Brief Report

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The expression of HER-2 in extramammary Paget's disease

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Summary **Extramammary Paget's disease (EMPD) is a rare intraepidermal adenocarcinoma. The common sites of EMPD involvement are the vulva, perineal, perianal, scrotal and penile skin. Several studies have shown that HER-2/neu, also known as c-erbB-2, is amplified and overexpressed in many cancers. In this study, we investigated the expression and clinical significance of HER-2 in Japanese patients with EMPD. Keratinocytes in epidermis were slightly positive for HER-2. As for EMPD, 19 of 31 EMPD were positive for HER-2 (61%). There is significant correlation between the presence of invasion and strong positivity (3+) for HER-2 ($p < 0.02$). Furthermore, there is significant correlation between the presence of lymph node metastasis and strong positivity (3+) for HER-2 ($p < 0.02$). These results suggest that patients with EMPD strongly positive for HER-2 may have high risk for lymph node metastasis and should be followed up carefully. The observed overexpression of HER-2 in EMPD presents a potential therapeutic target for adjuvant treatment of this disease. Treatment with trastuzumab is well established in breast cancer with HER-2 overexpression and is recommended by several consensus statements. The results of the present study indicate that targeting therapies for HER-2, such as trastuzumab, may be used for EMPD particularly in patients with invasive and/or metastatic EMPD.**

Keywords: Immunostaining, invasion, metastasis, overexpression, therapeutic target

1. Introduction

Extramammary Paget's disease (EMPD) is a rare intraepidermal adenocarcinoma with similar clinical features to inflammatory reactions. The common sites of EMPD involvement are the vulva, perineal, perianal, scrotal and penile skin. The diagnosis of EMPD is frequently delayed and there is a high incidence of associated invasive disease. EMPD presents with well-demarcated, erythematous or leucoplakic plaques. Most cases of EMPD appear eczematous but others are crusting, scaling, lichnoid, or ulcerated. Metastatic dissemination, especially to regional lymph nodes, can also occur. The diagnosis of EMPD rests on the histological identification of unique infiltrating intraepithelial neoplastic cells showing glandular differentiation. Paget's cells are large round cells with abundant pale cytoplasm and large vesicular nuclei. Mitotic figures are unusual and Paget's cells are distributed singly or in groups within the epidermis and epithelium of adnexal structures.

There are two explanations proposed for the pathogenic origin of EMPD. It is thought that Paget's cells either arise within the pore portion of an apocrine duct (*1*) or from the multipotential cells in the epidermis (*2*).

Immunohistochemical analyses have been used both to diagnose EMPD and to determine the origin of Paget's cells. Typically Paget's cells are known to be positive for apocrine and eccrine markers, such as low molecular weight cytokeratins (CK), gross cystic disease fluid protein-15 (GCDFP-15), periodic acid-Schiff (PAS), and carcinoembryonic antigen (CEA).

EMPD is histologically closely related to Paget's disease of the mammary gland, which is an adenocarcinoma affecting the skin of the nipple. Several studies have shown that HER-2/neu, also known as c-erbB-2, is amplified and overexpressed in almost all cases of mammary Paget's disease, in contrast to about 20% of invasive ductal breast carcinomas (*3,4*).

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HER-2 overexpression in breast cancer is correlated with a more aggressive behavior. Previous studies have investigated the expression of HER-2 in EMPD (*4-7*). However, inconsistent results and lack of a large number of cases have resulted in different outcomes among studies.

The HER-2 gene, located on chromosome 17q21, encodes for the 185-kD transmembrane glycoprotein growth factor receptor processing a tyrosine kinase domain (*8,9*). The HER-2 protein recognizes growth stimuli and acts by 2 principal pathways, phosphatidylinositol 3 kinase (PI3K) and extracellular signal-regulated kinase (ERK). Activation of both pathways is intimately correlated with aggressiveness in various cancers (*10,11*). In many human cancers, the HER-2 oncogene is involved in transformation and progression of many human cancer cells (*12*). HER-2 gene amplification and/or HER-2 protein overexpression are common in many human cancers, such as breast, ovarian, and gastric adenocarcinoma (*12).*

In this study, we investigated the expression and clinical significance of HER-2, PAS, CEA, CK7 and epithelial memebrane antigen (EMA) in Japanese patients with EMPD.

