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Loss of E-cadherin Activates EGFR-MEK/ERK Signaling, Promoting Cervical Cancer Progression

HEE YUN[1†], GWAN HEE HAN[2†], DANIEL J. WEE[3], DOO-BYUNG CHAY[4], JOON-YONG CHUNG[5], JAE-HOON KIM[6,7] and HANBYOUL CHO[6,7]

[1]Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea;
[2]Department of Obstetrics and Gynecology, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Republic of Korea;
[3]Department of Chemistry, College of Arts and Sciences, Case Western Reserve University, Cleveland, OH, U.S.A.;
[4]Department of Obstetrics and Gynecology, Sahmyook Medical Center, Seoul, Republic of Korea;
[5]Molecular Imaging Branch, Center for Cancer Research National Cancer Institute, National Institutes of Health, Bethesda, MD, U.S.A.;
[6]Department of Obstetrics and Gynecology, Yonsei University College of Medicine, Seoul, Republic of Korea;
[7]Institute of Women's Life Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea

Abstract

Background/Aim: This study investigated the relationship between E-cadherin down-regulation and enhanced pERK1/2 signaling in cervical cancer, evaluated their combined prognostic impact, and explored potential therapeutic targets.

Materials and Methods: We analyzed 188 cervical cancer specimens and 300 normal cervical tissue samples using tissue microarray and immunohistochemistry. Small interfering RNA transfection and western blotting were used to study molecular interactions in cervical cancer cell lines.

Results: We observed a significant inverse correlation between E-cadherin and pERK1/2 expression, as well as poor disease-free survival and overall survival. Additionally, molecular analysis indicated that E-cadherin silencing enhanced ERK signaling and promoted cancer cell proliferation.

Conclusion: The findings suggest that E-cadherin and pERK1/2 are crucial biomarkers for cervical cancer prognosis and their interaction provides a potential target for therapeutic interventions. Further studies are recommended to explore these pathways in the clinical setting.

Keywords: E-cadherin, pERK1/2 signaling, cervical cancer, EGFR-MEK/ERK pathway, biomarkers.

†These Authors contributed equally to this work.

Hanbyoul Cho, Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-Ro, Gangnam-Gu, Seoul 06273, Republic of Korea. Tel: +82 220193430, Fax: +82 234628209, e-mail: hanbyoul@yuhs.ac

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Introduction

Cervical cancer is one of the most common malignancies among women worldwide, with approximately 660,000 new cases and 350,000 deaths in 2022 (1). Epidemiological and biological studies have confirmed that the human papillomavirus (HPV) is a critical cause of cervical cancer, driving the introduction of HPV vaccination. However, persistent infection with high-risk HPV remains a major factor in the progression of cervical cancer (2). Despite significant advancements in the understanding and management of cervical cancer, mortality and morbidity rates remain alarmingly high (3). This issue underscores the urgent need to develop reliable biomarkers that can provide deeper and more objective insights into the pathological processes of the disease, facilitate early diagnosis, and enable personalized therapeutic strategies.

Emerging evidence indicates that the epithelial-mesenchymal transition (EMT) plays a crucial role in the progression of malignant tumors (4). EMT is a process in which epithelial tumor cells lose their polarity and cell-cell adhesion capabilities (5). A key component of this process is the down-regulation of E-cadherin, a critical calcium-dependent cell-cell adhesion molecule (6). Traditionally recognized as a tumor suppressor in various malignancies, including lung, gastric, laryngeal, breast and bladder cancers, E-cadherin promotes cell-cell adhesion and inhibits EMT, thereby helping to prevent tumorigenesis, invasion, and metastasis (4, 7-13). Yet, the specific mechanisms by which E-cadherin influences cervical cancer development, particularly its progressive loss during carcinogenesis, remain unclear (14, 15).

Compounding this issue, the loss of E-cadherin has been shown to activate intracellular signaling pathways that significantly contribute to cancer metastasis and progression (16, 17). Notably, the aberrant activation of the epidermal growth factor receptor (EGFR) signaling pathway, often observed in non-small cell lung and breast cancers, correlates closely with poor prognoses. This pathway is frequently activated by overexpressed ligands, such as epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α), enhancing oncogenic signaling (18-20). Moreover, recent studies have suggested that E-cadherin suppression might activate the extracellular signal-regulated kinase (ERK) cascade within the broader mitogen-activated protein kinase (MAPK) signaling pathway, which could promote cancer cell proliferation, tumor growth, and metastatic potential (21). Although ERK pathway activation is common in cervical cancer, the detailed interaction between ERK and E-cadherin remains poorly understood, highlighting a critical research gap (22).

Therefore, this study aimed to integrate *in vitro* and immunohistochemical analyses to explore the relationship between ERK and E-cadherin in cervical cancer. We aim to enhance our understanding of the molecular landscape of cervical cancer and identify novel therapeutic targets, ultimately leading to the development of more effective treatment strategies that can significantly impact patient outcomes.

Materials and Methods

Patients and tumor specimens. Between March 1996 and March 2010, 188 patients with cervical cancer who had undergone radical hysterectomy were recruited from the Department of Obstetrics and Gynecology at the Gangnam Severance Hospital, Yonsei University College of Medicine, Republic of Korea. Concurrently, 340 non-adjacent normal cervical epithelial tissue samples were collected. Additionally, formalin-fixed, paraffin-embedded (FFPE) blocks were obtained from the Korea Gynecologic Cancer Bank, supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) and funded by the Korean government (MIST) (NRF-2017M3A9B8069610). After a thorough pathological review, only specimens meeting the requisite criteria for histological integrity were included. Comprehensive clinicopathological data, including patient age, survival time and status, tumor characteristics, and treatment response, were extracted from the medical records. The response to therapy was evaluated using the Response Evaluation

Criteria in Solid Tumors (RECIST; version 1.1) using either magnetic resonance imaging (MRI) or computed tomography (CT). This study was rigorously reviewed and approved by the Institutional Review Board (IRB) of Gangnam Severance Hospital (No. 3-2024-0049). All procedures were conducted in strict accordance with international ethical standards for medical research.

