Original Research Article

The expression of Human Epididymis Protein 4[a] (HE4) in the normal gastric epithelia and its role in the development of intestinal metaplasia and gastric cancer

ABSTRACT

Background: To investigate the role of human epididymis protein 4 (HE4) in the development of intestinal metaplasia (IM) and gastric carcinoma.

Methods: A total of 41 patients with a diagnosis of gastric cancer (GC) and 48 patients with a diagnosis of intestinal metaplasia (IM were enrolled. Gastric cancer samples were taken from patients who underwent gastric resection due to GC. For IM, biopsies obtained from patients who underwent endoscopic examination for non-tumoral reasons were used. Intestinal metaplasia adjacent to GC was also examined separately. For HE4 expression, immunohistochemistry was used and the results were compared to demographic, clinic, pathologic and prognostic parameters.

Results: The patients were 38–90 years-old (mean: 65.6). Tumor localization were antrum in 37.8%, corpus in 27%, lesser curvature in 21.6%, greater curvature in 2 5.4%, and cardia in 3 8.1%. Tumor sizes ranged between 1-13 cm (mean 5.54). There were 37.8% well to moderately, and 62.2% poorly differentiated carcinoma. Pathological T evaluations were as follows: pT1=13.5%; pT2=8.1%; pT3=59.4%; pT4=18.9% patients. The expression of HE4 was seen in 50% of tumors. Among the stomach that harbors intestinal metaplasia next to tumor 61.1% of metaplastic cells expressed HE4. Diffuse type carcinoma expressed more HE4 and there was inverse correlation between depth of tumor invasion and the presence of HE4 (p=0.037).

Conclusions: Human epididymis protein 4 was expressed in oxyntic glands and metaplastic cells as well as GC cells but not in the surface foveolar epithelium. Loss of HE4 correlated with the depth of tumor invasiveness.

*Keywords: [*HE4, stomach, carcinoma, intestinal metaplasia*]*

1. INTRODUCTION

The role of histological parameters ie; atrophic gastritis, intestinal metaplasia (IM) and dysplasia in the development of gastric cancer is indisputable. Of these triple precursor lesion called Correa cascade, IM has a progression rate of 0.377% [1,2]. The development of intestinal metaplasia is actually a protective response to inflammation, but it increases the risk of neoplastic transformation as well [3.4]. The prevalence of intestinal metaplasia is high in countries where H. pylori infection and stomach cancer are common. As a consequence of this observation, IM is thought to be the result of a number of genetic events that are mostly caused by H. pylori infection, but the molecular mechanisms that transform normal epithelia to intestinal metaplasia is yet to be elucidated.

Human epididymal protein 4 (HE4) belongs to whey protein family that contains acidic 4-disulfide center [5,6]. It was discovered in the epididymis, but has been shown to be over-expressed in ovarian and lung cancers as well [7,8]. When ovarian cancer cell cDNA is hybridized and then compared to the sequences in the community, the HE4 gene is found to be present [9]. For ovarian cancer screening, HE4 has been registered as the equivalent of CA125: When the serum cut-off value is kept at 70 pmol/L, HE4 is found to be higher statistically in patients with ovarian cancer compared to patients with benign gynecological diseases [10]. The value of HE4 in ovarian cancer diagnosis is higher than that of CA125 in logistic regression analyzes [11].

In this study, we investigated the expression of HE4 in normal gastric epithelia, the cells of intestinal metaplasia and gastric carcinoma in order to elucidate the role of, if any, HE4 in the development of IM and gastric carcinogenesis. We also compared our findings with histological parameters and H. pylori presence.

2. MATERIAL AND METHODS

After obtaining approval from Antalya Education and Research Hospital Ethics Committee (#12/27, 30/06/2016), 48 gastric endoscopic antral biopsy that had IM graded as 3 positive (intense) (Group I) and one representative tumor block containing sufficient tumor tissue from 41 gastric adenocarcinoma patients (Group II) were chosen retrospectively. Exclusion criteria were tumors with <10 tumor cells, tumors from metastatic focuses and endoscopic biopsies without full thickness gastric biopsies.

