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두경부 종양에서 혈액과 임파선의 면역표지 발현의 비교

Comparison of immune cell marker expression of blood and lymph node in head and neck tumors

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Comparison of immune cell marker expression of blood and lymph node in head and neck tumors

by

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Abstract

Comparison of immune cell marker expression of blood and lymph node in head and neck tumors

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Introduction: Head and neck cancer is a common disease, and most of it is squamous cell carcinoma. The traditional therapy includes surgery, chemotherapy, and radiotherapy. But the five-year survival of head and neck cancer is 50-60%, which is relatively poor. Thus immunotherapy using targeting agents have been spotlighted in recent years. As preceding research for immunotherapy, the aim of this study is to analysis the immune markers which express in blood and lymph nodes of head and neck cancer.

Methods: Blood and lymph node were sampled from patients who underwent surgery for squamous cell carcinoma or a benign tumor in head and neck area. Using flow-cytometry analysis and Milliplexkit®, we compared immune markers in both groups.

Results: The expression ratio of CD8 and CD40 in serum was statistically lower in malignant group. IL-6 in plasma was statistically higher in malignant group. Those were same in subgroup analysis between the benign, low-stage, and high-stage group. Analysis of lymph node did not show any significant difference between two groups. But the expression of CD33 in lymph node was lower in extra-capsular spread(ECS) positive group than that in the ECS-negative group.

Conclusion: The difference of expression of CD40 and IL-6 in this study implies a possibility of immunotherapy targeting these immune markers in the treatment of head and neck cancer.

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Keywords : Head and neck tumor, immune marker, CD40, CD33, IL-6, lymph node

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LIST OF ABBREVIATIONS

HNC : Head and neck cancer

HNSCC : Head and neck squamous cell carcinoma

FACS : Fluorescence-activated cell sorting

ECS : Extra-capsular spread

APC : Antigen-presenting cell

TNF : Tumor necrosis factor

MDSC : Myeloid-derived suppressor cell

Introduction

Head and neck cancer (HNC) is a common disease, accounting for approximately 4% of all cancers in the United States(1). And about 90% of head and neck cancers are squamous cell carcinoma(2). Although traditional treatments for HNC including surgery, chemotherapy, and radiation have been developed for decades, the five-year survival rate of HNC is only 50-60%, resulting in relatively poor prognosis. Therefore, a novel approach like immunotherapy has been spotlighted for the treatment of HNC for better prognosis in recent years. Cetuximab is a well-known chimeric monoclonal antibody targeting epidermal growth factor inhibitor and was approved by the Food and Drug Administration of United States in 2006 for treating HNC. Currently, other new targeting agents, Nivolumab, Ipilimumab, or Pembrolizumab, for examples, are in ongoing clinical trials for HNC(3). Thus an understanding of molecular and immunologic characteristics of HNC is becoming more important in the era of cancer immunotherapy. So far, many articles comparing immune markers have been published, but most of them were comparisons of specimens from cancer.

However, to understand other therapy, immunotherapy, cytokine therapy or cancer vaccines, for examples, analysis of healthy tissue in patients with HNSCC is also necessary.

Thus, this study aims to compare expression of immune cell markers in blood and normal lymph node in head and neck squamous cell carcinoma(HNSCC) and find the way to applicate the results clinically.

Material and Methods

1) Patients

From August 2016 to August 2018, adult patients who underwent surgery for squamous cell carcinoma of head and neck area with neck lymph node dissection in Seoul National University Hospital and Seoul National University Bundang Hospital were prospectively enrolled. The exclusion criteria were patients who received concurrent chemoradiation therapy for head and neck cancer patients, who were suspicious for distant metastasis, and who are under age of eight-teen. The control group was defined as adult patients who had surgery for a benign tumor in head and neck area and also enrolled during the same period. The clinical data of both groups, such as sex, age, the pathologic result of surgery, and stage of cancer were collected.

All patients were informed about the benefits and risks of this study. Written and signed consents were obtained from all subjects. The study was approved by the Institutional Review Board of Seoul National University Hospital and Seoul National

University Bundang Hospital.

2) Collection of samples

Peripheral venous blood (5-10mL) was collected into anticoagulation tubes just after general anesthesia for the operation. After the end of surgery, a lymph node which is the farthest from tumor was collected from the removed specimen. The lymph node was collected without affecting diagnosis and treatment. If additional surgery was necessary for lymph node sampling after removal of tumor specimen, the lymph node was not harvested. A sampling of blood and a lymph node was same in the control group.