Tissue microarray (TMA) and immunohistochemistry (IHC). TMAs were constructed as previously described (23). For IHC, 5 μm sections were prepared from TMAs, deparaffinized in xylene, and rehydrated through a graded ethanol series. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide (H_2O_2) for 10 min. Antigen retrieval was performed using a steam pressure cooker (Pascal; Dako, Carpinteria, CA, USA) with a preheated pH 6 buffer (Dako) for 10 min. The primary antibodies used were anti-pERK1/2 (rabbit antibody, clone #20G11, 1:200; Cell Signaling Technology, Danvers, MA, USA) and anti-E-cadherin (mouse antibody, clone #ab1416,1:100; Abcam, Cambridge, UK). The Dako Autostainer Plus system was used for staining using the Dako EnVision+ Dual Link System-HRP, followed by development with 3,3-diaminobenzidine (DAB; Dako). The slides were counterstained with hematoxylin and coverslipped for microscopy.

Immunohistochemical analysis. High-resolution digital images of the stained TMA sections were captured using a NanoZoomer 2.0 HT (Hamamatsu Photonics K.K., Hamamatsu, Japan) with a 20× objective. Image analysis was performed using Visiopharm software (version 6.5.0.2303; Hørsholm, Denmark). The intensity of the brown DAB staining was semi-quantitatively assessed on a scale of 0 (negative) to 3 (strong), and the percentage of positively stained tumor cells was calculated. A histoscore was then computed by multiplying the intensity by the percentage of positive cells, providing a range from 0 to 300.

Cell culture and reagent preparation. Human cervical cancer cell lines, SiHa, HeLa, ME-180, and SUN-17 were acquired from the Korea Cell Line Bank (Seoul, Republic of Korea). These cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were maintained in a controlled environment at 37˚C with 5% CO_2 The MEK inhibitor, PD98059, was purchased from Selleck Chemicals (Houston, TX, USA) to assess its effects on ERK phosphorylation pathways.

Small interfering RNA (siRNA) transfection. siRNAs specific for E-cadherin were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). For gene silencing experiments, cells were transfected with 50 pmol of siRNA per well in 6-well plates utilizing Lipofectamine® RNAiMAX Reagent (Invitrogen, Gaithersburg, MD, USA), according to the manufacturer's protocols.

Western blot analysis and immunoprecipitation. For protein analysis, the cells were lysed using a Cell Lysis Buffer containing protease inhibitors (Cell Signaling Technology). Immunoprecipitation was conducted using Pierce™ IP Lysis Buffer (Thermo Fisher Scientific, Waltham, MA, USA), with proteins incubated with agarose-conjugated EGFR antibodies at 4˚C for 3 h (sc-373746 AC, Santa Cruz Biotechnology). After incubation, the complexes were washed, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membranes. Immunoblotting was performed using primary antibodies against E-cadherin, p-ERK, ERK, and EGFR (purchased from Cell Signaling Technology). Antibodies for EGFR-AC and α-actinin were purchased from Santa Cruz Biotechnology. Immunoreactive bands were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientific).

Cell growth assay. Cellular proliferation was quantified by seeding SiHa and SNU-17 cells at 2×10^5 cells/well in 6-well plates. Cells were dissociated and counted daily over a 72 h-period using a Bio-Rad TC20™ Automated Cell Counter (Bio-Rad Laboratories, Hercules, CA, USA). Growth curves were plotted based on live cell counts, with each

experimental condition replicated thrice to ensure statistical robustness.

Statistical analysis. Differential expression of E-cadherin and pERK1/2 was analyzed using the Mann–Whitney or Kruskal–Wallis test, as appropriate. The Kaplan–Meier method was employed to generate survival curves for patients, categorizing E-cadherin and pERK1/2 expression into high or low groups based on the optimal cutoff point determined using the MaxStat package in R software (version 4.2.0). Statistical analyses were carried out using SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA), with a significance threshold set at $p<0.05$.

Results

Comparative analysis of E-cadherin and pERK1/2 expression in normal and cervical carcinoma specimens with clinicopathological data. In agreement with the hypothesis concerning the roles of E-cadherin and pERK1/2 in various cancers, we conducted a comprehensive quantitative evaluation using automated digital image analysis to assess the expression levels of these biomarkers in relation to the clinicopathological characteristics. Given the technical complexities associated with staining and sectioning, and the inherent variability among samples, our analysis focused on a specific subset: 334 normal and 128 carcinoma tissues were analyzed for E-cadherin, and 311 normal and 129 carcinoma tissues were examined for pERK1/2. Figure 1A shows representative immunohistochemical images highlighting the differential expression of E-cadherin and pERK1/2 in normal cervical tissues and carcinoma specimens. These images reveal that E-cadherin is prominently expressed in the membranes of normal tissues, but its expression is substantially reduced in cervical cancer tissues. Conversely, the expression of pERK1/2 was minimal in normal cervical tissues but significantly increased in the cytoplasm of cervical cancer tissues, indicating its role in promoting oncogenic pathways. Table I further quantifies these observations, presenting the mean scores and confidence intervals for E-cadherin and pERk1/2 across various diagnostic categories. E-cadherin expression was significantly associated with advanced FIGO stage of cervical cancer ($p=0.006$, Table I), whereas a trend towards significance was observed with a good response to concurrent chemoradiation therapy (CCRT) ($p=0.08$, Table I). In case of pERK1/2, the expression level of pERK1/2 was significantly associated with advanced FIGO stage, squamous cell type, poor response to CCRT and positive squamosa cell carcinoma (SCC) antigen level ($p<0.001$, $p=0.008$, $p=0.006$ and $p=0.048$, respectively; Table I), whereas a trend towards significance was observed between pERK1/2 expression and tumor size larger greater than 4 cm ($p=0.055$; Table I). Next, we examined the relationship between E-cadherin and pERK1/2 protein expression and patient survival outcomes. Patients were grouped into either high or low expression groups with optimal cutoff points of 119 or 146. Kaplan–Meier plots demonstrated that patients with low E-cadherin expression displayed shorter disease-free survival (DFS) and overall survival (OS) ($p=0.016$, $p=0.013$), whereas low expression of pERK1/2 was significantly associated with longer DFS and OS ($p<0.001$, $p=0.002$) in patients with cervical cancer (Figure 1B).

