Expression of Ber-EP4 (EpCAM-1) in adenoid cystic carcinoma

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Expression of Ber-EP4 (EpCAM-1) in adenoid cystic carcinoma

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The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

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December 2017

연세대학교
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December 2017

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to Professor Sang Kyum Kim, my research supervisor, for her patient guidance, enthusiastic encouragement and useful critiques of this research work.

I would also like to acknowledge the support provided by my colleagues during the preparation of my project.

Finally, I wish to thank my parents for their support and encouragement throughout my study.

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ABSTRACT

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Adenoid cystic carcinoma (ACC) is an uncommon malignancy of the secretory glands with frequent local recurrence and a poor long-term prognosis. The mutational landscape of ACC is currently being revealed, but further studies are needed to identify therapeutic targets of ACC. Epithelial cell adhesion molecule (EpCAM) is a 40-kDa cell surface glycoprotein, which is overexpressed in epithelial cancers and is used for diagnostic and therapeutic purposes. We investigated the expression of EpCAM in ACCs. This study included 72 patients with ACC who underwent surgical resection. Using tissue microarray, we conducted immunohistochemical staining using the anti-EpCAM antibody. EpCAM expression was analyzed by intensity score analysis and the total immunostaining score. A higher histologic grade and specific tumor location (non-salivary gland origin) showed a correlation with higher EpCAM expression. Diffuse, strong EpCAM positivity was associated with distant metastasis. ACCs arising from the salivary gland and the non-salivary gland sites, respectively, might display different pathophysiologies in which EpCAM could play a role.

Key words: adenoid cystic carcinoma, EpCAM, Ber-EP4

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I. INTRODUCTION

Adenoid cystic carcinoma (ACC) is an uncommon malignancy of the secretory glands and often occurs in the areas of the head and neck, particularly in the salivary glands[1]. It is characterized by indolent, persistent growth and frequent perineural invasion, local recurrence and a poor long-term prognosis[1,2].

Immunohistochemical staining is often used to distinguish ACC from other malignancies, especially when histologic findings are confusing. Tumor cells are strongly positive for c-KIT regardless of the histologic grade, and many investigators have focused on this receptor as a therapeutic target[3,4]. However, clinical trials using imatinib, a c-KIT inhibitor, have been unsatisfactory, and several studies have shown that ACCs express wild-type c-KIT[5,6,7,8]. Strong nuclear MYB immunostaining is detected in ACCs regardless of the site of tumor origin[9,10]. MYB-NFIB translocation seems to be specific in ACC, and these aberrations may be a critical event in ACC pathogenesis[11]. However, further studies are needed to uncover the application of MYB-associated genes in therapy.

Epithelial cell adhesion molecule (EpCAM) is a 40-kDa cell surface glycoprotein that is overexpressed in epithelial cancers and, at lower levels, in normal epithelium; therefore it is considered a tumor marker[12]. It consists of a large extracellular domain of 242 amino acids (aa), a single-spanning transmembrane domain of 23 aa and a short cytoplasmic domain of 26 aa[12]. The function of

EpCAM includes intercellular adhesion, cell proliferation, signaling, migration and differentiation[12].

EpCAM is overexpressed in certain carcinomas, including colon, pancreas, and breast carcinomas[13,14,15]. The high levels of EpCAM in some cancers are related to a poor prognosis, and EpCAM can serve as a prognostic marker[13]. Therefore, the overexpression of EpCAM in tumors makes it an attractive therapeutic target. In the context of skin cancer, EpCAM has been used for diagnostic purposes: the anti-EpCAM antibody Ber-EP4 is a sensitive marker of basal cell carcinoma[16,17,18].

Studies regarding EpCAM expression in ACC are relatively scarce. Given the potential diagnostic and therapeutic applications of anti-EpCAM antibodies, we investigated the expression of EpCAM in ACCs. The purpose of this study was to demonstrate the expression of EpCAM in ACC and to verify the clinicopathologic features of ACC according to the expression level of EpCAM.