Patient information and histopathological parameters of each patient were obtained from the relevant pathology reports and from the hospital data basis. For tumor pathology reports, the AJCC cancer

staging system, 7th Ed and for endoscopic biopsy reports, Sydney System was used [12]. Immunohistochemical study was performed on paraffin embedded tissues that were fixed in formalin. HE4 immunostaining was applied manually. Tissue sections of normal human epididymis processed in a comparable manner provided as positive control. Negative controls were obtained by omitting the primary antibody. Cytoplasmic staining was graded for intensity (0, negative; 1, weak; 2, moderate; and 3, strong) and percentage of positive cells [0 (0%), 1 (1–24%), 2 (25–49%) and 3 (50–100%)]. Protein expression was then defined as negative, weak (score 1–2), moderate (score 3–4) or strong (score ≥5).

Immunohistochemical procedure

Formalin-fixed, paraffin-embedded sections were de-waxed with xylene and rehydrated through gradient ethanol into phosphate-buffered solution (PBS). Endogenous peroxidase activity was quenched with 0.3% H_2O_2 in methanol for 10 minutes at room temperature. Tris-EDTA buffer (ab93684; Abcam, Cambridge, MA, USA) was used for antigen retrieval in a domestic microwave. Protein block was applied for 5 minutes before application of the rabbit polyclonal antibody to HE4 [anti-HE4 antibody (EPR16658) (ab200828), 1:2,000 dilution]. After 2 h incubation with the primary antibody, biotinylated goat anti rabbit IgG secondary a.

Statistical Analysis

All data were evaluated by using SPSS for Windows ver. 11.5 (Chicago, INC.). We used Chi-Square test and Fisher-Exact test to compare groups for categorical data, Unpaired t test (if variable has normal distribution) and Mann-Whitney U test (if variable has abnormal distribution) for continuous variables.

Frequencies and percentages were given as descriptive statistics for categorical data, Mean±SD (Median) were given for continuous data. P values < 0.05 were considered to be significant in all tests.

3. RESULTS AND DISCUSSION

One case which was HE4 negative was neuroendocrine carcinoma and was excluded from the study. One cancer slide had no enough tumor cells but had IM. This case was included in Group II. During IHC staining, 3 samples from cancer slides and 7 samples from IM slides were washed off while antigen retrieval heating. There were not enough metaplastic cells (grades less than 1) in six cases. In total, 37 patients with a diagnosis of adenocarcinoma and 34 patients with a diagnosis of IM were eligible for the study. There were 14 female and 23 male patients, aged 38–90 years (mean: 65.6) in Group II. Tumor localization were antrum in 14 (37.8%), corpus in 10 (27%), lesser curvature in 8 (21.6%), greater curvature in 2 (5.4%), and cardia in 3 (8.1%) patients and tumor sizes ranged between 1-13 cm (mean 5.54). There were 3 (8.1%) well, 11 (29.7%) moderately, 13 (35.1%) poorly differentiated tumor and 10 (27%) signet cell carcinoma. Pathological T and N evaluations were as follows: pT1=5 (13.5%); pT2=3 (8.1%); pT3=22 (59.4%); pT4=7 (18.9%) patients and pN0=10 (27%); pN1=5 (13.5%); pN2=7 (18.9%); pN3=15 (40.5%) patients (Table-1). The expression of HE4 was seen in 18 (50%) tumors in which there were four 1+ (11.1%); nine 2+ (25%); and five 3+ (13.9%) staining (Figure-1 and 2). Interestingly, one endoscopic biopsy harbored displastic epithelia which expressed HE4 protein (Figure-3).

Table 1. Clinicopathological caracteristics and the expression of Human Epididymis Protein-4 in Group I (gastric cancer patients).

			HE4 expression		
			Present, n (%)	Absent, n (%)	p
Age	(Mean±SD)		64,17±16,58	67,17±17,78	0.559
gender	male	#23	13 (56,5)	10 (43,5)	0.298
	female	#13*	5 (38,5)	8 (61,5)	
tumor location	antrum	#14*	6 (46,2)	7 (53,8)	0.860
	corpus	#10	4 (40)	6 (60)	
	lesser curvature	#8	5 (62,5)	3 (37,5)	
	greater curvature	#2	1 (50)	1 (50)	
	cardia	#3	2 (66.7)	1 (33.3)	
tumor size	(Mean±SD)		5,14±2,35(5,50)	6,07±3,66(5,00)	0,830
tumor grade	well	#3	2 (66.7)	1 (33.3)	0.34
	moderately	#11	5 (45,5)	6 (54,5)	
	poor	#13*	4 (33,3)	8 (72,7)	
	signet	#10	7 (70)	3 (30)	
pT	1	#5*	4 (100)	0 (0)	0.037
	2	#3	0 (0)	3 (100)	
	3	#22	12 (54,5)	10 (45,5)	
	4	#7	2 (28,6)	5 (71,4)	
pN	0	#10	7 (70)	3 (30)	0.083
	1	#5	0 (0)	5 (100)	
	2	#7*	3 (50)	3 (50)	
	3	#15	8 (53,3)	7 (46,7)	

