의학석사 학위논문

Predictive Biomarkers of Tumor Cell and Host Immunity in Postoperative Prognosis of Oral Squamous Cell Carcinoma

구강 편평상피암 수술 후 예후에 있어 종양세포와 숙주면역의 예측생체지표들에 대한 연구

2014 년 8 월

서울대학교 대학원
의학과 이비인후과학 전공
안 재 철

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지도교수 안 순 현

이 논문을 안 재 철 석사학위논문으로 제출함

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안재철의 석사학위논문을 인준함

2014 년 7 월

위 원 장 이 재 서 (인)

부 위 원 장 안 순 현 (인)

위 원 정 진 행 (인)

ABSTRACT

Predictive Biomarkers of Tumor Cell and Host Immunity in Postoperative Prognosis of Oral Squamous Cell Carcinoma

Jae-Cheul Ahn

Department of Othrhinolaryngology,

The Graduate School

Seoul National University

Introduction: Oral squamous cell carcinoma (OSCC) is one of the common malignancies worldwide. While treatment modalities have been advanced, the five-year survival rate is about 50% in stationary for several decades. The TNM staging has been conventionally used to predict the prognosis but it does not give biological information of tumor cell and host immunity against tumor. Evaluating the biologic characteristics of the primary tumor and host immune defense system, our previous microarray analysis of mRNA found out 9 candidates of prognostic biomarkers; 5 for primary tumor tissue and 4 for host normal lymph nodes. This study aimed to evaluate the prognostic values of the biomarkers assessed by immunohistochemistry (IHC)

Methods: All patients with OSCC, who underwent successful

surgical resections from January 2003 to December 2011, were included in this study. Tissue arrays were constructed for 69 primary tumor tissues and 60 normal cervical lymph nodes not affected by tumor. IHC of the nine candidates were applied to corresponding tissue arrays; FAS, HIF1-alpha, Bcl2A, MST4, and ErbB3 to the primary tumors and STAG2, CD40L, CD80, and PTPRO to the lymph nodes. IHC was graded by semi-quantative histologic scoring system (H score) considering the extent and intensity of the staining. IHC results were correlated with clinicopathological characteristics and with clinical outcomes such as, relapse free, disease free, and overall survivals.

Results: In total 69 cases, Oral tongue was the most frequently affected primary site. In TNN staging, stage IV (33.3%) is most frequent followed by stage I (26.1%), II (26.1%), and III (14.5%). Despite of successful resection, there was 27.5% of recurrence. TNM stage IV was highly related with poor clinical outcomes ($p < 0.001$). Among the 9 biomarkers, the expression of FAS only showed strong positive correlation with the TNM staging ($p = 0.019$). The positive expression of FAS, HIF1-alpha, and ErbB3 in the tumor cells had correlation with

recurrence (p = 0.070, 0.070, and 0.039 respectively). The negative expression of CD40L in the lymph nodes had significant positive correlation with recurrence (p = 0.030). The multivariate Cox regression showed that TNM staging and H score of HIF1-alpha in primary tumors were independent poor prognostic factors in the relapse free survival while H score of CD40L in the lymph nodes was good prognostic factor (p < 0.001, HR=6.990, 95% CI 2.361-20.70 for TNM stage IV; p = 0.006, HR=1.020, 95% CI 1.006-1.034 for H score of HIF1-alpha; p = 0.020, HR=0.978, 95% CI 0.960-0.996 for H score of CD40L). In the overall survival, elevated H score of HIF1-alpha and decreased H score of CD40L were independent poor prognostic factors with TNM staging. (p = 0.008, HR=1.024, 95% CI 1.007-1.042 for HIF1-alpha; p = 0.022, HR=0.973, 95% CI 0.950-7.996 for CD40L).

Conclusions: The expressions of HIF1-alpha and CD40L, in the primary tumors and the normal lymph nodes respectively, could be used as postoperative prognostic biomarkers of tumor cell and host immunity against the tumor in OSCC.