Survival outcomes and correlation of E-cadherin and pERK1/2 expression levels in cervical cancer: A Spearman analysis. Despite extensive research delineating the distinct roles of E-cadherin and pERK1/2 in oncogenic pathways, their interactive dynamics and synergistic effects in cervical cancer remain underexplored. To address this gap, we employed Spearman's rank correlation analysis, revealing a notable negative correlation between E-cadherin and pERK1/2 expression in cervical cancers (Spearman's rho=–0.457, $p<0.001$, Figure 2A). Further analysis using Kaplan–Meier plots demonstrated significant disparities; patients characterized by low E-cadherin levels coupled with high pERK1/2 levels exhibited significantly poorer DFS and OS than others (both $p=0.013$, Figure 2B). In addition, FIGO stages III and IV were significantly associated with poor DFS and OS, which was expected and further validated in the present study (Figure 2B). Further elucidation of these findings was

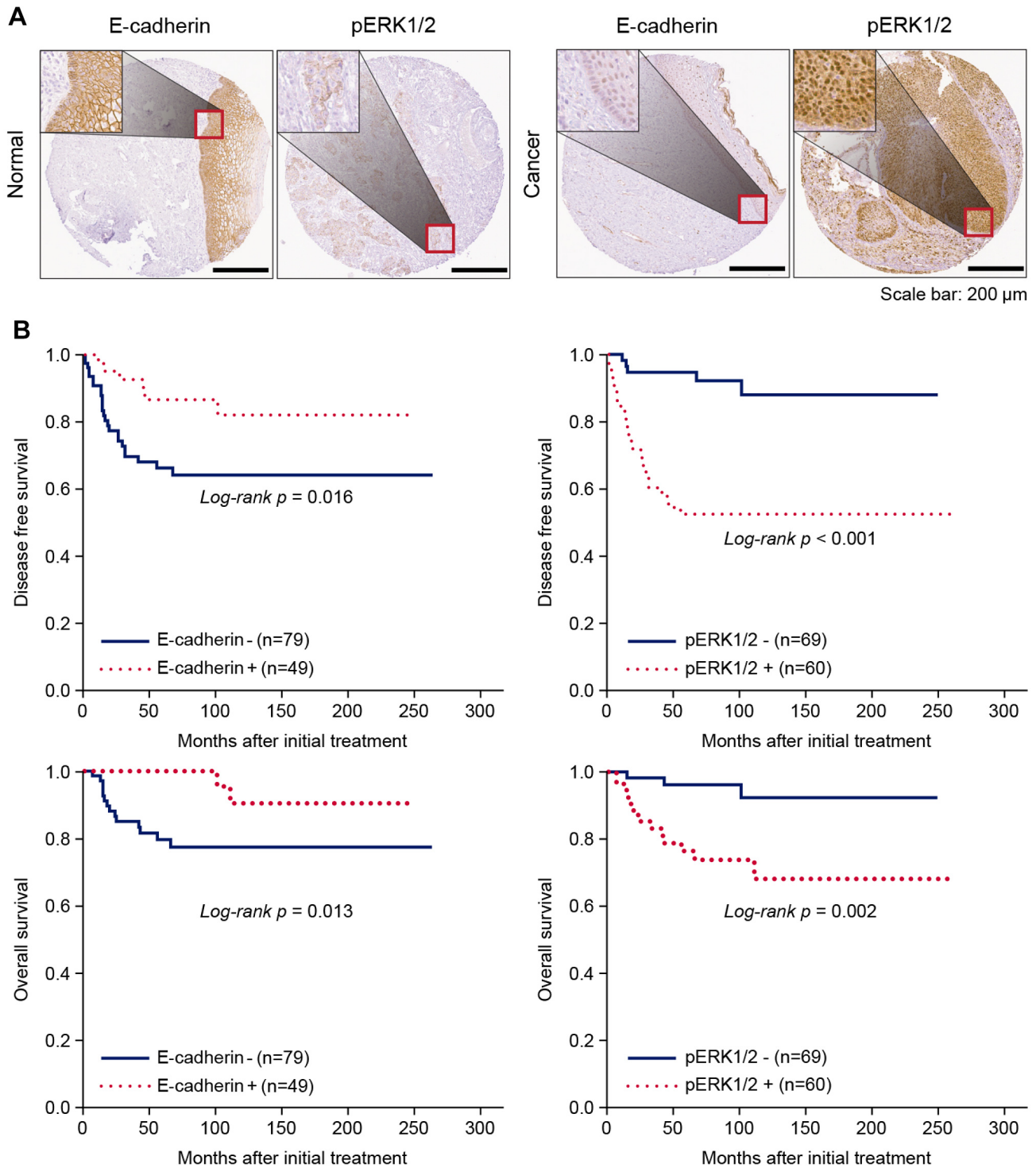


Figure 1. *Protein expression of E-cadherin and pERK1/2 in normal and cervical cancer tissues. (A) Immunohistochemistry images showing E-cadherin and pERK1/2 expression in normal versus cervical cancer tissues. Scale bar: 200 μm. (B) Kaplan–Meier survival plots comparing disease-free survival (DFS) and overall survival (OS) across groups defined by high versus low expression levels of E-cadherin and pERK1/2.*