*1 patient had less tumor cell but HE4 positive metaplastic cells

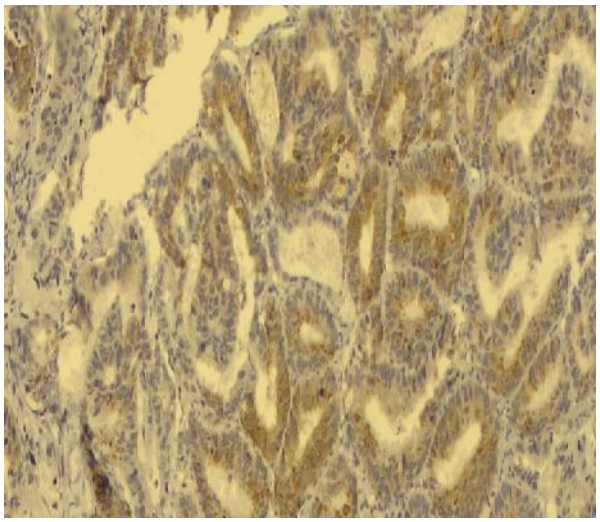

Figure-1: Strong and diffuse Human Epididymis Protein-4 expression in seen in this moderately differentiated adenocarcinoma cases (anti-HE4, x10).

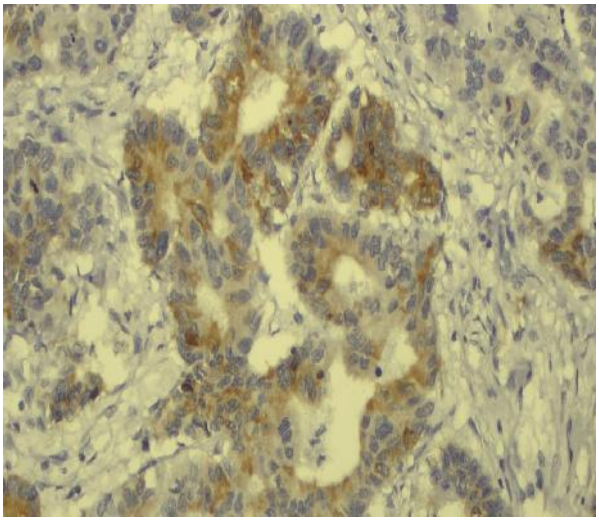

Figure-2: Closer view highlights luminal as well as cytoplasmic staining in tumor cells (anti-HE4, x20).

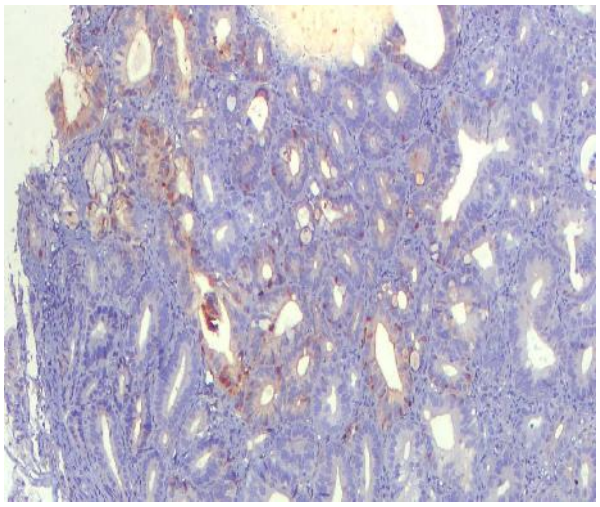

Figure-3: Back to back glands with crowded nuclei are the hallmark of this displastic focus. Note HE4 expression in preneoplastic cells (anti-HE4, x10).

