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# Tissue expression of human epididymal secretory protein 4 may be useful in the differential diagnosis of uterine cervical tumors

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#### ABSTRACT

**Objectives:** Human Epididymal Secretory Protein 4 was firstly described as an epididymis-specific protein but more recently it has been demonstrated to be a putative serum tumor marker for different malignancies, especially ovarian epithelial cancers. The aim of this study is to investigate the association between tissue Human Epididymal Secretory Protein 4 expression and the clinicopathological features of uterine cervical tumors.

Material and methods: This retrospective study was designed to evaluate the differences of tissue expressions of Human Epididymal Secretory Protein 4 protein in a spectrum of cervical neoplasms. One hundred and seven patients recently diagnosed as having cervical intraepithelial neoplasm or invasive squamous cell carcinoma, adenosquamous carcinoma and adenocarcinoma based on pathology databases.

**Results:** Decreased or negative Human Epididymal Secretory Protein 4 expressions were determined in both normal cervical epithelia and in intraepithelial carcinomas, while increased HE4 expression was observed in invasive tumors.

**Conclusions:** This study demonstrated that altered expression of Human Epididymal Secretory Protein 4 may involve in tumorigenesis in the uterine cervix. Our findings also suggested the presence of a correlation between Human Epididymal Secretory Protein 4 expression and the invasive potential of uterine tumors. Therefore it may be thought that the tissue expression of HE4 can be used to differentiate high grade intraepithelial tumors from carcinomas.

Key words: uterine cervix, Human Epididymal Secretory Protein 4, HE4, squamous cell carcinoma, adenosquamous carcinoma, adenocarcinoma

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#### INTRODUCTION

Human Epididymal Secretory Protein 4 (HE4) was discovered by Kirchhoff et al. [1] in 1991. HE4 contains four disulfide core proteins and due to similarities with other whey acidic protein family members, it functions as a proteinase inhibitor [2, 3]. Firstly, presence of HE4 in the distal regions of the epithelial cells of the epididymal duct, suggests its roles in sperm maturation [1–5]. Currently, it is known that HE4 is expressed in a large spectrum of normal and malignant tissues. Majority of secretory cells such as glandular epithelium of the breast and female genital tract, epididymis, vas deferens, distal renal tubules, respiratory epithelium and salivary glands show strong HE4 positivity [6]. Physiopathologically, HE4 probably involves in cancer progression and metastases [7]. In intact tissues of trachea and salivary gland highest levels of HE4 expression, while in lung, prostate, pituitary gland, thyroid and kidney lower HE4 positivity have been detected. Some studies have shown marked HE4 expressions in ovarian epithelial cancers, mesotheliomas, transitional cell carcinomas and tumors of lung, endometrium, breast, gastrointestinal system and kidney [6]. Ovarian epithelial cancers, especially serous and endometrioid ovarian cancers

**Corresponding author:** Gulden Diniz Kibris Sehitleri Cad. 51/11 Alsancak/Izmir 35220, Turkey tel.: 90 232 3627144 fax: 90 232 3625522 e-mail: agdiniz@gmail.com have demonstrated highest rates of HE4 positivity among these tumors [8].

In spite of frequent implementation of screening programs, uterine cervical cancers are still one of the main causes of cancer mortality among female population around the world [9, 10]. Squamous cell carcinoma (SCC) is the most frequent histological type accounting for 75-80% of invasive uterine cervical carcinomas [9-11]. Adenosquamous carcinomas (ASCs) can be defined as tumors that contain a mixture of malign squamous and glandular cells. ASC is the second frequent cervical cancer and its incidence is reported to range between 3.6 % and 25% among all cervical cancers. The prevalence of ASC is higher particularly in young women. It metastasizes to pelvic lymph nodes twice as frequently as SCC or adenocarcinomas [8-10]. Most authors have reported a poorer prognosis for patients with ASCs. Other type is pure adenocarcinoma (AC) of the uterine cervix. Squamous and glandular components of cervical cancers are monoclonal in origin and arise from the pluripotent subcolumnar reserve cells of the endocervical mucous epithelium [8–11].

In the past decade, HE4 emerged as a promising biomarker to address the unmet clinical needs in ovarian and endometrial cancer. Herein, we studied the immune expressions of HE4 in different uterine cervical malignancies. In addition, we aimed to determine both the role of HE4 in the differential diagnosis among uterine cervical tumors, and its prognostic value.

### **MATERIAL AND METHODS**

At Tepecik Training and Research Hospital 107 patients with the diagnoses of CIN, SCC, ASC and AC based on immunohistochemical analysis of pathology specimens were included in the study. The study was approved by the Local Ethics Committee of the Hospital.

