of lymphovascular space

invasion (LVSI) was associated with significantly elevated PERK-IRS [median, 8 (8-9) *vs*. 6 (4-8); p=0.016] but not IRE1-IRS (p=0.416). No significant differences in IRE1-IRS or PERK-IRS were noted based on the administration of adjuvant or neoadjuvant chemotherapy. Female patients receiving brachytherapy had significantly lower PERK-IRS [median, 4 (3-8) *versus* 8 (6-12); p<0.001], although no significant differences in IRE1-IRS were observed (p=0.165). Radiation therapy was not significantly associated with either IRE1-IRS or PERK-IRS scores. Furthermore, neither tumor grade (p=0.121 for IRE1-IRS; p=0.759 for PERK-IRS) nor FIGO 2023 stage (p=0.323 for IRE1-IRS; p=0.242 for PERK-IRS) was significantly associated with the immunoreactivity scores. Finally, risk stratification into low (32.9%), intermediate (23.3%), and high-risk (43.8%) groups was not associated with IRE1-IRS (p=0.862) or PERK-IRS (p=0.093). All the aforementioned results are presented in Table I.

IRS expression and EC survival. Regarding overall survival, the 73 cases were followed for a median of 27 months, during which 21 deaths occurred. Neither IRE1-IRS nor PERK-IRS modelling approaches (continuous, binary), and neither unadjusted nor adjusted models provided significant results. This implies that IRS expressions do not offer prognostic value in all-cause mortality after hysterectomy. For the DFS, 69 cases (n=4 were excluded for not being disease-fee at any time of follow-up) were followed for a median of 23 months, and 19 deaths occurred. In the binary IRE1-IRS unadjusted model a significant higher risk was observed in the group with the 12-score compared to the 0-9 group (HR=3.04; 95%CI=1.15-8.08; p=0.026) but not in the adjusted one (HR=2.18; 95%CI=0.56-8.47; p=0.260). PERK-IRS did not seem to be a significant predictor of DFS in any specification (unadjusted or adjusted). Interactions between the markers were not significant for DFS or OS and are not reported. Figure 3 and Figure 4 present Kaplan–Meier curves for the binary IRE1 and PERK scores, respectively. Results for all the Cox regression analyses are presented in Table II.

Discussion

The UPR is essential for maintaining cellular homeostasis by regulating ER stress, and its dysregulation has been implicated in the pathogenesis of several cancers, including EC (11, 20, 21). The IRE1 and PERK branches of the UPR are central players in regulating tumor biology through their distinct and sometimes overlapping functions (22). In our study of 73 EC survivors, we observed that IRE1-IRS and PERK-IRS are associated with a series of clinicopathologic characteristics, but not with OS or DFS, highlighting the complex role of these pathways.

Our findings show higher IRE1 and PERK expression in tumor tissues compared to controls, consistent with broader literature showing elevated UPR markers in various malignancies (23-25). Interestingly, while the IRE1-IRS did not differ significantly between endometrioid and non-endometrioid histologies, PERK-IRS was significantly higher in non-endometrioid tumors, suggesting a closer association of PERK activation with aggressive tumor subtypes. This finding aligns with previous reports linking PERK signaling to tumor aggressiveness, particularly in high-grade or more invasive cancers (20, 26, 27).

A significant positive correlation between IRE1-IRS and PERK-IRS was observed among EC survivors, supporting the hypothesis that these pathways may act in concert to modulate EC progression. This co-activation has been observed in other cancers, where both pathways respond to cellular stress (2, 28, 29), potentially reflecting an adaptive response to the stresses of tumor growth. Nevertheless, testing their interaction with cancer prognosis outcomes did not yield significant findings; however, this analysis was severely underpowered to detect any interaction effects.

Given that EC characteristics often determine the type of surgical approach (laparoscopy *vs*. laparotomy), no significant association with IRE1-IRSwas found, but women undergoing laparotomy had higher PERK-IRS. This may reflect systemic and tumor micro-environmental differences associated with these

Table I. *Clinicopathologic characteristics of endometrial cancer survivors and their association with IRE1-IRS and PERK-IRS expression levels. Statistical comparisons were performed to evaluate potential correlations between biomarker expression and clinical variables.*