3) Measurement

The blood tubes were left undisturbed at room temperature for 30 minutes and then centrifuged at 2,000 rpm for 10 minutes. The separated plasma was kept under refrigerated under minus 20 Celsius degree for later analysis of cytokines. The centrifuged white blood cells are analyzed with

Fluorescence-activated cell sorting(FACS). The expression of CD4, CD8, CD40L, CD40, CD33, CD19 of white blood cells was calculated. The harvested lymph node was put in RPMI media, and its tissue was ground through 40 μ m pore (Falcon™ cell strainer) with a plunger from 3 mL syringe. The extracted single cell suspension was analyzed with FACS. The expression of CD4, CD8, CD40L, CD40, CD33, CD19 of the lymph node was also calculated. Luminex cytokine bead array technology with a Millipore HCYTOMAG-60K kit according to the protocol established by Milliplex® was used to measure the levels of IFN-γ, IL-1β, IL-10, IL-2, IL-4, IL-5, IL-6, sCD40L, and TNF-α from refrigerated plasma.

4) Statistical analysis

The ratio of CD expressions and levels of cytokine are presented as the mean ± standard deviation and were compared between experimental group and control group by Student's t-test. The malignant patients were divided into low-stage (I-Ⅲ) group and high-stage (Ⅳ) group by the seventh edition of the American Joint Committee on Cancer. One-way ANOVA was

used to evaluate the ratio of CD expressions and levels of cytokine between control group, low-stage group, and high-stage group. Samples from malignant patients were also compared according to the extra-capsular spread (ECS) by Student' s t-test. Differences were considered significant if the p-value was less than 0.05. Statistical analysis was performed using software package SPSS® version 19.0 for Windows® (SPSS Inc., Chicago, IL, USA).

Results

1) Comparison of the distribution of immune cells using blood and lymph node tissue in patients with malignancy or benign tumor

Total 39 patients were analyzed. The number of malignant groups was twenty-six, and the number of control group (benign) was thirteen. The pathology of the experimental group was all squamous cell carcinoma. Anatomical sites of the experimental group included twelve cases of the oral cavity, nine cases of the oropharynx, three cases of the hypopharynx, and two cases of the larynx. By TNM stage, four cases were T1, sixteen cases were T2, one case was T3, and five cases were T4. Twelve cases were node-negative(N0). The number of N1 patients was five, N2a patients were eight, and the N1c patient was one. There was no case with N2a or N3 stage. The control group included benign tumors with ten parotid patients and three submandibular gland patients. Their pathologic reports were eight cases of pleomorphic adenoma, four cases

of Warthin's tumor, and one case of aspergilloma (table 1).

The ratio of CD4, CD8, CD40, CD40L, CD19(B cell), CD33 in macrophage/monocyte from blood or node was calculated using fluorescence-activated cell sorting. In the blood sample, the ratio of CD8 was significantly lower in malignant group(17.6±2.3) than that in the benign group(24.2±2.3) (p=0.001). And the ratio of CD40 of blood was also significantly lower in malignant group(3.9± 1.5) than that of the benign group(6.8±1.7) (p=0.022). Other antibodies in blood or node did not show any significance between two groups(table 2).

The results between benign, low-stage and high-stage group were similar. One-way ANOVA showed significant differences in the ratio of CD8 and CD40 between three groups of blood. The ratio of CD8+ cells was 24.2± 2.3 in the benign group, 17.5± 3.1 in stage I-III group, and 17.8±3.5 in stage IV group(p=0.004). The ratio of CD40+ cells was 6.8± 1.7 in the benign group, 4.8± 2.4 in stage IIII group, and 3.0±1.4 in stage IV group(p=0.037). The ratio of CD33+ and CD19+ in blood look like showing a numeric difference, but statistical significance was none (p=0.381, 0.063). And between stage I-III and stage IV, there was no significant difference of ratio. The ratio in node

group also did not show significance(table 3).

In contrast, the analysis between ECS positive and negative group showed the only significant difference of the ratio of CD33 in nodal cells. The CD33+ ratio (macrophage/monocyte) was lower in ECS positive group (9.5± 3.5) than ECS negative group (1.6± 0.9) statistically (p=0.004). There was no significance in the ratio of other antibodies(table 4).