Keywords: Oral cavity, Squamous cell carcinoma, Prognosis,

Immunohistochemistry, Tissue array

Student number: 2011-23747

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LIST OF ABBREVIATION

OSCC: oral squamous cell carcinoma

IHC: immunohistochemistry

FAS: fatty acid synthase

HIF1-alpha: hypoxia-inducible factor 1-alpha

Bcl2A: B-cell lymphoma 2A

MST4: mammalian serine/threonin protein kinase 4

ErbB3: erythroblastic leukemia viral oncogene homolog 3

STAG2: stromal antigen 2

CD40L: cluster of differentiation 40 ligand

CD80: cluster of differentiation 80

PTPRO: protein tyrosine phosphatase, receptor type, O

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is most frequent malignant neoplasm of oral cavity cancer which is the sixth most common malignancies worldwide (1). Despite progress of multimodality treatments and discovery of nature in malignancies, its prognosis remains poor with the five-year survival rate about 50% relatively unchanged for three decades (2). Tumor-node-metastasis (TNM) classification system has been conventionally used to evaluate the prognosis of the OSCC. However, TNM staging reflects neither the exact biologic aggressiveness of the tumor nor the host immune defense mechanism against to the tumor. Therefore, some of patients with early stages sometimes experienced poorer prognosis than the others. Therefore, it is important to discover the biomarkers explaining the biological information of the tumor cell and host immunity for the postoperative prognosis to assist clinician's decision of treatment modality and to develop the more effective treatment.

Recently, several biomarkers have been studied and most of studies adopt advanced technologies, such as microarray and

tissue array, to search and confirm the biomarkers in genomics and proteomics. Most of studies focused on the tumor behavior and they discovered prognostic biomarkers related to signaling pathways of hypoxic microenvironment or tumor growth including epidermal growth factor receptor (EGFR) (3, 4). In our previous study, we found out nine candidate biomarkers related with tumor aggressiveness as well as host immune defense system via mRNA microarray analysis of the primary tumor cells and host normal cervical lymph nodes. The 5 candidate biomarkers for tumor aggressiveness were fatty acid synthase (FAS), hypoxia-inducible factor 1-alpha (HIF1-alpha), B cell leukemia 2A (Bcl2A), mammalian STE20-like protein kinase (MST4), and human epidermal growth factor receptor 3 (HER3/ErbB3). The 4 candidate biomarkers for host immune defense system were stromal antigen 2 (STAG2), CD40 ligand (CD40L), CD80, and receptor-type tyrosine-protein phosphatase O (PTPRO).

FAS is responsible for endogenous production of saturated long-chain fatty acids. It is down-regulated in normal healthy person due to ingestion of sufficient dietary fatty acid (5). FAS is hyper-activated and over-expressed in some aggressive

tumors and associated with a poor prognosis (6). HIF1-alpha is a key regulator of response to hypoxia and a transcription factor that up-regulates the expression of genes involved in process related to tumor progression such as angiogenesis, anaerobic metabolism, cell proliferation, survival, and migration (7). Bcl2A is an anti-apoptotic protein expressed in cells, which protects against apoptosis. Bcl2A participates in human carcinogenesis especially in oral cavity (8). MST4, a member of sterile 20 serine/threonine kinase family has potential role in transduction pathway promoting growth of prostate cancer cells (9). ErbB3 is a transmembrane tyrosine kinase receptor of the ErbB family. Overexpression of ErbB family has been associated with regulation of cell proliferation, survival and differentiation (10).

STAG2 acts as a transcriptional co-activator and enhance the activity of tumor necrosis factor alpha and CD69 (11). CD40L is reported as an effective anti-tumor immune response with high level and a pro-tumor immune response with lower level (12). CD80 produces co-stimulatory signal for T lymphocyte activation and survival. CD80 was impaired in the esophageal cancer tissue and correlated with poor prognosis (13). PTPRO

regulates the proliferation, differentiation, and viability of lymphocytes by modulating their signal pathway (14).

The aim of this study is to evaluate whether expressions of FAS, HIF1-alpha, Bcl2A, MST4, and ErbB3 in the primary tumor tissues and expressions of STAG2, CD40L, CD80, and PTPRO in the lymph nodes predict postoperative prognosis, such as relapse free, disease free, and overall survivals.