Variable	Variable median [range] or n (%)	IRE1-IRS median [IQR]	*p*-Value	PERK-IRS median [IQR]	*p*-Value
Age (years)	64 [45-83]		0.750		0.511
Histology			0.585		0.017
Endometrioid	59 (80.8)	8 [4-9]		6 [4-8]	
Non-endometrioid	14 (19.2)	6 [4-8]		8 [8-12]	
Operation type			0.068		0.009
Laparoscopic	36 (49.3)	8 [4-8]		6 [4-8]	
Laparotomy	37 (50.7)	8 [4-12]		8 [4-9]	
Hypertension			0.161		0.601
No	41 (56.2)	8 [4-8]		8 [4-8]	
Yes	32 (43.8)	8 [5-9]		8 [4-9]	
Diabetes melitus			0.268		0.852
No	59 (80.8)	8 [4-8]		8 [4-8]	
Yes	14 (19.2)	8 [6-9]		6 [6-8]	
Sentinel node procedure			0.943		0.012
No	48 (65.8)	8 [4-8]		8 [4-9]	
Yes	25 (34.2)	8 [4-9]		6 [2-8]	
Sentinel node positive for malignancy			0.729		0.534
No	71 (97.3)	8 [4-9]		8 [4-8]	
Yes	2 (2.7)	8 [8-8]		8 [8-8]	
Pelvic lymphadenectomy			0.428		0.376
No	37 (50.7)	8 [4-8]		8 [4-9]	
Yes	36 (49.3)	8 [4-9]		7 [4-8]	
Positive for malignancy			0.503		0.233
No	65 (89.0)	8 [4-9]		6 [4-8]	
Yes	8 (11.0)	6 [4-8]		8 [8-8]	
Paraaortic lymphadenectomy			0.074		0.168
No	60 (82.2)	8 [5-9]		8 [4-9]	
Yes	13 (17.8)	4 [4-4]		6 [4-8]	
Para-aortic lymph nodes positive for malignancy			0.862		0.457
No	69 (94.5)	8 [4-9]		8 [4-8]	
Yes	4 (5.5)	6 [5-9]		8 [6-10]	
Omentectomy			0.214		<0.001
No	43 (58.9)	8 [4-9]		6 [4-8]	
Yes	30 (41.1)	8 [6-9]		8 [8-12]	
LVSI			0.416		0.016
No	51 (69.9)	8 [4-9]		6 [4-8]	
Yes	22 (30.1)	8 [6-8]		8 [8-9]	
Brachytherapy			0.165		<0.001
No	40 (54.8)	8 [5-9]		8 [6-12]	
Yes	33 (45.2)	6 [4-9]		4 [3-8]	
Radiation therapy			0.864		0.860
No	42 (57.5)	8 [4-9]		8 [4-8]	
Yes	31 (42.5)	8 [4-8]		6 [4-8]	
Adjuvant chemotherapy			0.892		0.144
No	35 (47.9)	8 [4-9]		6 [4-8]	
Yes	38 (52.1)	8 [4-8]		8 [4-9]	
Neoadjuvant chemotherapy			0.265		0.175
No	56 (78.9)	8 [4-9]		6 [4-8]	
Yes	15 (21.1)	8 [8-9]		8 [4-12]	

Table I. *Continued*

Table I. *Continued*

Variable	Variable median [range] or n (%)	IRE1-IRS median [IQR]	*p*-Value	PERK-IRS median [IQR]	*p*-Value
Tumor grade			0.121		0.759
1	10 (13.9)	9 [9-9]		6 [4-8]	
2	32 (44.4)	8 [4-8]		8 [3-9]	
3	30 (41.7)	8 [4-8]		6 [4-8]	
FIGO 2023 stage			0.323		0.242
1	33 (45.2)	8 [4-8]		6 [4-8]	
2	15 (20.5)	6 [4-8]		8 [6-9]	
3	18 (24.7)	8 [4-12]		8 [4-12]	
4	7 (9.6)	8 [8-8]		8 [4-8]	
Risk stratification			0.862		0.093
Low risk	24 (32.9)	8 [4-9]		6 [2-8]	
Intermediate risk	17 (23.3)	8 [4-8]		6 [6-8]	
High risk	32 (43.8)	8 [4-9]		8 [4-9]	

FIGO: International Federation of Gynecology and Obstetrics; IQR: interquartile range; IRE1: inositol-requiring enzyme 1; IRS: immunoreactivity score; LVSI: lymphovascular space invasion; PERK: protein kinase RNA-like endoplasmic reticulum kinase.

surgical approaches, potentially influencing the inflammatory and stress responses within the tumor. Similarly, PERK-IRS was significantly elevated in females undergoing omentectomy, which is typically conducted in non-endometrioid carcinomas (*e.g.,* serous EC). The increased PERK expression in these cases may indicate an enhanced stress response linked to cancer cell survival and treatment resistance (30). Interestingly, the presence of LVSI, a known marker of poor prognosis in EC, was associated with higher PERK-IRS, but not IRE1-IRS, supporting the notion that PERK contributes to the tumor's stress adaptation and metastatic potential (4, 31). Similar findings have been reported for the expression of coregulators in endometrial cancer (32).