2. Materials and Methods

2.1. *Patients*

Besides 5 normal skin samples, skin samples were obtained from 31 patients with EMPD. Institutional review board approval and written informed consent were obtained according to the Declaration of Helsinki. All patients with EMPD were diagnosed by clinical and histopathological findings. All samples were fixed in neutral buffered formalin, embedded in paraffin, and prepared for hematoxylin-eosin examination.

2.2. *Immunohistochemical stainings*

Immunohistochemical staining on paraffin-embedded sections was performed using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's recommendations. Serial 4 μm thick sections were mounted on silane-coated slides (Dako), then deparaffinized with xylene and rehydrated through a graded series of ethyl alcohol and PBS. The sections were then incubated with various antibodies overnight at 4°C. Antibodies against HER-2 (CB11, NCL; 1:50), CEA (A0115, Dako; 1:2,000), EMA (M0613, Dako; 1:200), and CK7 (M7018, Dako; 1:50) were used for primary staining. The immunoreactivity was visualized with Vector Red (Vector Labotatories). The sections were then counterstained with hematoxylin. We used the following grading system: 1+ for slight staining, 3+ for strong staining, and 2+ for staining between 1+ and 3+.

2.3. *Statistical analysis*

Statistical analysis was carried out with Fisher's exact probability test for the analysis of frequency. Two-tailed *p* values less than 0.05 were considered significant.

3. Results

3.1. *Immunoreactivity of PAS, CEA, CK7, and EMA with EMPD cells*

First, we investigated the immunoreactivity of PAS, CEA, CK7 and EMA with EMPD cells. The EMPD cells from all the patients included in this study were positive for PAS, CEA, CK7 and EMA (Figure 1). These results suggested that PAS, CEA, CK7 and EMA stainings were all useful for diagnosis of EMPD, but not for disease activity or severity.

3.2. *Immunoreactivity of HER-2 with EMPD cells*

We investigated the immunoreactivity of HER-2 with normal skin samples. Keratinocytes in epidermis were slightly positive for HER-2 (data not shown). As for EMPD, 19 of 31 EMPD were positive for HER-2 (61%, Table 1 and Figure 2). There is a significant correlation between the presence of invasion and strong positivity (3+) for HER-2 ($p < 0.02$). Furthermore, there is a significant correlation between the presence of lymph node metastasis and strong positivity (3+) for HER-2 ($p < 0.02$). However, there is no significant correlation between the presence of invasion and positivity (1+, 2+, or 3+) for HER-2, or between the presence of lymph node metastasis and positivity (1+, 2+, or 3+) for HER-2.

4. Discussion

The HER-2 gene, located on chromosome 17q21, encodes for the 185-kD transmembrane glycoprotein growth factor receptor processing a tyrosine kinase domain (*8,9*). The HER-2 protein recognizes growth stimuli and acts by 2 principal pathways, phosphatidylinositol 3 kinase (PI3K) and extracellular signal-regulated kinase (ERK). Activation of both pathways is intimately correlated with aggressiveness in various cancers (*10,11*).

In many human cancers, the HER-2 oncogene is involved in transformation and progression of many human cancer cells (*12*). HER-2 gene amplification and/or HER-2 protein overexpression are common in many human cancers, such as breast, ovarian, and gastric adenocarcinoma (*12*).

It is generally agreed that overexpression of proto-oncogenes including myc and HER-2 occurs late in the progression of many human tumors (*13,14*). In this study, there was significant correlation between the presence of invasion and strong positivity (3+)