MATERIALS AND METHODS

1. Subjects and tissue samples

From July 2003 to December 2011, 71 consecutive OSCC patients who underwent successful surgical resection in Seoul National University Bundang Hospital were enrolled in this study. This study was approved by the institutional review board at Seoul National University Bundang Hospital (No. B-1205/153-304). The paraffin blocks of the surgical specimens were examined by two pathologists, Paik JH and Kim HJ. Since paraffin blocks of primary tumor tissues were not available in 2 patients, the paraffin blocks of 69 patients were selected to make a tissue array of primary tumor tissues. In addition, cervical lymph nodes dissection was not performed in 4 patients and paraffin blocks of lymph nodes were not available in 5 patients. Therefore, the lymph nodes of 60 patients were selected to develop tissue array of lymph nodes. To evaluate host immune defense system, the normal lymph nodes were selected in the cervical side without evidence of tumor invasion.

2. Tissue array

After review of the 69 primary tumor tissues and 60 lymph nodes, the representative core tissue sections (2 mm in diameter) were taken from paraffin blocks and arranged in new tissue microarray (TMA) blocks using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). The detailed description of the tissue array construction can be found at homepage of the company (http://www.tissue-array.com). After the tissue array blocks were constructed, the array blocks were sectioned with thickness 4 ㎛ for immunohistochemistry (IHC).

3. IHC staining

This study evaluated the expressions of five proteins for the primary tumor tissues and four proteins for the lymph nodes; FAS, HIF1-alpha, Bcl2A, MST4, and ErbB3 for the tumors and STAG2, CD40L, CD80, and PTPRO for the lymph nodes

For IHC, Antibodies for the following molecules were used in this study; FAS (1:100, Cell Signaling Technology Inc, Danvers,

MA, USA), HIF1-alpha (1:10, abcam, Cambridge, MA, USA), Bcl2A (1:100, abcam, Cambridge, MA, USA), MST4 (1:100, abcam, Cambridge, MA, USA), ErbB3 (1:25, Cell Signaling Technology Inc, Danvers, MA, USA), STAG2 (1:25, abcam, Cambridge, MA, USA), CD40L (1:10, abcam, Cambridge, MA, USA), CD80 (1:10, abcam, Cambridge, MA, USA), and PTPRO (1:10, abcam, Cambridge, MA, USA). Anti-FAS, anti-Bcl2A, anti-MST4, anti-ErbB3, and anti-CD80 were rabbit monoclonal antibodies. Anti-CD40L and anti-PTPRO were rabbit polyclonal antibodies. Anti-HIF1-alpha and anti-STAG2 were mouse monoclonal antibodies.

The sectioned slides of the tissue arrays underwent the process of deparaffinization, rehydration, antigen retrieval, antibody binding, dehydration and mounting. From the tissue microarray blocks, 4 ㎛ thick section were transferred to poly-L-lysine-coated glass slides and incubated in a dry oven at 60℃ for 1 hour. These sections were then dewaxed in xylene (three changes), rehydrated in a graded series of ethanol solutions with decreasing concentrations and rinsed in Tris-buffered saline (TBS; pH 7.4). The endogenous peroxidase activity was inactivated with 3% hydrogen peroxide

in methanol for 15 minutes at 37℃. The slides were then placed in citrate buffer (10% citrate buffer stock in distilled water, pH 6.0) and microwaved for 25 minutes. Non-reactive staining was blocked using 1% horse serum in TBS (pH 7.4) for 3 minutes.

The IHC staining divided into probings of the primary and secondary antibodies. The over-night probing of primary antibody were conducted in 4℃. After probing primary antibodies, the slides were soaked into 0.1% PBST twice for 5 minutes each. The biotinylated secondary probing was done in RT for 30 minutes. The slides were incubated with ABC kit (Vector laboratories, Burlingame, CA, USA) in RT for 30 minutes, were soaked into 0.1% PBST twice for 5 minutes each, and were incubated again with the ABC kit in humid chamber. After two times of the incubation, the slides were soaked into 0.1% PBST twice for 5 minutes each again. Then, Diaminobenzidine (Dako, Glostrup, Denmark) was applied on the slide and the staining was closely monitored with following five times of washing for 3 minutes each.

After IHC staining, the slides underwent dehydration and mounting. The dehydration was conducted as the exact reverse

process of the rehydration procedure described before. After mounting, the slides were dried up in RT for several hours.