For immunhistochemistry (IHC), hematoxylin and eosin (HE) staining was used to select appropriate paraffin blocks and to identify the viable tumor areas. For the assessment of HE4 expression in normal cervical epithelium, we also selected specific paraffin blocks which contained both tumor areas and surrounding normal tissues. IHC was performed using streptavidine biotin peroxidase method (Invitrogen, Camarillo, 85–9043, CA, USA). Serial 5-µm-thick sections were obtained and these slides were baked overnight at 60°C, dewaxed in xylene, hydrated with distilled water, then treated with decreasing concentrations of alcohol. All slides were subjected to heat- induced epitope retrieval procedure in the microwave (in 10 mM/L citrate buffer, pH 6.0, for 20 minutes, followed by cooling at room temperature for 20 minutes) so as to block endogenous peroxidase and biotin activities. The purified monoclonal mouse antibodies against Human Epididymal protein 4 (Anti-HE4 Ab, Abcam, ab24480) were used at a dilution of 1:40. Laboratory tests were evaluated by a researchers (GD, SS, DA) blinded to the clinical features of the patients. Strong and diffuse cy-toplasmic staining indicated presence of HE4 expression. *Chi*-square test was performed for statistical analysis using SPSS 15.0. software statistical package program. P values less than 0.05 were considered to be statistically significant.

## RESULTS

This series consisted of 30 (28%) low (CIN1), 29 (27.1%) high grade CINs (CIN2 and CIN3), 27 (25.2%) SCCs, 15 (14%) ASCs and 6 (5.6%) ACs. Mean age of the patients was 47.9  $\pm$  12.8 years (ranging from 20 to 80 years). The cases with invasive carcinomas (52.6  $\pm$  13.2 years/30 to 80 years) were older than cases with intraepithelial neoplasms (44.01  $\pm$  11.1 years/20 to 66 years). Contrarily, the mean age was similar in all invasive tumor groups. For example the mean age for SCC was 52.8  $\pm$  14.8, while it was 52.9  $\pm$  12.1 and 51.5  $\pm$  9.2 for ASC and AC, respectively.

Decreased or negative HE4 expressions were detected both in normal cervical epithelia and in most intraepithelial carcinomas (Fig. 1). HE4 expression was determined in 45 of 48 invasive carcinomas (93.8%), and in only 34 of 59 noninvasive neoplasms (57.6%) Fisher's Exact Test, revealed a statistically significant correlation between cellular invasion and HE4 expression (p < 0.001) (Fig. 2). Conversely, there was no association between subtypes of invasive tumors and expressions of HE4 (p = 0.595) (Fig. 3).

# DISCUSSION

HE4 is a novel biomarker that is expressed in most of the epithelial ovarian cancers but not in normal surface epithelium [12]. HE4 is expressed in the Mullerian type epithelium of ovarian cortical inclusion cysts which suggested that HE4 might be a significant predictor for Mullerian- derived tumors [13]. A prominent upregulation of HE4 immune expression has been seen in female genital cancers, especially in ovarian serous and endometrioid carcinomas. Detection of HE4 expression with immunohistochemical staining in several studies has revealed that HE4 is expressed in all endometrioid adenocarcinomas, 90% of serous adenocarcinomas and 50% of clear cell epithelial ovarian cancers [12, 13]. Interestingly, high HE4 tissue expression is mostly found in epithelial ovarian adenocarcinomas [13]. Conversely, HE4 overexpression has not been detected in ovarian mucinous tumors [4-6], non-epithelial ovarian cancers, sex cord stromal tumors and germ cell tumors. However overexpression of HE4 has been detected in primary tuba uterina carcinomas and endometrial malignancies [2-7]. Among non-gynecological cancers, HE4 expression has been also demonstrated in various subtypes of lung cancer [12]. The lowest HE4 expression has been detected in small-cell car-



Figure 1 A. Note HE4 positivity in invasive component of a SCC and tHE4 negativity in normal cervical epithelium on the left of field, B. strong HE4 expression in an adenosquamous carcinoma, and C. weak HE4 expression in an adenocarcinoma (DABX 100)



**Figure 2.** Higher HE4 expression in invasive carcinomas, relative to lower HE expression in intraepithelial tumors (p < 0.001)

cinomas when compared with non-small cell carcinomas such as squamous cell carcinomas, adenocarcinomas and large-cell lung cancers [4, 14–16]. In the gastrointestinal system, HE4 tissue overexpression was found in stomach [18], pancreatic, colorectal , and hepatocellular carcinomas [4, 14, 18, 19]. In the urological tract, varying HE4 expression levels were showed in clear cell carcinomas, papillary renal cell carcinomas, and chromophobe carcinomas [4, 12, 18–20]. Although HE4 expression has been extensively studied in several carcinomas, there are little or no data on the expression and significance of HE4 in the uterine cervical tumors. In this study, majority of uterine cervical carcinomas demonstrated HE4 expression without any statistical significant difference among subtypes of carcinomas.