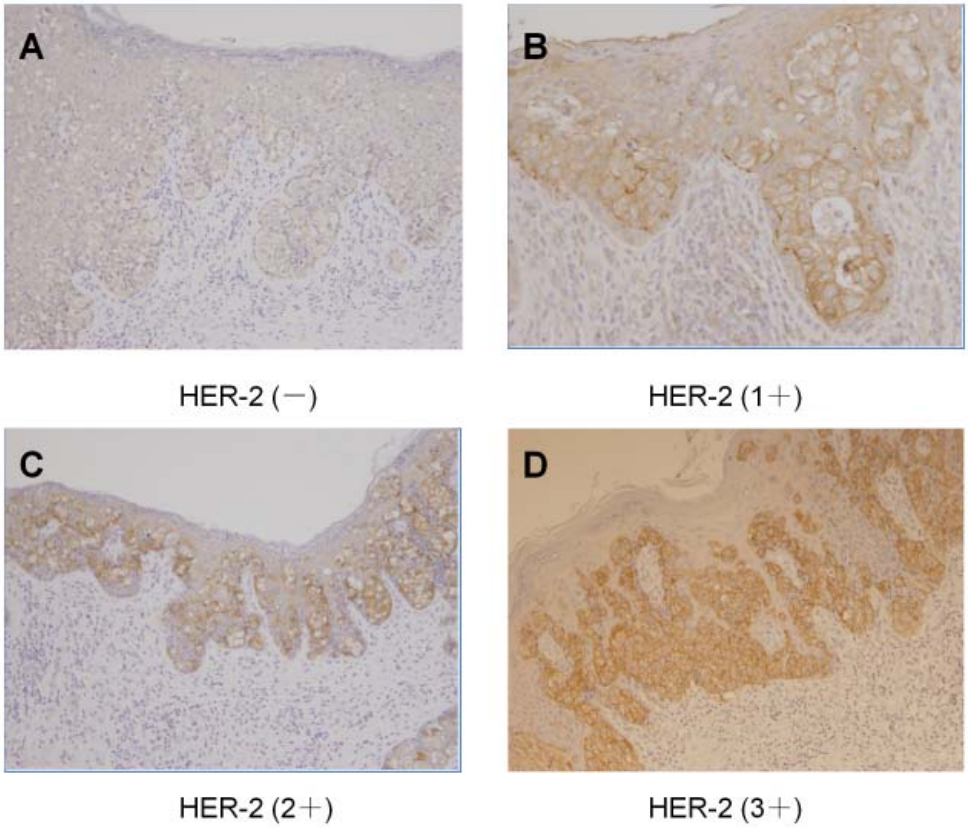

Figure 1. Immunohistochemical staining of HER-2 in extramammary Paget's disease cells. The membrane of tumor cells are clearly stained. **(A)** HER-2 (-); **(B)** HER-2 (1+); **(C)** HER-2 (2+); **(D)** HER-2 (3+).

Table 1. Immunohistochemical staining of HER-2 in extramammary Paget's disease

Items	HER-2				*p* Values
	-	1+	2+	3+	
Age (from 48 to 93)					
Sex					$p < 0.337$
M ($n = 18$)	6	8	2	2	
F ($n = 13$)	6	6	1	0	
Location					$p < 0.128$
Vulva ($n = 13$)	6	6	1	0	
Scrotum ($n = 16$)	4	8	2	2	
Perianal ($n = 2$)	2	0	0	0	
Ca. or *in situ*					$p < 0.02$
Ca. ($n = 4$)	0	2	0	2	
in situ ($n = 20$)	11	6	3	0	
Microinvasion ($n = 7$)	1	6	0	0	
CK7					
+ ($n = 26$)	12	11	1	2	
PAS					
+ ($n = 19$)	7	8	3	1	
CEA					
+ ($n = 27$)	11	12	3	1	
EMA					
+ ($n = 21$)	10	9	1	1	
LN meta. ($n = 4$)	0	2	0	2	$p < 0.02$
Death ($n = 1$)	0	0	0	1	