In addition, we also explored the prognostic value of IRE1 and PERK expression in EC. Neither IRE1-IRS nor PERK-IRS was associated with OS or DFS in any model, indicating that while both scores are up-regulated in EC, their role as standalone prognostic markers in EC remains unclear. This finding is consistent with the dual, context-dependent nature of UPR signaling, which may vary with tumor stage, grade, and microenvironment (33). The lack of significance in our study may also reflect the heterogeneity in UPR activation in EC, underscoring the need for more refined prognostic biomarkers.

IRE1 and PERK exhibit context-dependent roles in cancer progression and therapy resistance (34). IRE1 has been shown to promote tumor progression in various cancers, such as breast, multiple myeloma, and glioblastoma (28, 35), as chronic IRE1 activation can promote cancer cell survival and resistance to apoptosis under prolonged stress (36). However, it has also been associated with improved prognosis in lung cancer, suggesting a tumor-suppressive role in specific contexts (20). PERK's prognostic role may also be context specific. High PERK expression has been associated with poor prognosis in cancers like breast cancer, low-grade glioma, kidney papillary carcinoma, and thyroid carcinoma (37), but also with improved prognosis in cancers like head and neck squamous carcinoma, possibly due to its ability to induce growth arrest or enhance tumor immunogenicity (38, 39). In breast cancer, PERK activation enhances resistance to radiation and chemotherapy, while its inhibition in colorectal cancer increases oxidative stress-induced cell death (36, 40). The context-dependent nature of these pathways highlights the complexity of targeting them therapeutically.

Limitations of our study include the relatively small sample size that could potentially mask some associations, the retrospective nature of the study, as well as the short

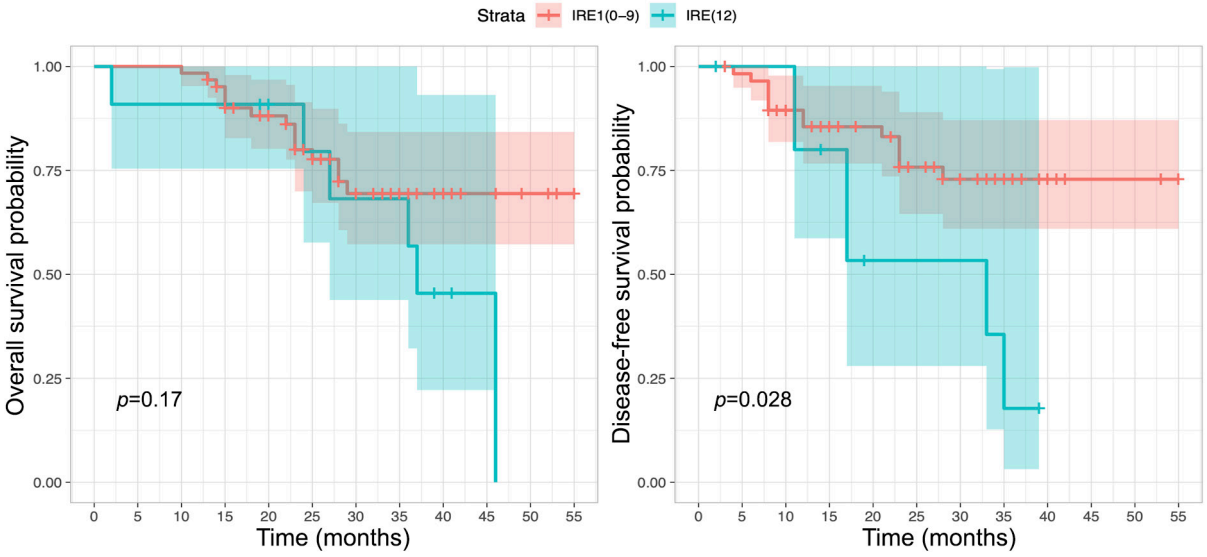

Figure 3. *Kaplan-Meier curves for binary IRE1 with 95% confidence intervals for overall (left) and disease-free (right) survival. Crosses denote censoring events. p-Values were calculated using log-rank tests.*