Currently HE4 levels have been measured both in tissue sections, and sera. The potential use of HE4 as a tumor marker has been supported by an increasing number of studies demonstrating an upregulation of HE4 in a range of malignant neoplasms, particularly of gynecological, pulmonary



Figure 3. A diagram demonstrating lackof any correlation between subtypes of invasive tumors and HE4 expressions of (p = 0.595)

and gastrointestinal origin [3, 12, 13, 16, 17, 21]. Serological tests have revealed that HE4 has increased sensitivity and specificity in the detection of ovarian cancer relative to CA 125, which is the current gold standard serum biomarker for ovarian carcinomas [3, 12]. Several studies examining multiple serum biomarkers for epithelial ovarian carcinomas have found the combination of HE4 and CA125 had greater sensitivity and specificity than either marker alone. Because CA 125 is often elevated in benign conditions or as a result of physiologic changes in premenopausal women, it has not any confirmed role in the early detection of gynecologic cancers. Since abnormal levels of HE4 have been less frequently encountered in benign gynecological conditions HE4 may have increased specificity in differentiating between benign and malignant conditions [2-7]. In the present study, we have found that HE4 tissue expression increased in carcinomas, but decreased in intraepithelial neoplasia. We thought that the screening test for serum HE4 level may be also clinically useful in the differential diagnosis between invasive and intraepithelial cervical neoplasms. Despite the fact that our data require further evaluation on a large scale population, it can be suggested that the tissue expression of HE4 can be used to differentiate the CIN from carcinomas.

In this study, the higher HE4 expression was found mainly in invasive carcinomas of the uterine cervix compared to non-invasive carcinomas. The relationship between uterine cervical neoplasms and HE4 expression has not been investigated in the literature up to now. However similar results were reported about ovarian epithelial neoplasms [14, 20]. For example Georgakopoulos et al. [14] determined that HE4 protein was significantly upregulated in epithelial carcinomas compared with benign or borderline tumors. In this study, we compared HE4 immune expression levels in several subtypes of cervical uterine tumors and found significant differences in HE4 expression levels between invasive carcinomas and CINs. Since comparative studies on this issue has not been cited in the literature we haven't enough data to discuss HE4 immune expression levels in uterine cancer subtypes.

Guo et al. [21] reported that HE4 was significantly upregulated in human gastric cancer and correlated with some prognostic factors such as TNM stage, tumor size, and survival. The overall survival rate of patients without HE4 expression was significantly higher than those with HE4 overexpression. In addition, the authors demonstrated that silencing HE4 expression inhibited celluar proliferation, migration and enhanced apoptosis. They also suggested that HE4 might regulate proliferation, migration, and apoptosis through Src/Fak, Akt, and Erk1/2 signaling in gastric cancer cells [21]. For these reasons, HE4 may not be only a useful diagnostic marker, but also it might have therapeutic implications for several cancer types [21]. To the best of our knowledge, the present study was the first to determine the differential diagnostic value of HE4 tissue expression for uterine cervical cancers. Unfortunately it has certain limitations. Its most evident limitation was its small sample size. In future studies, it may be appropriate to compare expression levels HE4 in tissue samples , and sera in patients with carcinoma in situ and invasive tumors of uterine cervix. The observations of the present study should be confirmed by future studies using a larger cohort and additional serum analyses.

Herein, we compared HE4 expression patterns in both non-invasive and invasive cervical neoplasms. HE4 protein was significantly upregulated in different subtypes of invasive cervical neoplasms compared with intra-epithelial neoplasms. In addition, we have demonstrated for the first time, that high HE4 expression correlates with an aggressive phenotype and may constitute an independent prognostic factor for uterine cervical neoplasms. In conclusion, the altered expression of HE4 may play a role in tumorigenesis in most SCCs, ASCAs and ACs of uterine cervix. Therefore HE4 expression may be important for the prediction of the tumor progression. In ddition, determination of serum HE4 could be clinically useful in identifying high-risk patients for a more aggressive adjuvant therapy. Finally, the issue whether determination of tissue expression of HE4 can provide prognostic benefit for the patients with uterine cervical neoplasms or it can be used for tumor screening should be investigated in larger series.

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