2) Comparison of the level of cytokines using plasma in patients with malignancy or benign tumor

Total 42 patients were analyzed. The number of malignant groups was twenty-seven, and the number of control group (benign) was fifteen. The anatomical site, pathologic results, T and N stage of cases are as in the following (table 5). In control group, one case was diagnosed as acinic cell carcinoma of the parotid gland. But the lymph node was not metastatic, and the purpose of this study was to analysis the immunologic characteristics of squamous cell carcinoma in head and neck area. So it was regarded as the control group. The level of IFN-γ , IL-1β , IL-10, IL-2, IL-4, IL-5, IL-6, sCD40L, and

TNF-α from plasma was calculated using Milliplex map kit. Compared to control group (1.9±1.0 pg/mL), the level of IL-6 was statistically higher in the malignancy group (9.3±2.7 pg/mL) (p=0.014). Other cytokines in plasma did not show any significance between two groups (table 6).

The comparison between benign, low-stage and high-stage group was similar. One-way ANOVA showed significant differences in the level of IL-6 between three groups (p=0.035). The levels of IL-6 were 1.9±1.0 pg/mL in the benign group, 6.0± 2.5 pg/mL in the lowstage group, 13.0±4.8 pg/mL in the high-stage group. Other cytokines in plasma did not show any significance between three groups (table 7).

The analysis between extra-capsular spread (ECS) positive and negative group showed no significant difference in levels of all cytokines (table 8).

Discussion

CD40, a costimulatory protein found on antigen presenting cells(APC), is a member of the tumor necrosis factor (TNF) receptor superfamily and binds to T cell express CD40-ligand (CD40L)(4). Without proper antigen-presenting function of CD40+ APC and CD8+ T cells, pre-malignant cells can evade immune surveillance and progress to cancer(4). Thus the expression of CD40+ monocyte is related to anti-tumor immune response. Several studies showed that the levels of CD40 expression influenced the nature of the antitumor immune response in a mouse tumor model(5). They also showed that lower expression of CD40 in monocytes in peripheral blood of patients with HNSCC is related with higher stage and its expression increased after removal of tumor(6). Most of immunotherapy is to block negative regulatory receptor on lymphocyte. But recently, another way which enhances and triggers positive, co-stimulatory signals using agonistic antibody has emerged(4, 7). Dacetuzumab is one of an agonistic CD40 antibody which triggers CD40-mediated signaling in various cells. AntiCD40-mediated cancer immunotherapy using it had

been ongoing trials for the treatment of hematological malignancies, but not yet for HNSCC(8). Such an agonistic antibody agent of TNF receptor superfamily co-stimulating T and NK cell can be tried for the treatment of HNSCC. Our data demonstrated lower expression of CD40 and CD8 in the blood of HNSCC group similarly. So this result can be a background to formulate CD40-targeted anti-tumor immunotherapy for HNSCC in future.

CD33 is a myeloid surface antigen, which is also expressed in Myeloid-derived suppressor cells(MDSC). MDSCs are heterogeneous subset of immune-regulatory cells present in most patients and animals with various types of cancer including HNSCC(9, 10). In the aspect of immune escape of cancer, MDSCs suppress the function of T cell and NK cell, and induce T-reg cell, so that they decrease antitumor immunity. Many studies have shown that circulating MDSCs in peripheral blood significantly increase in cancer patients and correlate with high stage and metastasis(11). MDSCs are derived from bone marrow, circulate in peripheral system, and infiltrate to a tumor and its draining lymph node(12). Our data showed slightly higher expression of the CD33 ratio of peripheral blood in the malignant

group than that in control group. In contrast, node analysis showed lower CD33 expression in malignant group. According to ECS, ECS-positive subgroup showed lower expression of CD33 in a normal lymph node than that of the ECS-negative subgroup. Though all of these results were not statistically significant, these can be interpreted that MDSCs in circulation infiltrates highly to a tumor and metastatic nodes, not to a benign node. In other words, lower expression of CD33 of a benign node in the ECS-positive group would be because of consumed MDSCs by high tumor burden. This implies that there might be a change of host immunity toward immune escape even in normal lymph nodes depending on the presence of ECS, which is an important prognostic factor. Further studies are necessary to subtype or investigate the function of MDSCs in a benign node.