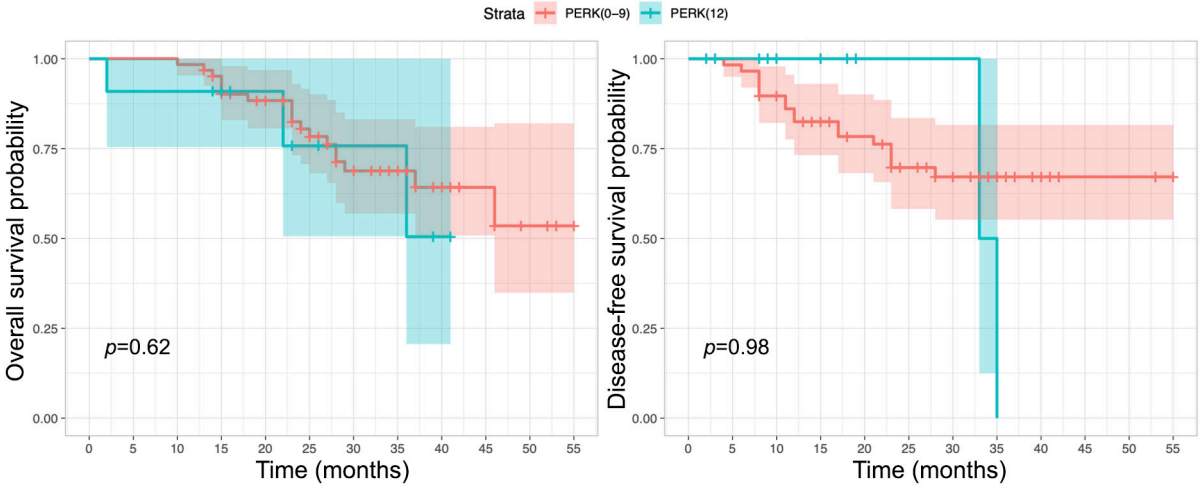

Figure 4. *Kaplan-Meier curves for binary PERK with 95% confidence intervals for overall (left) and disease-free (right) survival. Crosses denote censoring events. p-Values were calculated using log-rank tests.*

follow-up time. Furthermore, differential censoring or measurement errors in survival times cannot be ruled out. Considering these limitations, our findings indicate that IRE1 and PERK may serve as potential biomarkers in EC, given their elevated expression in tumor tissues and association with aggressive histologic features. Therapeutically targeting these pathways may enhance tumor sensitivity to chemotherapy or radiotherapy and impair cancer cell survival (12, 14, 22). However, due to their dual roles in cancer progression, further research is needed to optimize therapeutic strategies (18, 19, 35). Future research should explore the efficacy of combining UPR-targeted therapies with standard treatments to improve therapeutic outcomes and reduce resistance.

Table II. *Regression results from Cox- proportional hazard models for the association of IRE1-IRS and PERK-IRS expression with survival outcomes in endometrial cancer.*

	Overall survival (73 obs. 21 events)		Disease-free survival (69 obs. 19 events)	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Unadjusted				
IRE1-IRS (continuous)	1.06 (0.92, 1.22)	0.411	1.08 (0.93, 1.26)	0.295
IRE1-IRS (0-9)	Reference		Reference	
IRE1-IRS (12)	1.90 (0.73, 4.94)	0.186	3.04 (1.15, 8.08)	0.026
Adjusted*				
IRE1-IRS (continuous)	0.96 (0.81, 1.14)	0.675	0.96 (0.79, 1.17)	0.662
IRE1-IRS (0-9)	Reference		Reference	
IRE1-IRS (12)	1.52 (0.44, 5.31)	0.510	2.18 (0.56, 8.47)	0.260
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Unadjusted				
PERK-IRS (continuous)	1.09 (0.95, 1.25)	0.204	1.05 (0.91, 1.20)	0.535
PERK-IRS (0-9)	Reference		Reference	
PERK-IRS (12)	1.35 (0.40, 4.63)	0.631	1.10 (0.25, 4.80)	0.902
Adjusted*				
PERK-IRS (continuous)	1.05 (0.89, 1.24)	0.538	1.05 (0.82, 1.11)	0.544
PERK-IRS (0-9)	Reference		Reference	
PERK-IRS (12)	1.10 (0.18, 6.85)	0.921	0.32 (0.05, 2.05)	0.230

CI: Confidence interval; HR: hazard ratio; IRE1: inositol-requiring enzyme 1; IRS: immunoreactivity score; PERK: protein kinase RNA-like endoplasmic reticulum kinase. *Adjusted for age, FIGO classification, and tumor grade.

Stratifying EC survivors based on IRE1 and PERK expression may also help identify those most likely to benefit from such interventions.

In conclusion, our study provides evidence that the IRE1 and PERK pathways are up-regulated in EC and are associated with tumor physiology, particularly in aggressive histological subtypes. The lack of prognostic significance suggests that more work is needed to fully understand their role in EC. Further investigation into the molecular mechanisms linking UPR activity to cancer prognosis may provide deeper insights into ER stress-driven tumor progression and therapy resistance in EC.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

Conceptualization: SF; Data Curation: All Authors; Formal Analysis: GM, SF and KC; Investigation: SF, CG, CMS, GMS, AT, MK, KF, ES, ELS, IN, AA, AS, EAA, CK, KP, KD, and SP; Methodology: SF, GM, KC and SP; Project Administration: SF, AS, GM, KD and SP; Resources Software: GM and KC; Supervision: AS, CMS, KD and SP; Validation: All Authors; Visualization: GM, SF and KF and KC; Writing – Original Draft Preparation: SF; Writing – Review & Editing: CMS, GM, KC, AS and SP.

Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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