4. IHC grades

The stained slide was evaluated by the extent and intensity of the staining. We adopt semi-quantative histologic scoring system (H score) to compare the stained slides. The intensity divided into 4 grade; no, weak, moderate and strong straining. The extent were reviewed from 0% to 100% according to the intensity under lower power field (x200 magnification); the sum of extents of the four intensity become 100%. H score was calculated by the following formula (Figure 1).

H score = 1x(extent percent of weak staining) + 2x(extent percent of moderate staining) + 3x(extent percent of strong staining)

The positive expression of IHC was defined by H score more than or equal to the median of the H scores in the IHC; the negative expression by H score less than the median.

5. Statistical analysis

Univariate Pearson's chi-square test was used to analyze the relationship between each H score and clinicopathological characteristics. The relapse free, disease free, and overall survivals were investigated with positive and negative expressions of IHC by univariate log rank test in Kaplan Meier survival analysis. The IHC stainings with $p \leq 0.2$ in the univariate log rank test were evaluated for their hazard ratio in the survivals by multivariate Cox regression; Cox regression was performed with semi-quantative H score rather than dichotomous expression. All statistical tests were two tailed test with the statistical significance at $p < 0.05$ and the marginal significance at $p < 0.10$. SPSS (Version 18.0; SPSS, Inc., Chicago, IL) was used for the statistical analysis.

RESULTS

1. Clinicopathological characteristics

Patients consisted of 45 (63.4%) male and 24 (33.8%) female with a median age of 57.7 years (range, 23-84) (Table 1). Median follow up duration was 40.9 months. Most common primary site of the tumor was oral tongue (58.0%) followed by buccal mucosa (18.8%), floor of the mouth (11.6%), retromolar trigone (10.1%), and alveolar ridge (1.4%) (Table 1). According to TNM staging system, there were 18 patients in stage I, 18 in II, 10 in III, and 23 in IV (Table I). Lymph node metastasis was pathologically confirmed in 26 patients. Angiolymphatic invasion and perineural infiltration was confirmed in 19 patients; 11 patients with both angiolymphatic and perineural infiltrations, 8 with angiolymphatic invasion only, and the other 8 only with perineural infiltration. Extracapsular spread (ECS) could be evaluated in 56 patients and 2 patients with ECS were confirmed. There were 19 patients with tumor recurrence.

2. IHC of the primary tumors

FAS

The FAS expression in the primary tumor appeared in the form of a cytoplasmic staining pattern. The median H score of FAS was 130. For the statistical analysis, the cytoplasmic expressions of FAS were divided into 2 categories: negative (H score < 130) and positive (H score ≥ 130) expressions. The FAS expression was associated with advanced TNM stage (p=0.019) and marginally significantly associated with older age and recurrence (p=0.065 and p=0.070 respectively) (Table 2).

HIF1-alpha

The HIF1-alpha expression in the primary tumor appeared in the form of a nuclear staining pattern. The median H score of HIF1-alpha was 70. For the statistical analysis, the nuclear expressions of HIF1-alpha were divided into 2 categories: negative (H score < 70) and positive (H score ≥ 70) expressions. The male patients tended to have positive expression of HIF1-alpha (Table 3). With marginal significance, less expression of HIF1-alpha was associated with recurrence

(Table 3).

Bcl2A

The Bcl2A expression in primary tumor appeared in the form of cytoplasmic staining pattern. The median H score of Bcl2A was 50. For the statistical analysis, the cytoplasmic expressions of Bcl2A were divided into 2 categories: negative (H score < 50) and positive (H score ≥ 50) expressions. The older age showed more positive expression in IHC staining of Bcl2A (Table 4). With marginal significance, increased expression of Bcl2A was associated with angiolymphatic invasion and recurrence (Table 4).

MST4

The MST4 expression in the primary tumor appeared in the form of cytoplasmic staining pattern. The median H score of MST4 was 30. For the statistical analysis, the cytoplasmic expressions of MST4 were divided into 2 categories: negative (H score < 30) and positive (H score ≥ 50) expressions. The female patients tended to have increased expression of MST4 (Table 5).

ErbB3

The ErbB 3 expression in the primary tumor appeared in the form of membrane staining pattern. The median H score of ErbB3 was 60. For the statistical analysis, the membranous expressions of ErbB3 were divided into 2 categories: negative (H score < 60) and positive (H score ≥ 60) expressions. The expression was associated with recurrence (Table 6).

