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# The role of *EpCAM* in tumor progression and the clinical prognosis of endometrial carcinoma

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## HIGHLIGHTS

- Downregulation of EpCAM favors a poor prognosis and cancer cell invasion of EC.
- ERα-induced EpCAM expression suppresses the dissemination of EC cells.
- Transamin is a potential inhibitor of highly invasive EC cells.

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## ABSTRACT

*Objective.* *EpCAM* is a transmembrane glycoprotein that functions as an epithelial marker in endometrial tissues. However, the correlation between *EpCAM* and endometrial carcinoma (EC) is not clear.

*Methods.* This study investigated the association between *EpCAM* and EC. Immunohistochemistry staining and bioinformatics analysis disclosed the clinical importance of low *EpCAM* expression. The migratory ability of cells expressing low *EpCAM* levels was studied in transwell invasion assays *in vitro* and an orthotopic intrauterine tumor injection model *in vivo*. The Connectivity MAP was used to identify drugs that effectively inhibit cells with low *EpCAM* expression.

*Results.* According to immunohistochemistry analysis results, low *EpCAM* expression was associated with an advanced stage and lymph node metastasis in patients with endometrioid EC, and high *EpCAM* expression favored survival. *EpCAM* silencing promoted cell invasion, and *EpCAM* re-expression in *EpCAM*-silenced EC cells attenuated their invasiveness. *EpCAM* suppression in an orthotopic uterine implantation model promoted the lymph node metastasis of EC cells. According to quantitative PCR and promoter reporter analyses, estrogen receptor alpha signaling regulated *EpCAM* expression by enhancing its promoter activity. As shown in the Connectivity MAP analysis, transamin inhibited the invasiveness of *EpCAM*-silenced EC cells.

*Conclusions.* The loss of *EpCAM* may increase the malignancy of EC, and these findings provide new insights into the prognostic role of *EpCAM* in patients with EC.



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## 1. Introduction

Endometrial carcinoma (EC) arises from the uterine endometrium and is the most common gynecological malignancy in developed countries [1]. The prognosis of patients with EC primarily relies on information obtained during the staging surgery, which includes total hysterectomy, bilateral salpingo-oophorectomy, and pelvic/para-aortic lymph node dissections [2]. Most patients are diagnosed with EC at an early stage, which portends a good prognosis. However, patients with EC who are diagnosed with a late-stage tumor have limited therapeutic options and often experience treatment failure [2,3]. Patients with EC whose tumors have metastasized to the lymph nodes or distant organs often exhibit poor survival outcomes [2]. Therefore, the discovery of reliable biomarkers and therapeutic targets for patients with EC who are susceptible to relapse or metastasis is urgently needed.

Uterine tumors are categorized into two types based on histology. Type I EC exhibits an endometrioid histology with a low histological grade (mostly grade 1 or 2) and a favorable survival outcome, whereas type II EC typically exhibits a non-endometrioid histology with a high histological grade and a poor prognosis. The uterine endometrium is exquisitely sensitive to estrogen, which acts through the estrogen receptors ERα and ERβ to drive cell proliferation. Endometrioid EC is associated with hyperestrogenic risk factors, including estrogen hormone replacement therapy and obesity [4], but non-endometrioid EC is not sensitive to estrogen. Moreover, the absence of *ERα* expression in endometrioid EC correlates with an advanced grade and stage and with lymph node metastasis [2]. ER functionality/expression status has not been applied to patients with EC in the clinic, unlike patients with breast cancer. Although *ERα* expression inversely correlates with lymph node metastasis in patients with endometrioid EC, whether ERα signaling affects adhesion molecule expression, and thus has an inhibitory effect on EC cell invasiveness, needs to be further elucidated.

Increased invasion and migration are the defining characteristics of metastatic cancer cells [5]. EpCAM is a glycosylated type I membrane protein that is expressed in a variety of normal human epithelial cells and functions as a cell-cell adhesion molecule to maintain cell polarity [6]. EpCAM is involved in the proliferation and differentiation of the mouse endometrial epithelium, and it serves as a biomarker for the selection of endometrial epithelial progenitors [7]. Although EpCAM is overexpressed in certain cancers, its role in cancer progression is controversial [8].

The biological and clinical relevance of *EpCAM* overexpression in EC progression and tumor metastasis is largely unknown. Therefore, this study investigated the role of EpCAM in EC dissemination and found that ERα signaling regulated *EpCAM* expression and that the downregulation of *EpCAM* expression facilitated cancer cell dissemination.