II. MATERIALS AND METHODS

1. Patient selection

We included 72 patients with ACC who underwent surgical resection from 1996 to 2013 at Severance Hospital, Korea. The clinical data, including gender, age at diagnosis, tumor location, disease recurrence, metastasis, and survival of the patients, were obtained from the patients' medical records. The histologic grades of the tumor and perineural invasion were noted from the review of slides and pathology reports. All slides were retrospectively reviewed by two pathologists (SJ Lee and SK Kim), and histological evaluation was conducted on hematoxylin- and eosin-stained (H&E) slides. Histologic grade was evaluated using the grading system of Perzin/Szanto as follows: 1, predominantly tubular and cribriform component, no solid pattern; 2, predominantly cribriform component or mixed, <30% solid component; 3, marked predominance of the solid component[19,20]. All methods and experimental protocols using human tissue (formalin-fixed paraffin-embedded, FFPE tissue) were carried out in accordance with relevant guidelines and regulations approved by the Institutional

Review Board of Severance Hospital, Yonsei University Health System (4-2015-0872). The informed consent was waived because the IRB decided that this retrospective study showed minimal risk to the patients (risk level I).

2. Tissue microarray analysis

A representative area was selected on each H&E slide, and a corresponding spot was marked on the surface of the FFPE block. Using a biopsy needle, the selected area was punched out, and a 3-mm tissue core was placed into a 6 × 5 recipient block. Each tissue core was assigned to a unique tissue microarray location number linked to a database containing other clinical data.

3. Immunohistochemical staining

FFPE tissue blocks were cut into 4-μm sections. Immunohistochemical staining was performed using a Ventana XT automated stainer (Ventana Corporation, Tucson, AZ, USA) with antibodies against EpCAM (clone VU-1D9, 1:1,000; Calbiochem, San Diego, CA, USA) according to the manufacturer's instructions. Negative control samples were processed without the primary antibody. Positive control tissue was used as per the manufacturer's recommendation.

4. Interpretation of immunohistochemical staining

The expression of EpCAM was semiquantitatively evaluated by intensity score (IS) analysis and by calculating the total immunostaining score (TIS), the product of the intensity score and proportion score (PS). IS represents the estimated staining intensity compared with that of control cells (0, no staining; 1, weak; 2, moderate; 3, strong), and PS describes the estimated area of positively stained tumor cells (0, none; 1, <10%; 2, 10-50%; 3, 51-80%; 4, >80%). TIS (IS*PS) ranges from 0 to 12 with only nine possible values (0, 1, 2, 3, 4, 6, 8, 9 and 12). Using TIS, we defined the low-expression group (TIS 0-6) and high-expression group (TIS 8-12).

5. Statistical analysis

All statistical analyses were performed using SPSS software, version 21.0 for Windows (IBM Corp. Released 2012, Armonk, NY, USA). To analyze each clinicopathologic parameter, Student's t-test, Fisher's exact test and Pearson's χ^2-test were used, depending on the purpose. Patient survival statistics were analyzed using the Kaplan–Meier method and log-rank test, and uni- and multivariate analyses were performed using the Cox regression model. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were presented. $P<0.05$ was considered to indicate statistical significance.

III. RESULTS

1. Subject characteristics

This study included 72 patients with ACC: 48 cases (66.7%) arose from the salivary gland (42 cases from the major salivary gland and six cases from the minor salivary gland), and 24 cases (33.3%) arose from the non-salivary gland site, including the lacrimal gland, orbit, nasal cavity, pharynx, larynx, auditory canal, breast and trachea. The mean age of the patients was 53.6 years (age range: 20 to 81 years), and the male-to-female ratio was 1:1.8. According to the histologic grade, 17 cases (23.6%) demonstrated grade 1, 40 cases (55.6%) demonstrated grade 2, and 15 cases (20.8%) demonstrated grade 3.

2. EpCAM expression in ACC and correlation with clinicopathologic parameters

The expression of EpCAM in normal salivary gland tissue was negative or weakly positive in acinar and ductal cells, as previously reported[21]. Positive staining of EpCAM occurred primarily on the cell membrane (Fig. 1). The positivity was 97.2% (70/72 cases), regardless of the intensity score. Negative expression of EpCAM was identified in two cases (2.8%), weak expression in 54 cases (75%), moderate expression in nine cases (12.5%), and strong expression in seven cases (9.7%). The number in the low-expression group by TIS was 57 (79.2%) and that in the high-expression group was 15 (20.8%). The correlation between

EpCAM expression by IS and clinicopathologic parameters is listed in Table 1. No significant difference was identified between EpCAM staining and certain clinicopathologic parameters such as age at diagnosis, gender, perineural invasion, local recurrence and distant metastasis. However, tumor location (non-salivary gland origin, P=0.042) and higher histologic grade (P=0.002) were related to higher expression of IS. By TIS, the histologic grade was also related to a higher EpCAM expression level (P=0.005) and tended to show a specific tumor location (non-salivary gland origin, P=0.065) (Table 2). Once the tumor displayed strong EpCAM expression (IS=3), its PS was always 4 (diffuse strong positivity). The diffuse and strong EpCAM positivity (IS=3 or TIS=12) was related to distant metastasis (P=0.037).