Table I. *Clinicopathological characteristics of E-cadherin and pERK in cervical cancer.*

Variables	No	E-cadherin Mean score (95% CI)	*p*-Value	No	pERK1/2 Mean score (95% CI)	*p*-Value
Diagnostic category						
All	462			440		
Normal	334	134.93 (128.34-141.51)	<0.001	311	114.56 (108.11-121.00)	<0.001
Cancer	128	110.26 (95.61-124.90)		129	153.59 (141.72-165.46)	
FIGO stage						
I-IIA	93	123.67 (105.67-141.66)	0.006	96	137.65 (124.90-150.39)	<0.001
IIB-IV	35	74.63 (53.66-95.60)		33	199.97 (177.97-221.97)	
Grade						
Well/Moderate	82	120.60 (101.28-139.92)	0.096	38	145.59 (131.01-160.18)	0.079
Poor	43	88.09 (65.52-110.66)		86	165.58 (143.74-187.41)	
Histology type						
Squamous	109	105.78 (90.35-121.21)	0.177	106	160.89 (147.65-174.12)	0.008
Others	19	135.95 (90.13-181.76)			119.96 (96.17-143.75)	
Tumor size						
≤4	81	117.06 (98.79-135.34)	0.165	83	143.36 (129.60-157.12)	0.055
>4	47	98.53 (73.53-123.54)		46	172.04 (150.16-193.93)	
LN metastasis						
No	78	117.12 (97.91-136.32)	0.875	80	143.29 (128.83-157.75)	0.547
Yes	25	118.88 (84.09-153.67)		25	158.44 (127.37-189.51)	
Response to CCRT						
Good	40	128.00 (100.78-155.22)	0.08	39	142.26 (121.85-162.67)	0.006
Poor	18	89.06 (52.74-125.37)		18	190.00 (162.03-217.97)	
SCC antigen						
Negative	59	117.49 (94.66-140.32)	0.114	47	150.59 (134.00-167.19)	0.048
Positive	39	88.36 (66.50-110.21)		53	178.19 (154.77-201.62)	

SCC, Squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; LN metastasis, lymph node metastasis; CCRT, concurrent chemoradiation therapy. Protein expression was determined through analysis of an immunohistochemically stained tissue array, as described in the 'Materials and Methods' section.

provided by Cox proportional hazards modeling. While the univariate analysis underscores the prognostic relevance of each biomarker, the multivariate analysis indicated that E-cadherin alone did not achieve statistical significance in influencing DFS or OS [hazard ratio (HR)=0.58 (95% CI=0.20-1.73), p=0.331] and OS [HR=0.32 (95% CI=0.06-1.59), p=0.163] (Table II). However, the combination of low E-cadherin and high pERK1/2 expression was significantly associated with deleterious outcomes, markedly elevating both DFS and OS risks [HR=3.65 (95% CI=1.52-8.74), p=0.004] and OS [HR=7.51 (95% CI=2.09-27.03), p=0.002] (Table II). Importantly, these results not only underscore the necessity of multidimensional biomarker evaluation in prognostic assessment but also reveal the critical interdependence of E-cadherin and pERK1/2 in influencing patient survival.

In vitro analysis of inverse expression dynamics between E-cadherin and phosphorylated ERK in cervical cancer cell models. The expression levels of E-cadherin were examined in four cervical cancer cell lines: SiHa, HeLa, ME180, and SNU-17 (Figure 3A). HeLa cells exhibited undetectable E-cadherin expression. To determine whether E-cadherin expression has any effect on ERK phosphorylation and the mechanism underlying the possible regulation of ERK signaling by E-cadherin, we transfected SiHa, ME180, and SNU-17 cervical cancer cells with siRNA against E-cadherin. Western blotting was performed to measure changes in ERK