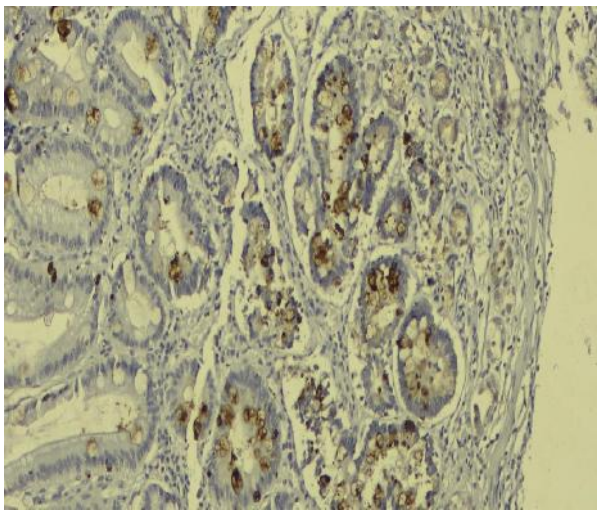

Figure-4: Metaplastic cells in the form of Goblet cells in the foveolar epithelium and deep gastric glands have strong HE4 expression (anti-HE4, x10).

Of the 34 endoscopic biopsies in which metaplastic cells successfully stained with HE4, there were six 1+ (17.6%); nine 2+ (26.5%) and eleven 3+ (32.4%) staining (Figure-4). Overall HE4 expression was 76.5%, whereas 8 cases (23.5%) were negative with HE4 (Table-2). Pretty much close HE4 expression rate was seen in the vicinity of tumor: Eleven out of 18 cases (61.1%) (Table-3). Besides metaplastic cells, we observed that while superficial foveolar tall columnar mucous epithelium almost never expressed HE4 protein, the long tubular gastric glands usually expressed HE4 protein (Figure-5).

Table-2: Human Epididymis Protein-4 expression in the metaplastic cells obtained from endoscopic biopsies.

			HE4 expression		
			Present, n (%)	Absent, n (%)	p
chronicity	yes	#33	25 (75,8)	8 (24,2)	0.765
	no	#1	1 (100)	0 (0)	
activity	yes	#26	19 (73,1)	7 (26.9)	0.615
	no	#5	4 (80)	1 (20)	
H. Pylori	yes	#18	14 (77.8)	4 (22,2)	0.583
	no	#16	12 (75)	4 (25)	

Table-3: Human Epididymis Protein-4 expression in the metaplastic cells in the vicinity of the tumor.

			HE4 expression in metaplastic cells		
			present n (%)	absent n (%)	p
tumor location	antrum	#11	6 (54,5)	5 (45,5)	0.478
	corpus	#5	3 (60)	2 (40)	
	cardia	#2	2 (100)	0 (0)	

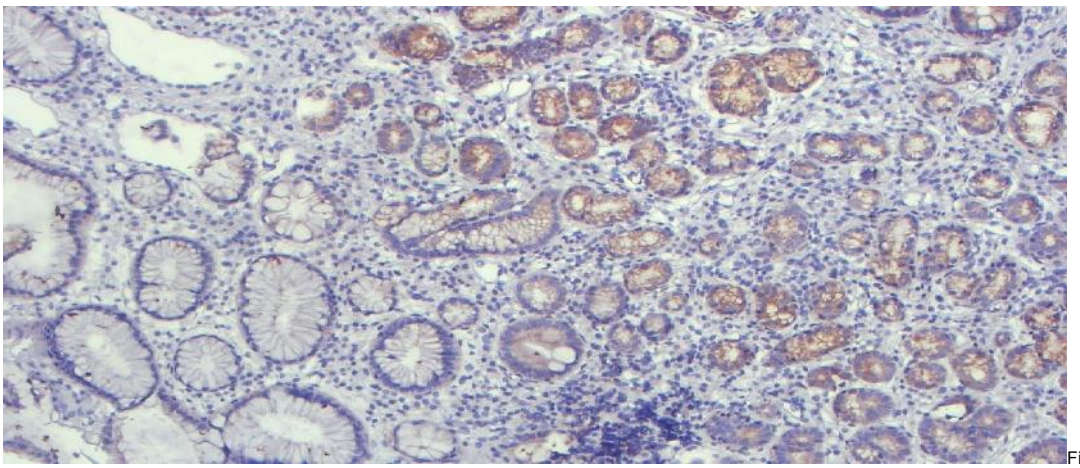

Figure-5: Superficial foveolar epithelium is devoid of HE4 expression, while the long tubular gastric gland have diffuse and strong expression. Some metaplastic cells also have weak expression (arrow) (anti-HE4, x10).