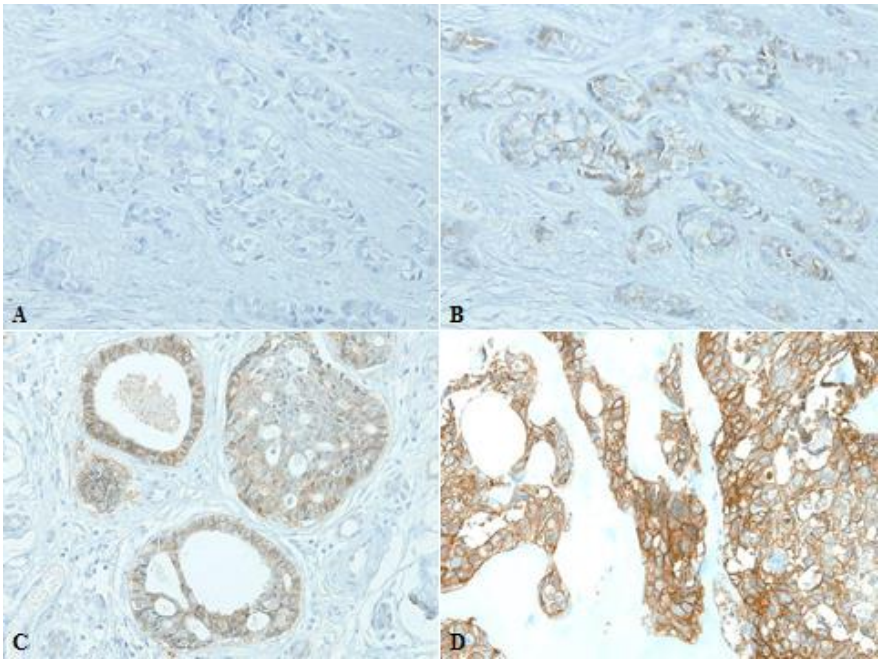

Fig. 1. Examples of the intensity levels of EpCAM in adenoid cystic carcinoma. (A: negative (0); B: weak (1+); C: moderate (2+); D: strong (3+)).

Table 1. Correlation between EpCAM expression by intensity score (IS) analysis and clinicopathologic parameters

		EpCAM expression (intensity score)				
		Negative (0)	Weak (1+)	Moderate (2+)	Strong (3+)	
	n	n (%)	n (%)	n (%)	n (%)	P-value
Age (years)	72					0.152
≤50	26	1 (3.8)	23 (88.5)	1 (3.8)	1 (3.8)	
>50	46	1 (2.2)	31 (67.4)	8 (17.4)	6 (13.0)	
Gender						0.078
Male	26	2 (7.7)	18 (69.2)	5 (19.2)	1 (3.8)	
Female	46	0 (0)	36 (78.3)	4 (8.7)	6 (13.0)	
Location						**0.042**
Salivary gland	48	0 (0)	40 (83.3)	4 (8.3)	4 (8.3)	
Major (parotid, submandibular, subligual)	42					
Minor (lip, hard palate)	6					
Others	24	2 (8.3)	14 (58.3)	5 (20.8)	3 (12.5)	
Histologic grade						**0.002**
Grade 1	17	2 (11.8)	12 (70.6)	3 (17.6)	0 (0)	
Grade 2	40	0 (0)	35 (87.5)	3 (7.5)	2 (5.0)	
Grade 3	15	0 (0)	7 (46.7)	3 (20.0)	5 (33.3)	
Perineural invasion						0.325
Present	54	1 (1.9)	39 (72.2)	7 (13.0)	7 (13.0)	
Absent	18	1 (5.6)	15 (83.3)	2 (11.1)	0 (0)	
Local recurrence						0.323
Present	16	0 (0)	10 (62.5)	3 (18.8)	3 (18.8)	
Absent	56	2 (3.6)	44 (78.6)	6 (10.7)	4 (7.1)	
Distant metastasis						0.120
Present	24	1 (4.2)	16 (66.7)	2 (8.3)	5 (20.8)	
Absent	48	1 (2.1)	38 (79.2)	7 (14.6)	2 (4.2)	