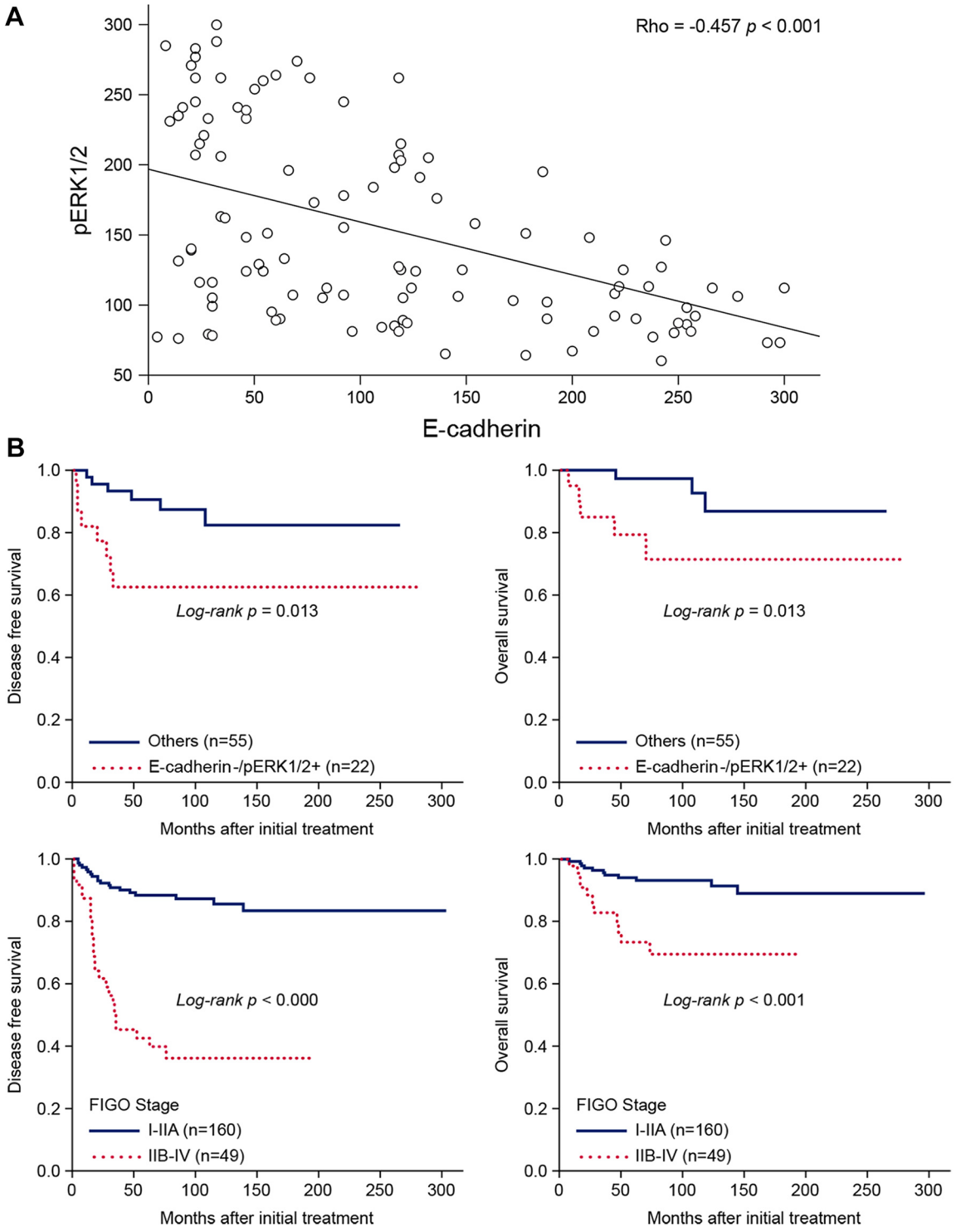


Figure 2. *Correlation analysis of E-cadherin and pERK1/2 expression in cervical cancers. (A) The scatter plot illustrates the Spearman rank correlation between E-cadherin and pERK1/2 expression levels in cervical cancer tissues. (B) Kaplan–Meier plots show disease-free survival (DFS) and overall survival (OS) for groups categorized by high and low expression of E-cadherin and pERK1/2.*

Table II. *Univariate and multivariate analyses of disease-free survival or overall survival in cervical cancer patients.*

Variables	Disease-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Age (>50)	1.49 (0.83-2.67)	0.181	NA		0.78 (0.33-1.82)	0.567	NA	
FIGO stage (>IIB)	6.72 (3.59-12.58)	<0.001	10.99 (2.56-47.22)	0.001	4.15 (1.84-9.38)	0.001	4.54 (0.78-26.61)	0.093
Grade (poor)	1.73 (0.96-3.12)	0.07	NA		2.03 (0.9-4.61)	0.09	NA	
Cell type (non-SCC)	1.03 (0.48-2.21)	0.939	NA		2.48 (1.06-5.8)	0.036	5.47 (0.87-34.42)	0.07
Tumor size (>4 cm)	2.31 (1.27-4.18)	0.006	0.77 (0.26-2.25)	0.628	1.96 (0.85-4.51)	0.114	NA	
LN metastasis	3.97 (2-7.88)	<0.001	1.79 (0.61-5.21)	0.287	2.69 (1.06-6.86)	0.038	1.22 (0.3-4.98)	0.787
SCC Ag^+	2.38 (1.26-4.52)	0.008	1.01 (0.37-2.81)	0.979	2.78 (1.18-6.55)	0.02	2.7 (0.61-11.91)	0.189
E-cadherin^{+a}	0.35 (0.14-0.85)	0.021	0.58 (0.2-1.73)	0.331	0.19 (0.04-0.82)	0.027	0.32 (0.06-1.59)	0.163
pERK1/2^{+b}	7.01 (2.69-18.28)	<0.001	4.75 (1.45-15.6)	0.01	5.69 (1.63-19.82)	0.006	6.21 (1.16-33.21)	0.033
E-cadherin+ pERK1/2- *vs.* Others	3.48 (1.69-7.14)	0.001	3.65 (1.52-8.74)	0.004	6.28 (2.17-18.16)	0.001	7.51 (2.09-27.03)	0.002

[a]cut-off value of E-cadherin$^+$ is over 119 of IHC score; [b]cut-off of pERK1/2 is over 146 of IHC score; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; NA, not applicable.

phosphorylation levels. We found that the knockdown of E-cadherin by siRNA increased ERK phosphorylation in SiHa, ME180, and SNU-17 cervical cancer cells (Figure 3B). We investigated whether E-cadherin regulates ERK signaling by modulating the activity of other upstream signaling molecules, such as EGFR. EGRF is known to co-localize with E-cadherin in the basolateral areas of epithelial cells and form complexes with E-cadherin (24-26). First, we performed co-immunoprecipitation to confirm E-cadherin/EGFR heterocomplex formation in cervical cancer cells. In SiHa and SNU-17 cells, the proteins co-immunoprecipitated in the absence of EGF, but treatment with 50 nM EGF for 15 min significantly decreased the complex levels (Figure 3C). Binding of EGFR to its ligand, EGF, induces the dimerization of EGFR, resulting in the activation of multiple signaling cascades. These data indicate that EGF stimulation disrupts E-cadherin–EGFR complexes in cervical cancer cells. Next, we assessed the effects of E-cadherin knockdown on the association between E-cadherin/EGFR heterocomplexes and ERK signaling. As shown in Figure 3D, the co-precipitation of E-cadherin with EGFR was completely abolished when cervical cancer cells were transfected with siE-cadherin. We also observed up-regulation of pERK, which is a downstream effector of EGFR. These results demonstrate that the loss of E-cadherin leads to EGFR mobility, which may stimulate EGFR dimerization and further boost EGFR-ERK activation.