IL-6, a multi-functional and pro-inflammatory cytokine, is secreted by T cells and macrophage to stimulate an immune response. Many studies have shown that the IL-6 serum level is higher in HNSCC patients than healthy people(13, 14). Moreover, many previous studies also implicated that expression of IL-6 is related to tumor progression (14), high stage(15), metastasis(16), and low survival rate of various kinds of cancers

including HNSCC(14, 17-19). The reason of elevated level of IL-6 seems to be a secretion from a tumor itself(20, 21). IL-6 binds to an IL-6 receptor, induces transient phosphorylation of STAT3, and triggers signaling cascades through the STAT3 pathway(22, 23). Activation of STAT3 inhibits dendritic cell maturation and NK cell, T cell, neutrophil, and macrophage activation, so it contributes to oncogenesis in various kinds of cancer(18, 24, 25). Recently, IL-6 receptor inhibitor was shown to suppress metastases in breast cancer cell in vivo(26). Our results showed the increase of IL-6 in plasma in malignant group, and that corresponds with the above studies. Though there are several anti-IL-6 agents, their usage in cancer therapy is still limited. Tocilizumab is an antibody against the IL-6 receptor, and siltuximab is an antibody targeting IL-6 itself. Both of them are approved for only a few kind of rheumatologic disease(27, 28), and they were used in only phase I or II clinical for multiple myeloma, prostate, lung and ovarian cancer(29). Based on these studies, our results also can be a clue to investigate the effect of the anti-IL-6 agent in HNSCC, but further research will be necessary.

To date, many studies already analyzed biomarkers in serum

between HNSCC and control group. However, to our knowledge, this study shows the analysis of immune marker from a benign lymph node between HNSCC patients and patients with benign tumor for the first time.

One of limitation of this study is that the control group is patients with the benign head and neck tumors. The ideal control group would be healthy people without any tumor, like the way Kaskas et al. did(30). However, obtaining samples of a lymph node in such group is almost impossible practically or ethically. Thus, we think that samples of the control group in our study are optimal.

Another limitation of our study is that we did not check whether the subjects were taking drugs which can influence the cytokines or immune cells. There are many pharmacological agents which can change the levels of cytokine or suppress MDSC. Various kinds of chemotherapeutic agents can affect cytokines and MDSCs. And other common drugs, COX2 inhibitor, amino-bisphosphonate, phohsphodiesterase-5 inhibitor, nitroaspirin, 25-hydroxyvitamin D3, for examples, can also alter the function of MDSCs(31-37). Even though we excluded patients with the history of chemotherapy and did not administer

drugs except prophylactic antibiotics to both groups preoperatively, but their drug history was not assessed. This would be a possible misinterpretation of our results.

Conclusion

In conclusion, we found the change of immune markers that means immune escape of HNSCC. And these differences of expression of CD40, CD33, and IL-6 implies a possibility of immunotherapy targeting these immune markers in the treatment of head and neck cancer

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TABLES

Table 1. Demographic Data of flow cytometry

	Benign (N=13)	Malignant (N=26)
Male:Female	12:1	22:4
Age (mean)	47.5	57.9
Diagnosis	Parotid: 10 Submandibular gland: 3	Oral cancer: 12 Oropharynx cancer: 9 Hypopharynx cancer: 3 Larynx cancer: 2
Pathology	Pleomorphic adenoma: 8 Warthin's tumor: 4 Aspergilloma: 1	Squamous cell carcinoma: 26
T Stage		T1: 4 T2: 16 T3: 1 T4: 5
N stage		N0: 12 N1: 5 N2b: 8 N2c: 1

Table 2. Comparison of Samples from Malignant vs. Benign Patients (student t-test)