Table 2. Correlation between EpCAM expression by total immunostaining score (IS*PS) and clinicopathologic parameters

		EpCAM expression (IS*PS)		
		Low (0-6)	High (8-12)	
	n	n (%)	n (%)	P-value
Age (years)	72			**0.039**
≤50	26	24 (92.3)	2 (7.7)	
>50	46	33 (71.7)	13 (28.3)	
Gender				0.801
Male	26	21 (80.8)	5 (19.2)	
Female	46	36 (78.3)	10 (21.7)	
Location				0.065
Salivary gland	48	41 (85.4)	7 (14.6)	
Major	42			
(parotid, submandibular, subligual)				
Minor (lip, hard palate)	6			
Others	24	16 (66.7)	8 (33.3)	
Histologic grade				**0.005**
Grade 1	17	15 (88.2)	2 (11.8)	
Grade 2	40	35 (87.5)	5 (12.5)	
Grade 3	15	7 (46.7)	8 (53.3)	
Perineural invasion				0.327
Present	54	41 (75.9)	13 (24.1)	
Absent	18	16 (88.9)	2 (11.1)	
Local recurrence				0.084
Present	16	10 (62.5)	6 (37.5)	
Absent	56	47 (83.9)	9 (16.1)	
Distant metastasis				0.218
Present	24	17 (70.8)	7 (29.2)	
Absent	48	40 (83.3)	8 (16.7)	

3. Survival analysis

Kaplan–Meier and Cox regression analyses were performed to discover a link between EpCAM expression in ACC and patient survival. In the Kaplan–Meier analysis, there was a tendency that the patients with diffuse strong EpCAM positivity (TIS 12) had poorer overall survival (OS) than those with a lower TIS level (P=0.078) (Fig. 2). According to Cox regression analysis, the variables with a significant difference in OS rates were histologic grade (P=0.019) and distant metastasis (P=0.013) in the univariate analysis (Table 3). EpCAM

expression did not affect the OS of ACC patients in the univariate and multivariate analyses. In univariate analysis for disease-free survival (DFS), female gender (P=0.01), a high histologic grade (P=0.037), perineural invasion (P=0.005), local recurrence (P=0.002) and distant metastasis (P<0.001) were statistically significant (Table 4). In the univariate and multivariate analyses, EpCAM expression did not affect the DFS. Local recurrence and distant metastasis were identified as independent prognostic factors for DFS. All the statistically significant variables were subjected to the Cox proportional hazards regression model.

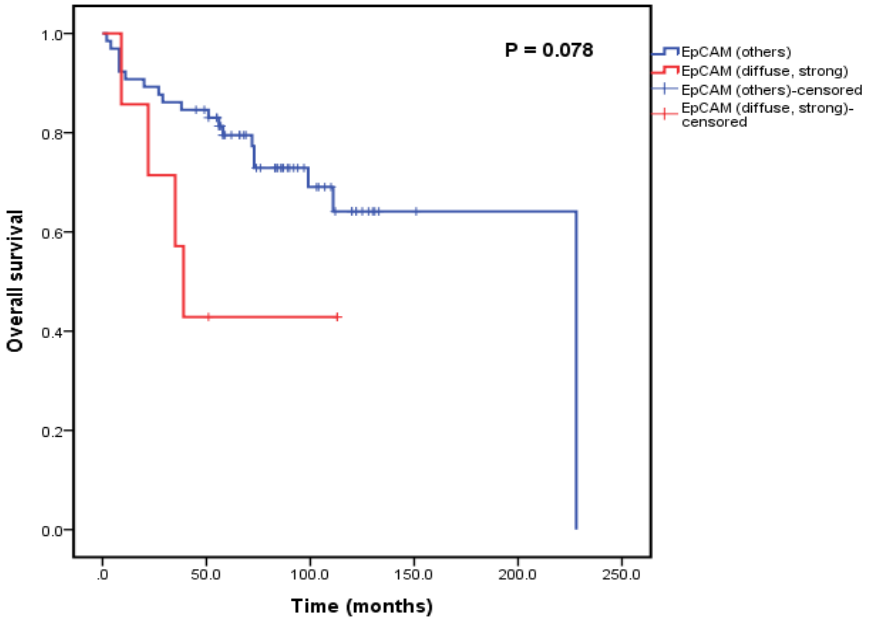

Fig. 2. Survival analysis according to the EpCAM expression pattern (diffuse strong or not) in adenoid cystic carcinoma patients.
The overall survival rates were decreased more sharply with diffuse strong EpCAM positivity cases, but no statistically significant difference was identified (P=0.078).