Dependence of cellular proliferation on ERK phosphory-lation following E-cadherin knockdown in cervical cancer cell lines. E-cadherin-mediated adherent junctions are hubs of intracellular signaling that regulate cell proliferation, survival, invasion, and migration (27). Elevated ERK activity is associated with hyperproliferation. Therefore, we analyzed the effects of E-cadherin knockdown on the proliferation of SiHa and SNU-17 cervical cancer cells. Compared to that of siN.C transfected cells, cell proliferation was increased in siE-cadherin transfected SiHa and SNU-17 cells (Figure 4A). To determine whether the effect of E-cadherin knockdown on cervical cancer cell proliferation depends on ERK signaling, we used an inhibitor of the MEK/ERK pathway, PD98059. PD98059 treatment markedly inhibited ERK phosphorylation (Figure 4B). PD98059 treatment clearly reduced the promoting effect of

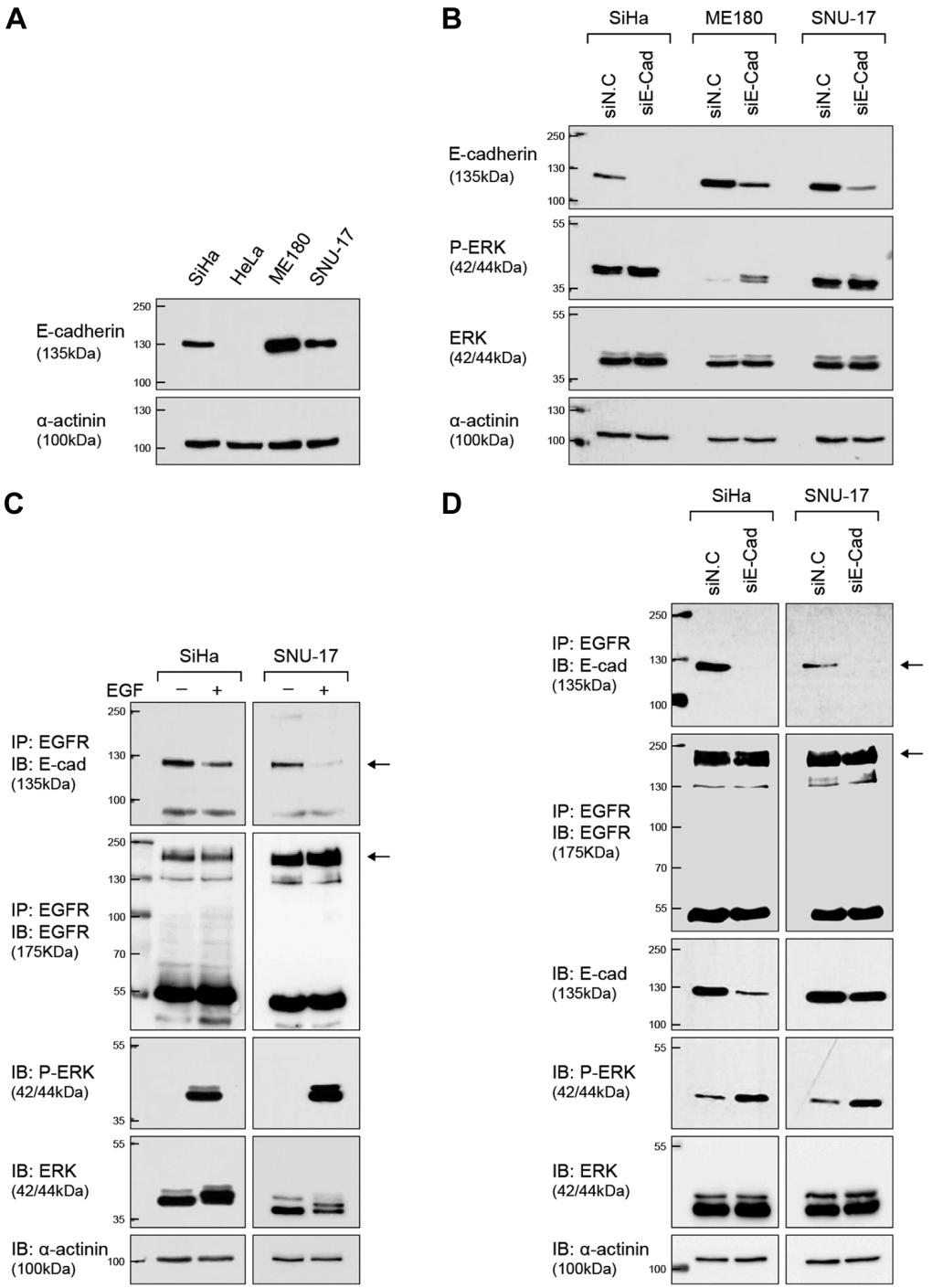


Figure 3. *Inverse correlation of E-cadherin expression with ERK phosphorylation. (A) Expression of E-cadherin in 4 cervical cancer cell lines determined via western blot analysis. α-actinin was used as the loading control for quantitative western blotting. (B) SiHa, ME180, and SNU-17 cells were transfected with siRNA against E-cadherin for 48 h. Protein expression of E-cadherin, P-ERK, ERK, and α-actinin was analyzed by western blot. (C) 50 nM EGF was added to SiHa and SNU-17 cells for 15 min, and lysates were immunoprecipitated with an EGFR antibody followed by immunoblotting with E-cadherin antibody. The blot was re-probed with anti-EGFR antibody. (D) SiHa and SNU-17 cells were transfected with siRNA against E-cadherin for 48 h. Lysates were immunoprecipitated with an EGFR antibody followed by immunoblotting with E-cadherin antibody. The blot was re-probed with anti-EGFR antibody.*

E-cadherin on cell proliferation (Figure 4C). These *in vitro* data suggest that E-cadherin knockdown in cervical cancer cells may affect cell proliferation by inversely regulating ERK phosphorylation levels.