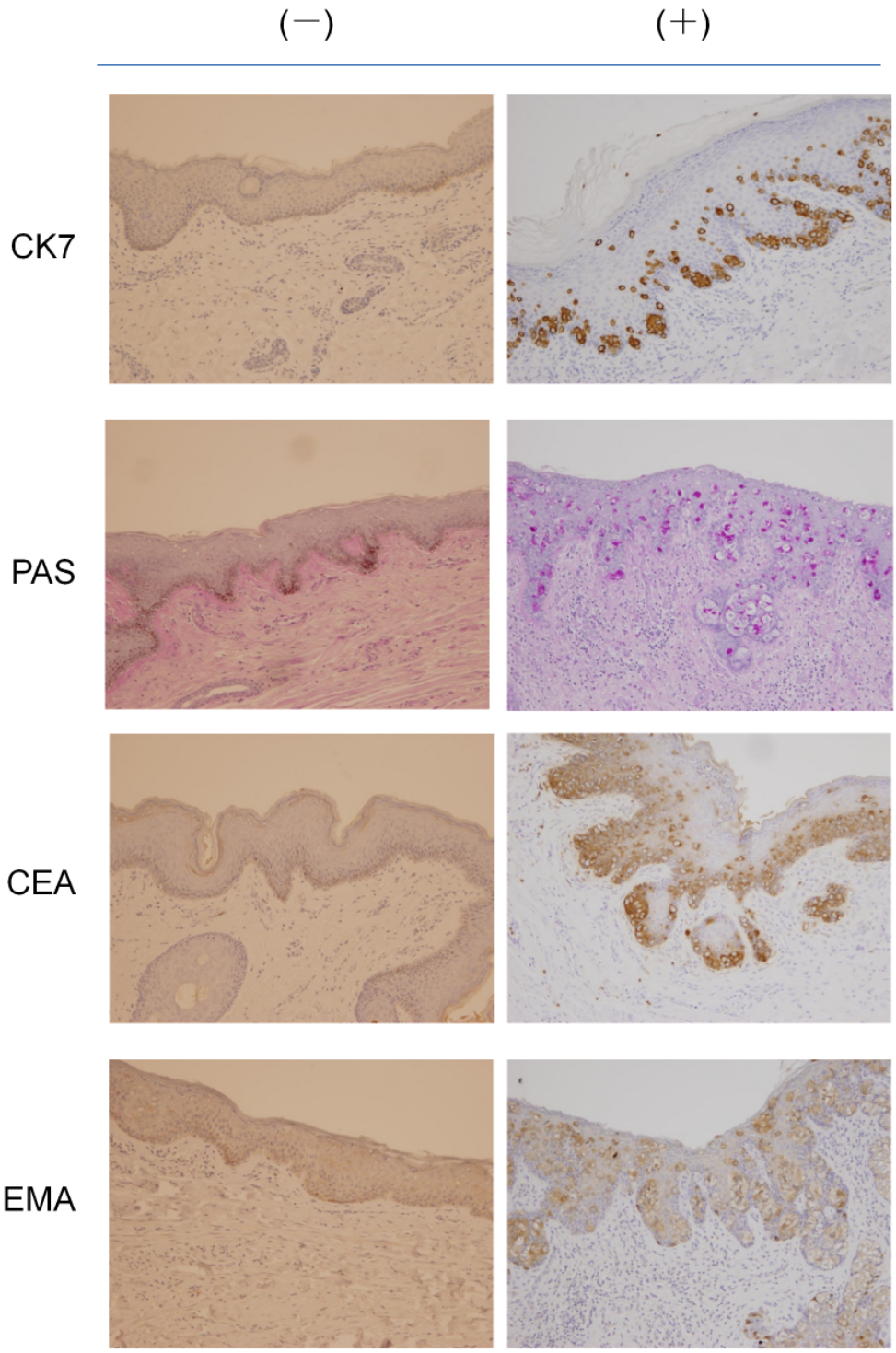

Figure 2. Immunohistochemical staining of cytokeratin 7 (CK7), periodic acid-Schiff (PAS), carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) in extramammary Paget's disease (EMPD) cells.

for HER-2 ($p < 0.02$). Furthermore, there was a significant correlation between the presence of lymph node metastasis and strong positivity (3+) for HER-2 ($p < 0.02$). This indicates that HER-2 overexpression plays a crucial role in the invasion and lymph node metastasis of EMPD. This observation is similar to several breast cancer studies showing enhancement of metastatic potential by HER-2 overexpression (*15*), the demonstration of potentially metastatic cell subpopulations expressing HER-2 within the individual cancer tissue (*16*), and correlation with HER-2 overexpression and random cell migration (*17*). These results suggest that patients with EMPD strongly positive for HER-2 may have a high risk of lymph node metastasis and should be followed up carefully.

As described above, HER-2 overexpression in breast cancer is correlated with more aggressive behavior. However, inconsistent results and lack of

a large number of cases have resulted in different outcomes among previous studies investigating the expression of HER-2 in EMPD (*4-7*). This is probably because of a small number of patients included in the previous studies. There is also another possibility that previous studies included a small number of patients with invasion or those with lymph node metastasis.

The observed overexpression of HER-2 in EMPD presents a potential therapeutic target for adjuvant treatment of this disease. Treatment with trastuzumab is well established in breast cancer with HER-2 overexpression and is recommended by several consensus statements (*18*). The results of the present study indicate that targeting therapies for HER-2, such as trastuzumab, may be used for EMPD, particularly in patients with invasive and/or metastatic EMPD.

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