Table 3. Univariate and multivariate analyses of clinicopathological variables and EpCAM expression in relation to overall survival in patients with adenoid cystic carcinoma

	Univariate analysis			Multivariate analysis		
Risk factors	HR	95% CI	P-value	HR	95% CI	P-value
Overall survival						
Age (≤50/>50)	1.585	0.619-4.056	0.337	0.725	0.253-2.077	0.549
Gender (Male/Female)	0.280	0.117-0.668	**0.004**	0.342	0.142-0.824	**0.017**
Location (others/salivary)	1.015	0.442-2.327	0.973	1.695	0.671-4.286	0.265
Higtologic grade (1+2/3)	2.697	1.179-6.172	**0.019**	2.215	0.968-5.070	0.060
Perineural invasion (N/P)	7.109	0.955-52.903	0.055	2.161	0.233-20.071	0.498
Local recurrence (N/P)	1.923	0.806-4.587	0.140	1.272	0.421-3.844	0.670
Distant metastasis (N/P)	2.948	1.259-6.904	**0.013**	2.324	0.983-5.497	0.055
EpCAM IS (0-2/3)	2.565	0.861-7.639	0.091	2.778	0.688-11.219	0.151

Table 4. Univariate and multivariate analyses of clinicopathological variables and EpCAM expression in relation to disease-free survival in patients with adenoid cystic carcinoma

	Univariate analysis			Multivariate analysis		
Risk factors	HR	95% CI	P-value	HR	95% CI	P-value
Disease free survival						
Age (≤50/>50)	1.224	0.614-2.438	0.565	0.600	0.272-1.326	0.207
Gender (Male/Female)	0.427	0.223-0.817	**0.010**	1.086	0.456-2.587	0.853
Location (others/salivary)	0.938	0.483-1.821	0.851	1.749	0.849-3.600	0.129
Higtologic grade (1+2/3)	2.076	1.044-4.128	**0.037**	0.996	0.466-2.130	0.992
Perineural invasion (N/P)	5.422	1.660-17.703	**0.005**	3.117	0.839-11.577	0.089
Local recurrence (N/P)	2.795	1.446-5.401	**0.002**	2.915	1.494-5.684	**0.002**
Distant metastasis (N/P)	6.403	3.186-12.870	**0.000**	6.625	3.264-13.449	**0.000**
EpCAM IS (0-2/3)	1.828	0.712-4.699	0.210	0.804	0.241-2.683	0.723

IV. DISCUSSION

Several studies have investigated the expression of EpCAM in various carcinomas. Although EpCAM might be expressed weakly on normal epithelium, it is overexpressed in tumors, including colorectum, esophagus, liver, prostate, lung, pancreas and breast tumors[15]. EpCAM overexpression is associated with poor the prognosis in carcinomas of the breast, gallbladder, ovary, ampulla and

esophagus because the molecule functions as an inhibitor of E-cadherin; therefore, it is believed that EpCAM plays a role in metastasis[22,23,24,25,26,27,28]. On the other hand, EpCAM can participate in cell adhesion, and its overexpression is suggested to be linked to better survival in patients with colorectal carcinoma, gastric carcinoma, clear cell renal cell carcinoma and non-small cell lung cancer[29,30,31,32,33].

We performed immunohistochemical staining of EpCAM on the tissues of 72 ACC samples. Some reports have suggested that the cellular distribution of EpCAM varies by tumor type and histologic differentiation of carcinoma; therefore, it might have diagnostic value[34,35]. However, we identified both membranous and cytoplasmic expression in cancer cells of ACC.

In this study, we found that the overexpression pattern of EpCAM in ACC is associated with a higher histologic grade. Furthermore, we could confirm that EpCAM plays a role in tumor distant metastasis in the context of ACC, as previous studies have suggested in other tumors[22,23,24,25,26,27,28].