Discussion

This study elucidated the interaction between E-cadherin down-regulation and enhanced pERK1/2 signaling in cervical cancer, a connection that was previously underexplored. We demonstrated a significant inverse correlation between these biomarkers, and directly linked their combined expression levels to survival outcomes, marking a substantial advancement in our understanding of cervical carcinogenesis. The coupling of low E-cadherin expression with high pERK1/2 expression significantly exacerbates disease progression, highlighting its potential as a biomarker for aggressive cancer phenotypes. These insights suggest that therapeutic strategies targeting specific molecular interactions can disrupt key oncogenic pathways and improve patient prognosis. Moreover, our results support the implementation of a dual biomarker strategy for more precise prognostic assessment in clinical settings. Integrating this approach could transform the current treatment paradigms, enabling personalized therapies that are better aligned with the molecular profiles of individual tumors. The results of this study highlight the significant prognostic implications of E-cadherin and pERK1/2 expression in cervical cancer and establish their correlations with various clinicopathological parameters. E-cadherin, traditionally viewed as a tumor suppressor, is expressed at lower levels in cervical cancer tissues than in normal tissues. This decrease was statistically significant and strongly associated with poor DFS and OS, highlighting its importance as a marker of tumor aggressiveness and potential metastasis. These findings are consistent with those of previous studies, showing that E-cadherin can be used as a prognostic marker in gastric cancer, head and neck squamous cell carcinoma, and endometrial cancer in clinical practice (12, 28-30). Additionally, a recent study showed that low E-cadherin expression was correlated with an increased risk of recurrence in breast cancer patients (29). The analysis also revealed that high pERK1/2 expression was significantly correlated with unfavorable clinicopathological features, including larger tumor size and advanced clinical stage, underscoring its role in promoting oncogenic signaling. These results align with findings in breast and hepatocellular carcinomas, suggesting that pERK1/2's role as a prognostic biomarker extends across different cancer types (31, 32). Notably, the interaction between these two biomarkers was particularly revealing, as patients with low E-cadherin and high pERK1/2 expression demonstrated a markedly worse survival outcome, with multivariate analysis confirming a compounded negative effect on patient survival. This synergy suggests that the loss of the tumor-suppressive effect of E-cadherin, coupled with the activation of ERK signaling, propels tumor progression more significantly than either factor alone. Importantly, this mechanism is supported by findings in various cancers, such as breast and non-small cell line cancers, where E-cadherin depletion induces EMT and facilitates invasion in a matrix metalloproteinase -2 (MMP2)-dependent manner with aberrant activation of ERK signaling (33). This evidence highlights the universality of this molecular interaction across different types of cancers.

To further underscore the potential targeting of this pathway in cervical cancer, we observed an inverse correlation between E-cadherin and pERK1/2 expression in the cervical cancer cell lines SiHa, ME-180, and SNU-17. In addition, they were inversely associated with the proliferation of cervical cancer cells. Coimmunoprecipitation experiments showed that EGF stimulation or E-cadherin silencing disrupted the E-cadherin/EGFR complex, suggesting that the loss of E-cadherin expression could lead to EGFR mobility, which could further promote cell proliferation by activating the EGFR/ERK signaling pathway. Moreover, the EGFR/ERK signaling pathway is abnormally activated when E-cadherin is reduced, and is also strongly associated with invasion and migration, which depends on MMP in cancers. Consistently, our data showed that E-cadherin silencing led to increased cell proliferation, accompanied by the activation of the MAPK/ERK signaling pathways. Similar to our own study, the creation of an EMT-