Regarding inflammatory activity and H. Pylori status, all but one of the biopsies had chronic (97%) and 26 (76.5%) had active inflammation. Helicobacter pylori were seen in the 18 (53%) biopsy.

Level of tumor invasion was inversely correlated with the HE4 expression (*P*= .037) but there was no statistical relationships between the presence of HE4 and age (*P*= .56), gender (*P* = .3), tumor location (*P* = .86), tumor size (*P* = .83), tumor grade (*P*=.34), nodal status (0.083), chronicity (*P* = .76), activity (*P* = .6), and the presence of H. pylori (*P* = .58).The data of our cases were given collectively in Table-1.

Discussion

Human epididymis 4 (HE4) protein is a secretory protease inhibitor [13] and involves in the innate immunity of the respiratory tract and nasal cavity [6]. It was originally discovered in the epididymis but is now shown to over-express in different types of carcinoma: The proliferation of HE4 silenced ovarian cancer cells are inhibited and the ability of invasion is decreased [14,15]. On the other hand, over-expression of HE4 yields cancer cells that have greater adhesion and migration. Besides these in-vitro studies, increased levels of serum HE4 in ovarian cancer patients is significantly associated with worse progression-free survival (PFS), and it is an independent prognostic parameter for PFS [16]. Besides ovarian carcinoma patients, HE4 is also detected in the serum or pleural effusion of patients with lung cancer and its utility for lung cancer diagnosis has been investigated in different studies [17-19].

There are few studies conducted on gastric cancer: Gastrin- deficient mice has little HE4 expression in the normal gastric mucosa [20]. Silencing of HE4 expression in gastric cancer cells leads increased apoptosis and decreased proliferation [21].

Two types of gastric metaplasia develop in the human stomach and considered putative pre- neoplastic lesions: Goblet cell intestinal metaplasia and pseudopyloric metaplasia [22]. In intestinal metaplasia, metaplastic epithelium is seen morphologically as goblet cells and absorptive enterocytes and according to enzyme histochemistry, sulfomucin-containing type is associated with increased risk of gastric cancer [23]. Pseudopyloric metaplasia, also called fundic antralisation or spasmolytic polypeptide expressing metaplasia (SPEM), on the other hand is characterized by the antral type metaplastic transformation of the oxyntic mucosa. Contrary to true pyloric glands present in the antrum, these cells does not produce gastrin (hence the name pseudopyloric) but expresses spasmolytic polypeptide [24].

To our knowledge, localization of HE4 in the oxyntic mucosa has never been observed before.

Nozaki et al. indicate that HE4 is not detected in the normal human fundic (oxyntic) mucosa but it is positive in all metaplastic lesions, including pseudopyloric metaplasia and intestinal metaplasia [25]. Our study also confirmed its presence in the metaplastic cells but contrary to their observation, we detected diffuse and strong HE4 expression in oxyntic mucosa (Figure-5). Even IM cells in cardiac mucosa expressed HE4.

Absence of HE4 on surface epithelium but the presence of HE4 in metaplastic cells as seen in our study is consistent with the proposal that the origin of metaplastic cells are the HE4 positive stem cells located in the base of the gland instead of surface foveolar cells [26-29].

Although a correlation between HE4 expresion and survival was not found, high HE4 expression in intestinal and diffuse type adenocarcinoma was 25% and 61% respectively [30]. Higher HE4 expression in diffuse type compared to intestinal type adenocarcinoma was also observed in our study (41% vs 70%) but it was not significant statistically. On the other hand, we also observed that HE4 expression was inversely correlated with invasiveness and it has been statistically significant ($p< 0.05$). Opposite results were seen in studies conducted on endometrial cancer: Serum HE4 level had been correlated with myometrial invasion [31,32]. Higher serum level of HE4 is expected parallel to higher tumor volume in the endometrial, ovarian or lung cancer patients, as tumor cells produce HE4. In the stomach, on the other hand, if normal oxyntic mucosa possesses this protein, it is expected that pT1 (less invasive) tumors as seen in our study would have higher HE4 expression compared to more invasive tumors.

Our study has limitation. Besides study population, we didn't investigate HE4 expression in the cell of SPEM, a novel candidate for gastric cancer development that gives rise to IM [33,34].

4. CONCLUSION

HE4 is expressed in oxyntic mucosa and the cells in intestinal metaplasia. It is expressed in diffuse type carcinoma compared to intestinal type carcinoma and the expression is correlated with pT1.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Ethics committee approval was obtained for this study.

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