Phattarataratip E et al. studied epithelial cell adhesion molecule expression in various salivary gland neoplasms, including mucoepidermoid carcinoma, adenoid cystic carcinoma, pleomorphic adenoma, and polymorphous low-grade adenocarcinoma[36]. They included EpCAM molecule and showed different EpCAM expression patterns among salivary gland neoplasms. They demonstrated that decreased EpCAM expression was associated with aggressive features in mucoepidermoid carcinoma, and the ACCs showed negative or weakly positive immunoreactivity to EpCAM, contrary to our result. However, that previous study possessed some limitations in that the number of ACC cases involved in the study was too small and they found that the solid growth area of ACC showed diffuse and strong immunoreactivity to EpCAM[36].

Interestingly, we newly found that higher EpCAM expression was more frequently observed in ACCs arising from non-salivary gland sites. Lin et al. compared the ACCs of salivary and non-salivary origin, and they found sinonasal, lacrimal, and tracheobronchial ACCs had significantly worse outcomes than ACCs of the major salivary glands[37]. From these two studies, we infer that ACCs arising from

the salivary gland and non-salivary gland sites, respectively, might have different pathophysiologies in which EpCAM molecule could play a role.

A previous study investigated the expression of tumor-associated calcium signal transducer 2 (TACSTD2, Trop2), a homolog of EpCAM, in salivary ACC[38]. Similar to our result of EpCAM in ACC, TACSTD2 overexpression was related to a poor prognosis in patients, although the molecule did not reflect the histologic subtype.

The possible prognostic significance of EpCAM overexpression in various cancers has been raised by several investigators[27,39,40,41]. Although the overexpression of EpCAM was correlated with higher histologic grade, we could not demonstrate EpCAM as an independent factor affecting the ACC patient's survival in the statistical evaluation. We assume that, due to the frequently recurrent but indolent behavior of ACC, it would be difficult to make a significant difference affecting the patient's survival by EpCAM expression. Nevertheless, we could see a tendency that the patients with higher EpCAM expression had a poorer overall survival than those with lower EpCAM expression.

Several clinical trials using various anti-EpCAM antibodies have been investigated despite the controversy. Catumaxomab was approved in Europe to treat malignant ascites in patients with EpCAM-positive carcinomas[42,43]. Additionally, a few studies using anti-EpCAM antibody showed that it might have an anti-tumor effect[44,45,46]. However, the validation of EpCAM as a therapeutic target remains unexplored.

V. CONCLUSION

We demonstrated the expression of EpCAM in ACC according to the various clinicopathologic conditions. A higher histologic grade and specific tumor location (non-salivary gland site) showed higher EpCAM expression, and diffuse and strong EpCAM positivity was associated with distant metastasis. We believe that this study will strengthen the basis for understanding the pathophysiology of ACC and suggests EpCAM as a candidate molecule for a diagnostic and therapeutic biomarker.

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ABSTRACT (IN KOREAN)

선양낭포암에서 Ber-EP4 (EpCAM-1)의 발현

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이 석 주

선양낭포암은 분비선에 발생하는 드문 종양으로, 잦은 국소재발과 나쁜 예후를 보인다. 선양낭포암의 유전자지도가 밝혀지고 있지만, 치료표적을 발견하기 위한 추가적인 연구가 필요하다. Epithelial cell adhesion molecule (EpCAM)은 40 kDa 세포표면 당단백으로, 암종에서 과발현되며 진단적, 치료적 목적으로 이용되고 있다. 이 연구의 목적은 선양낭포암에서의 EpCAM 발현을 보기 위함이다. 72명의 환자로부터 얻어진 조직으로 tissue microarray를 제작하여, 항 EpCAM 항체를 이용하여 면역조직화학염색을 시행하였다. Intensity score와 total immunostaining score를 이용하여 염색을 분석하였다. 그 결과, 높은 조직학적 등급과 특정한 종양의 발생 위치 (비침샘)가 강한 EpCAM 발현과 관계가 있었다. 종양에서 광범위에, 그리고 강하게 염색되는 EpCAM은 원격전이와 관계가 있었다. 침샘, 그리고 비침샘 기원의 선양낭포암은 서로 다른 병태생리를 갖는 것으로 생각되고, EpCAM이 이에 역할을 할 것이라 생각된다.

핵심되는 말 : adenoid cystic carcinoma, EpCAM, Ber-EP4