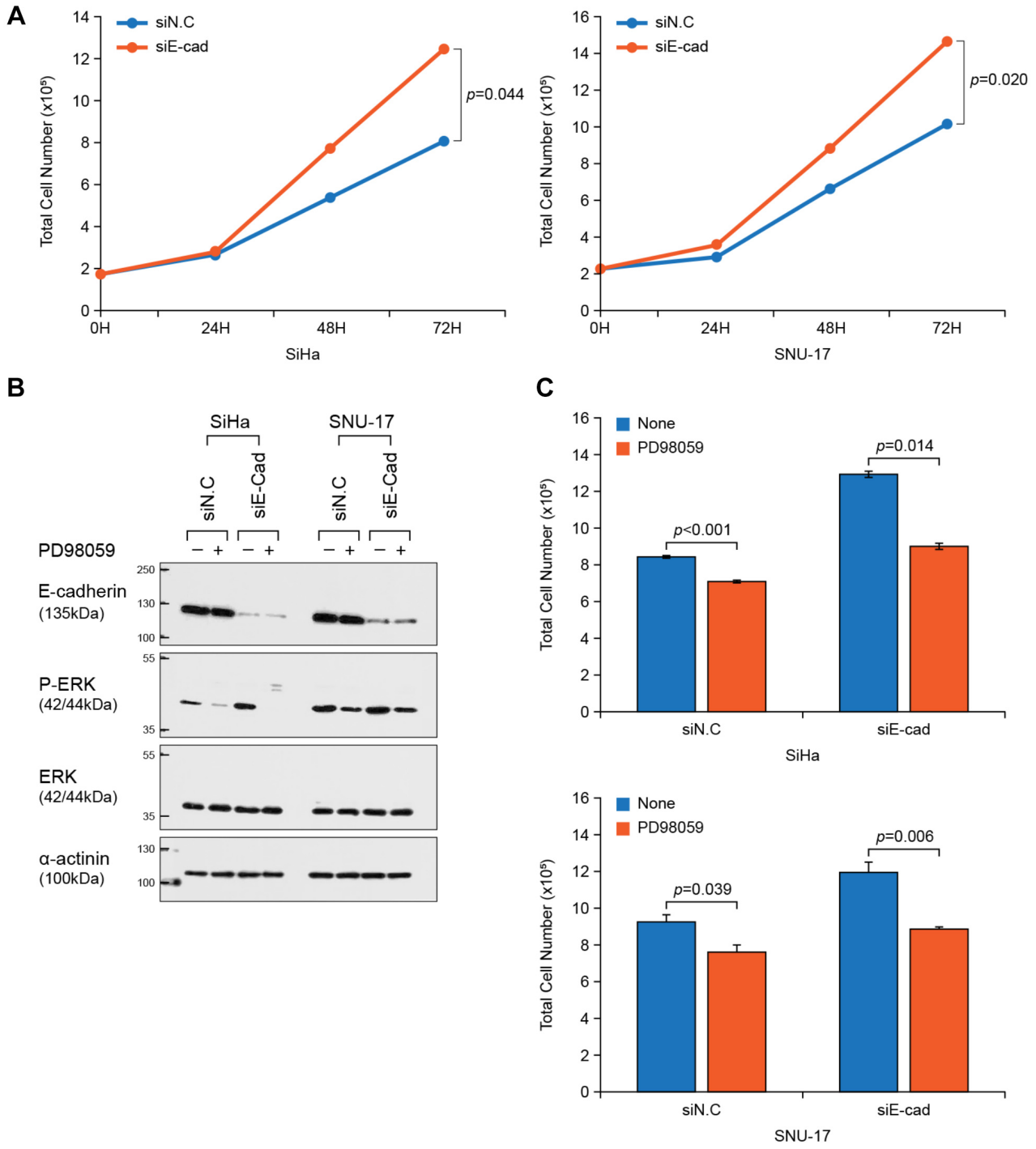


Figure 4. *Knockdown of E-cadherin expression promotes cervical cancer cell proliferation through ERK signaling. (A) SiHa and SNU-17 cells (2×10^5) were plated on six-well plates and then cells were transfected with siRNA against E-cadherin. At 24 h intervals, cells were harvested and counted. (B) SiHa and SNU-17 cells transfected with siE-cadherin were treated with DMSO or 20 μM PD98059 for 24 h. Protein expression of E-cadherin, P-ERK, ERK, and α-actinin was analyzed by western blot. (C) SiHa and SNU-17 cells were transfected with siRNA against E-cadherin for 24 h and then treated with DMSO or 20 μM PD98059 for an additional 24 h. 72 h after seeding the cells, cells were dissociated and counted.*

induced cell line through E-cadherin knockdown in non-small cell cancer revealed significant alterations in EGFR-dependent signaling *via* multiple key signaling kinases, including pERK1/2 (33). In particular, ERK activation through EMT induction promotes the imitation of epithelial tube development *via* EMT association morphological changes and contributes to metastasis and invasion through EMT induction in various human malignant cell lines (34-36).

In our investigation, targeted inhibition of ERK signaling *via* the MEK/ERK inhibitor, PD98059, significantly reduced pERK levels, effectively mitigating the hyperproliferative effects observed in E-cadherin-negative cervical cancer cells *in vitro*. This substantiates the potential of MEK/ERK pathway modulation as a strategic therapeutic intervention for cervical cancer characterized by E-cadherin deficiency. Notably, ONC201 (TIC10/NS350625), which was approved by the FDA in 2020 for the treatment of metastatic lung cancer following resistance to platinum-based therapies, is a promising therapeutic candidate for similar applications in cervical cancer given its mechanism of action (37). Moreover, Rineterkib, also known as LTT-462 or ERK-IN-1, a dual RAF and ERK1/2 inhibitor, has demonstrated significant preclinical efficacy in various MAPK-activated cancers, thereby extending its potential utility to a broader range of malignancies (38). Ongoing clinical evaluations, including a Phase I trial (NCT02711345) assessing its safety and efficacy across several advanced solid tumors and subsequent phase Ib/2 trials (NCT02974725 and NCT04417621) exploring its use in combination with other targeted agents for NSCLS and melanoma, underscore the critical role of ERK inhibition in oncology (39, 40). The integration of rineterkib into clinical trials for cervical cancer could therefore offer a novel approach for managing this malignancy, particularly in patients with low E-cadherin and high pERK1/2 expression.

It should be noted that this study has several limitations. Firstly, while we established a strong correlation between E-cadherin and pERK1/2 through statistical analysis and *in vitro* experiments, we did not perform co-expression analysis, which may have provided further insights into the spatial dynamics of their interaction. Secondly, this was a retrospective study with inherent biases, including a relatively small sample size and a broad timeline of case inclusion, which could influence the generalizability of the results. Additionally, although *in vitro* experiments were conducted to validate certain findings, no cellular experiments were performed specifically to further explore the mechanistic interactions of these biomarkers in the clinical context. Despite these limitations, our findings underscore the potential of E-cadherin and pERK1/2 as significant biomarkers in cervical cancer prognosis.

Conclusion

In conclusion, this study elucidated the pivotal roles of E-cadherin down-regulation and enhanced pERK1/2 signaling in exacerbating cervical cancer progression, establishing their combined expression as a significant prognostic indicator. The observed synergistic effect of these biomarkers highlights their potential in identifying aggressive cancer phenotypes and suggests that targeting the MEK/ERK pathway, particularly with inhibitors, such as PD98059, could provide a promising therapeutic strategy for E-cadherin-deficient tumors. Encouraged by the efficacy of MEK/ERK inhibition in clinical trials for other cancers, our results advocate for further research on integrating this pathway into cervical cancer treatment protocols, paving the way for more personalized and effective therapeutic strategies. Future studies should focus on clinical validation and exploration of the underlying molecular mechanisms to enhance the precision of targeted therapies in oncology.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization, H.Y., G.H.H., J.-Y.C. and H.C.; methodology, G.H.H., J.-H.K., J.-Y.C. and H.C.; data curation,

G.H.H., H.Y. D.W. and H.C.; investigation, H.Y. D.W. and D.C.; writing-original draft preparation, H.Y. and G.H.H; writing-review and editing, J.-Y.C., J.-H.K. and H.C. All Authors read and approved the final manuscript.

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