Sample	Antibody	Benign (N)	Malignant (N)	P-value
Blood	CD4	26.6±4.0 (13)	21.3±4.0 (25)	0.113
	CD8	**24.2±2.3 (13)**	**17.6±2.3 (25)**	**0.001**
	CD40L	33.6±4.1 (13)	28.3±4.2 (25)	0.118
	CD40	**6.8±1.7 (13)**	**3.9±1.5 (25)**	**0.022**
	CD33	12.7±7.0 (13)	13.4±2.8 (24)	0.819
	CD19	8.3±2.2 (13)	5.6±2.4 (24)	0.151
Node	CD4	57.8±5.6 (10)	52.3±6.3 (15)	0.249
	CD8	11.1±2.2 (10)	11.4±2.5 (15)	0.876
	CD40L	56.0±5.8 (10)	51.1±5.9 (16)	0.277
	CD40	24.2±7.1 (10)	23.8±6.8 (16)	0.931
	CD33	11.8±12.5 (11)	7.5±3.5 (12)	0.506
	CD19	25.3±6.8 (11)	26.3±6.8 (12)	0.847

Table 3. Comparison of Samples from Benign, Stage I-III, and Stage IV patients (One-wayANOVA)

Sample	Antibody	Benign (N)	Stage I-III (N)	Stage IV (N)	P-value
Blood	CD4	26.6±4.0 (13)	20.4±5.5 (13)	22.4±5.9 (12)	0.252
	CD8	**24.2±2.3 (13)**	**17.5±3.1 (13)**	**17.8±3.5 (12)**	**0.004**
	CD40L	33.6±4.1 (13)	27.5±6.8 (13)	29.1±4.8 (12)	0.276
	CD40	**6.8±1.7 (13)**	**4.8±2.4 (13)**	**3.0±1.4 (12)**	**0.037**
	CD33	12.7±7.0 (13)	10.8±2.2 (12)	16.1±4.8 (12)	0.381
	CD19	8.3±2.2 (13)	7.6±4.2 (12)	3.6±1.6 (12)	0.063
Node	CD4	57.8±5.6 (10)	49.8±9.8 (9)	56.1±5.5 (6)	0.305
	CD8	11.1±2.2 (10)	11.4±3.6 (9)	11.4±3.3 (6)	0.988
	CD40L	56.0±5.8 (10)	48.6±8.6 (9)	55.3±5.8 (6)	0.285
	CD40	24.2±7.1 (10)	27.3±7.8 (9)	17.9±12.3 (6)	0.375
	CD33	11.8±12.5 (11)	10.3±4.8 (7)	3.6±2.5 (5)	0.614
	CD19	25.3±6.8 (11)	26.5±6.8 (7)	25.9±4.7(5)	0.978

Table 4. Comparison of Samples from malignant patient according to the Extracapsular spread (independent sample t-test)

Sample	Antibody	ECS- (N)	ECS+ (N)	P-value
Blood	CD4	19.4±4.9 (17)	25.4±6.3 (8)	0.179
	CD8	17.8±2.9 (17)	17.3±5.4 (8)	0.857
	CD40L	26.2±11.4 (17)	32.7±5.2 (8)	0.107
	CD40	3.5±1.7 (17)	4.9±2.8 (8)	0.38
	CD33	14.0±3.5 (16)	12.3±4.9 (8)	0.596
	CD19	5.4±3.1 (16)	5.9±3.5 (8)	0.850
Node	CD4	51.6±8.3 (11)	54.3±8.1 (4)	0.730
	CD8	12.5±3.1 (11)	8.5±1.8 (4)	0.170
	CD40L	50.3±7.4 (12)	53.5±8.5 (4)	0.661
	CD40	24.6±7.8 (12)	21.3±15.8 (4)	0.702
	CD33	**9.5±3.8 (9)**	**1.6±0.9 (3)**	**0.004**
	CD19	25.3±7.9 (9)	29.1±15.6 (3)	0.657

Table 5. Demographic Data of cytokine analysis

	Benign (N=15)	Malignant (N=27)
Male:Female	13:2	23:4
Age (mean)	44.4±15.2	58.6±15.8
Diagnosis	Parotid: 12 SMG: 3	Oral cavity: 12 Oropharynx: 10 Hypopharynx: 3 Larynx: 2
Pathology	Pleomorphic adenoma: 9 Warthin's tumor: 5 Acinic cell carcinoma: 1	Squamous cell carcinoma: 27
T stage		T1: 4 T2: 15 T3: 2 T4: 6
N stage		N0: 13 N1: 5 N2b: 8 N2c: 1

Table 6. Comparison of samples from benign vs malignant patients (student t-test)

