The expression of EpCAM in extramammary Paget's disease

Saori Yamada-Kanazawa‡, Yukino Tasaki‡, Ikko Kajihara*, Ryoko Sakamoto, Saki Maeda-Otsuka, Hironobu Ihn

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Honjo, Kumamoto, Japan.

Summary Extramammary Paget’s disease (EMPD) is a rare skin malignant tumor. The prognosis of EMPD with distant metastasis is poor, however an effective therapy has not yet established. Recently, EpCAM (epithelial cell adhesion molecule, CD326) has attracted attention as both prognostic marker and therapeutic target in several cancers. Besides, EpCAM is an important surface marker of circulating tumor cell (CTC) in the collection of CTC. Thus, the purpose of our study was to examine the expression levels of EpCAM and evaluate the correlation between its intensity of EpCAM and the clinical characteristics of EMPD. The expression of EpCAM in EMPD was examined using immunohistochemistry. Skin samples were obtained from 32 patients with EMPD. We found that almost all EMPD tissues (90.6%, 29/32) were positive for EpCAM. Furthermore, the staining intensity of EpCAM protein negatively correlated with the presence of distant metastasis. Overexpression of EpCAM in EMPD cells suggests that EpCAM may be a novel therapeutic target and the research of CTC may be newly developed in EMPD. Based on these findings, EpCAM may be a meaningful molecule in EMPD.

Keywords: Extramammary Paget's disease, epithelial cell adhesion molecule, CD326, distant metastasis, immunohistochemistry, circulating tumor cell

1. Introduction

Extramammary Paget's disease (EMPD) is a rare skin malignant tumor that shows erythematous plaques and erosions in pubic or axillary lesions. Although the prognosis of EMPD with distant metastasis is poor, an effective therapy has not yet established. Thus, there is need to investigate molecular targets for EMPD.

Recently, EpCAM (epithelial cell adhesion molecule, CD326) has attracted attention as both prognostic marker and therapeutic target in several cancers (1). EpCAM is involved in not only cell adhesion but also cellular signaling, cell migration, proliferation and differentiation (2). EpCAM is overexpressed in many malignant tumors, e.g. prostatic, ovarian, urothelial and breast carcinoma (2). The anti-EpCAM antibody (catumaxomab) has been authorized for treatment of malignant ascites in cancer patients (3). On the other hand, EpCAM has been reported to be a tumor suppressive protein in certain types of cancers such as oral, rectal and endometrial carcinoma (4-6) although the molecular mechanism of the tumor suppressive function of EpCAM in several cancers has not been found yet (7). Moreover, EpCAM is an important surface marker of circulating tumor cell (CTC) (8), and the CellSearch system (CTC detector of circulating EpCAM+CD45− cells) is approved by FDA (9).

Thus, the purpose of our study was to examine the expression levels of EpCAM and evaluate the correlation between its intensity of EpCAM and the clinical characteristics of EMPD.

2. Materials and Methods

2.1. Patients

Skin samples were obtained from 32 patients with EMPD. All patients with EMPD were diagnosed by clinical and histopathological findings. These
findings were comprehensively analyzed by more than five dermatologists. Paraffin-embedded sections were collected from patients undergoing operation or mapping biopsy in Kumamoto University Hospital between 2011 and 2018. All patients underwent imaging tests for the presence or absence of metastasis. Most of EMPD patients were treated with definitive operation or radiotherapy although EMPD patients with unresectable and metastasis received chemotherapy or palliative radiotherapy. Institutional review board approval and written informed consent were obtained according to the Declaration of Helsinki.

2.2. Immunohistochemical staining

Paraffin-embedded sections were mounted on glass slides, then dewaxed in Clear Plus (FALMA, Japan) and rehydrated in graded alcohols. Immunohistochemistry (IHC) antigen activation was performed by incubation with antigen retrieval solution (pH9, Nichirei, Japan) for 10 min at 121℃. The sections were then incubated overnight with the antibody against EpCAM (Dako, CA, USA, 1:50) at 4℃. The immunoreactivity was visualized with DAB. We used the following grading system: - negative staining, + for slight staining, ++ for strong staining, as described previously (10).

2.3. Statistical analysis

Correlations were assessed according to Fisher’s correlation coefficient and its statistical analyses were performed using GraphPad Prism version 7 (MDF, Tokyo, Japan). A p value less than 0.05 was considered significant.

3. Results and Discussion

First, we investigated EpCAM protein expression in EMPD using immunohistochemical staining. Immunohistochemistry of EpCAM revealed that EMPD cells had more intense staining than the normal keratinocytes cells in same tissue samples (Figure 1). Almost all EMPD tissues (90.6%, 29/32) were positive for EpCAM.

Next, to analyze the relationship between the intensity of EpCAM protein expression levels and clinical findings of patients with EMPD, we classified semi-quantitative scoring of immunoreactivity ([−], [+], [+++]) (Figure 1 and Table 1). The expression of EpCAM in EMPD patients with distant metastasis was significantly decreased compared to that in patients without distant metastasis (p = 0.036), although it did not correlate any other clinical findings (age: p = 0.365, sex: p = 0.087, the degree of invasiveness: p = 0.826, the presence of lymph node metastasis: p = 0.132) (Table 2).

In this study, we presented two novel findings: first, we found that almost all EMPD tissues were positive for EpCAM. Furthermore, the staining intensity of EpCAM protein negatively correlated with the presence of distant metastasis.

EpCAM is overexpressed in several cancers such as adenocarcinomas of colon, stomach, pancreas and lung (11-13). As with these cancers, our results showed that almost all EMPD tissues were positive for EpCAM. It suggests that EpCAM may be a novel therapeutic target in EMPD.

Overexpression of EpCAM was associated with an advanced stage of the disease and linked to worse overall survival in certain tumor types (2). On the other hand, EpCAM could be a tumor suppressive protein in certain types of cancers (7). In gastric cancer, EpCAM appeared to be associated with a more favorable prognosis (14). Besides, the loss of EpCAM in rectal cancer was associated with the reduction of cell–cell adhesion and the augmentation of migration function (15). Our results showed that the EpCAM expression negatively correlated with the presence of distant metastasis. Taken together, these findings suggest that
these findings, EpCAM may be a meaningful molecule in EMPD although further investigations are needed.

Acknowledgements

This study was supported in part by a grant for scientific research from the Japanese Ministry of Education, Science, Sports and Culture and by project research from the Japanese Ministry of Health, Labour and Welfare.

References

13. Brezicka T. Expression of epithelial-cell adhesion molecule (Ep-CAM) in small cell lung cancer as defined


(Received January 26, 2019; Revised February 14, 2019; Accepted February 19, 2019)