## 2. Materials and methods

### 2.1. Patients and tumor samples

Tumor tissues from 55 female patients diagnosed with endometrioid-type uterine EC were obtained as paraffin blocks from the surgical archives of the Department of Pathology and the Department of Gynecology and Obstetrics, Taipei Veterans General Hospital. Patients from the Taipei Veterans General Hospital were diagnosed between 1998 and 2008. The surgical stage was determined after a staging surgery and revised according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO staging system for uterine cancer, 2009) [9]. Clinical data for 55 patients, including age, serum level of the tumor marker CA-125, overall survival, recurrent/alive status, and disease-free survival, were retrieved from their medical records. The duration of overall survival was recorded from the date of diagnosis to death or the date of the last follow-up. Disease-free survival was recorded as the time between the completion of primary therapy and disease recurrence. Staging surgery, including total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymph node sampling, was performed on all patients [10]. Clinical remission was defined as a lack of a palpable tumor and a normal CA-125 level after the completion of chemotherapy. Patients were followed every 3 months during the first 2 years and every 6 months in subsequent years. Recurrence was defined as suspicion of a tumor in an imaging study or pathologically proven adenocarcinoma after complete recovery from cancer. The uterine adenocarcinoma tissue samples obtained at the primary surgery were stained with hematoxylin and eosin and reviewed to confirm the histopathological diagnosis and tumor grade. All specimens were collected under protocols approved by the institutional review board of the Taipei Veterans General Hospital. Additional immunohistochemistry specimens (N = 97 carcinoma cases) from the commercial EMC1021 human endometrial cancer tissue array were obtained (US Biomax, Inc.) with complete clinical data, including clinical stage and grade.

### 2.2. Cell lines

The Ishikawa and RL95-2 cell lines were kindly provided by Dr. Ching-jang Huang (National Taiwan University) and the Taiwan Bioresource Collection and Research Center, respectively. The $ER^+$ Ishikawa cells (human Asian endometrial adenocarcinoma, European Collection of Cell Cultures, ECACC, No. 99040201) and $ER^-$ Ishikawa02 cells (human endometrial adenocarcinoma, ECACC No. 98032302) were kindly provided by Dr. Yi-Jen Chen (Taipei Veterans General Hospital). All cells were cultivated in DMEM supplemented with 10% fetal bovine serum as previously described [11].

### 2.3. Gene expression analysis of uterine carcinoma

The public gene expression profiling datasets used in this study were analyzed using a previously described method [12]. Gene expression, survival, and clinicopathological data were obtained from bioinformatics datasets, including the cBioPortal TCGA Cancer Genomics browser (https://www.cbioportal.org) and the Oncomine (https://www.oncomine.org) Bittner pan-cancer microarray database. Moreover, the Cancer Cell Line Encyclopedia (CCLE) dataset of endometrial cancer cell lines was also analyzed in this study. EMT signature genes were derived from published information [13,14].

### 2.4. Immunohistochemistry

Immunohistochemical procedures were performed using previously described methods [15,16]. Immunostaining was performed using a

**Fig. 1.** Correlation of EpCAM expression with the survival of patients with EC. (A) Immunohistochemical staining representing weak, moderate, and strong EpCAM expression in endometrial tumors from patients at Taipei Veterans General Hospital. (B) The Kaplan-Meier analysis assessed the correlation between EpCAM expression and progression-free survival (left panel) and overall survival (right panel) in patients with endometrial tumors from Taipei Veterans General Hospital. Different groups were compared using the log-rank test. (C) Comparison of the death rates between groups with low and high *EpCAM* expression in endometrioid endometrial tumors by a tercile approach. Kaplan-Meier analysis assessed the correlation of *EpCAM* expression with the overall survival of patients with endometrioid endometrial tumors; data were from the TCGA database (cBioPortal EC genomics). A log-rank $p$-value < 0.05 suggests a significant difference in overall prognosis ($^*p < 0.05$). (D) Death rates were compared between patients with low and high *EpCAM* expression in non-endometrioid (mixed or pure serous histology) endometrial tumors by a tercile approach. The Kaplan-Meier analysis assessed the correlation between *EpCAM* expression and the overall survival of patients with non-endometrioid endometrial tumors; data were from the TCGA database. Different groups were compared using the log-rank test.

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