Cytokine (pg/mL)	Benign (N=15)	Malignant (N=27)	P-value
IFN-γ	15.9±3.1	19.1±3.1	0.511
IL-1β	3.5±0.7	5.0±1.0	0.318
IL-10	6.0±2.1	27.9±16.2	0.314
IL-2	1.4±0.5	5.2±3.2	0.384
IL-4	32.7±9.1	30.1±7.1	0.826
IL-5	1.2±1.0	0.4±0.2	0.246
IL-6	1.9±1.0	9.3±2.7	**0.014**
sCD40L	996.9±360.8	983.6±312.8	0.979
TNF-α	16.1±2.1	19.2±1.9	0.313

Table 7. Comparison of samples from benign, stage I-III, and stage IV patients (one-way ANOVA)

Cytokine (pg/mL)	Benign (N=15)	Stage I-III (N=14)	Stage IV (N=13)	P-value (benign vs Stave IV)
IFN-γ	15.9±3.1	22.4±5.1	15.5±3.4	0.997
IL-1β	3.5±0.7	5.5±1.5	4.4±1.4	0.866
IL-10	6.0±2.1	7.9±2.6	49.3±33.0	0.205
IL-2	1.4±0.5	7.6±6.1	2.7±1.5	0.966
IL-4	32.7±9.1	35.6±11.4	24.2±8.3	0.975
IL-5	1.2±1.0	0.5±0.3	0.4±0.2	0.372
IL-6	1.9±1.0	6.0±2.5	13.0±4.8	0.035
sCD40L	996.9±360.8	704.7±246.3	1284.0±596.0	0.877
TNF-α	16.1±2.1	18.0±2.1	20.6±3.4	0.433

Table 8. Comparison of samples from malignant patients according to the Extracapsular spread (student t-test)

Cytokine(pg/mL)	ECS- (N=8)	ECS+ (N=19)	P-value
IFN-γ	22.1±4.3	17.8±4.1	0.542
IL-1β	5.8±2.1	4.6±1.2	0.613
IL-10	5.8±1.9	37.1±22.8	0.386
IL-2	4.3±2.2	5.6±4.5	0.855
IL-4	28.8±12.2	30.6±8.9	0.909
IL-5	0.5±0.3	0.4±0.2	0.735
IL-6	11.7±5.1	8.4±3.2	0.585
sCD40L	1254.8±442.4	869.4±408.0	0.584
TNF-α	20.7±3.0	18.6±2.5	0.632

국문 초록

두경부종양에서 혈액과 임파선의 면역표지 발현의 비교

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서론: 두경부암은 비교적 흔한 질환으로 대부분이 편평상피세포암종이다. 기존의 치료방법으로 수술, 항암치료, 방사선 치료 등의 방법이 있지만 5년 생존율이 50-60%로 높지 않은 편으로 그에 따라 표적치료 등의 면역치료가 수 년 전부터 각광받고 있다. 이에 본 연구에서는 두경부암의 면역치료에 대한 선행연구로서 두경부암 환자의 혈액과 림프절에 발현하는 면역표지를 분석하고자 한다.

방법: 두경부암으로 수술받는 환자를 실험군으로, 양성 종양으로 수술받는 환자를 대조군으로 삼아 두 집단의 정상 림프절과 혈액을 채취한 후 유세포분석기와 Milliplex® 분석 키트를 이용해 면역항체와 사이토카인을 비교 분석하였다.

결과: 악성종양 집단의 혈액에서 CD8과 CD40의 발현이 유의하게 대조군에 비해 낮게 나타났으며 실험군을 낮은 병기와 높은 병기로 나눠서 세 집단을 비교했을 때도 동일한 결과가 나타났다. 림프절에서는 유의한 차이가 없었다. 혈장의 사이토카인은 악성종양 집단에서 IL-6가 유의하게 수치가 높았으며 실험군을 낮은 병기와 높은 병기로 나눠서 세 집단을 비교했을 때도 동일한 결과가 나타났다. 실험군을 피막외침범 여부로 나누어 분석했을 때 림프절의 CD33이 피막외침범 양성군에서 낮게 나타났으며 혈액에서 유의한 차이는 없었다.

결론: 본 연구에서 나타난 CD40과 IL-6의 발현 차이는 향후 두경부암에서 CD40과 IL-6을 표적으로 한 표적치료의 가능성을 보여준다.

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주요어 : 두경부암, 면역표지, CD40, CD33, IL-6, 림프절

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