3. IHC of the lymph nodes

STAG2

The STAG2 expression in the lymph nodes appeared in the form of nuclear staining pattern. The median H score of STAG2 was 50. For the statistical analysis, the nuclear expressions of STAG2 were divided into 2 categories: negative (H score < 50) and positive (H score ≥ 50) expressions. The expression was found in buccal cancer (Table 7).

CD40L

The CD40L expression in the lymph nodes appeared in the

form of cytoplasmic staining pattern. The median H score of CD40L was 70. For the statistical analysis, the cytoplasmic expressions of CD40L were divided into 2 categories: negative (H score < 70) and positive (H score ≥ 70) expressions. While the perineural infiltration was observed more frequently in positive CD40L expression, the recurrence occurred more in negative CD40L expression (Table 8). Additionally, more advanced age had more positive expression (Table 8).

CD80

The CD80 expression in lymph nodes appeared in the form of cytoplasmic staining pattern. The median H score of CD80 was 60. For the statistical analysis, the cytoplasmic expressions of CD80 were divided into 2 categories: negative (H score < 60) and positive (H score ≥ 60) expressions. Lymph node metastasis occurred more in positive CD80 expression (Table 9).

PTPRO

The PTPRO expression in lymph node appeared in the form of cytoplasmic staining. The median H score of PTPRO was 40.

For the statistical analysis, the cytoplasmic expressions of PTPRO were divided into 2 categories: negative (H score < 40) and positive (H score ≥ 40) expressions. The expression was not associated with clinicopathological characteristics (Table 10).

4. Relapse free survival

Relapse free survival curves were shown in Figure 2 according to the expression of the candidates. In univariate log rank test of Kaplan Meier survival model, TNM stage IV, positive expressions of FAS and ErbB3 in primary tumors and loss of CD40L in the lymph nodes were statistically significantly related to poor relapse free survival (Table 11 and 12). However, multivariate Cox regression analysis showed that TNM stage IV, the elevated H score of HIF1-alpha, and the decreased H-score of CD40L had statistically significant risks in poor relapse free survival (Table 13).

5. Disease free survival

Disease free survival curves were shown in Figure 3 according to the expression of the candidates. In univariate log rank test of Kaplan Meier survival model, TNM stage IV, positive expressions of FAS, Bcl2A, and ErbB3 in the primary tumors and loss of CD40L in the lymph nodes were statistically significantly related to poor disease free survival (Table 14 and 15). However, multivariate Cox regression analysis showed that TNM stage IV, the elevated H score of HIF1-alpha, and the decreased H score of CD40L had statistically significant risks in poor disease free survival (Table 16).

6. Overall survival

Overall survival curves were shown in Figure 4 according to the expression of the IHC staining. In univariate log rank test of Kaplan Meier survival model, TNM stage IV and the positive expressions of FAS, Bcl2A and ErbB3 in the primary tumors were statistically significantly related to poor overall survival (Table 17 and 18). However, multivariate Cox regression analysis showed that TNM stage IV and the elevated H score of HIF1-alpha, and the decreased H score of CD40L had

statistically significant risks in poor overall survival (Table 19).

DISCUSSION

TNM staging is one of the most important standards for the initial treatment of OSCC. Considering that the TNM stage is drawn from the preoperative imaging studies, pathological findings of surgical specimen provides more important information postoperatively than the TNM staging. TNM staging could provide the accurate information of size and location of tumor but does not predict the potential of metastasis. Therefore, the tumor biology and host immunity against tumor would be supplement to the TNM staging.

Positive expression of HIF1-alpha in the primary tumor was independently raising risks 4 to 5 times of relapse free, disease free, and overall survivals. HIF1-alpha is a transcription factor controlling several target genes such as vascular endothelial growth factor (VEGF), glucose transporters 1 and 3 (GLUT 1, GLUT3), and insulin-like growth factor 2 (IGF2) (7). VEGF, controlled by HIF1-alpha, is a signaling protein promoting angiogenesis. To be survived in rapid growing condition of cancer, tumor cell needs neovascularization to obtain the oxygen and glucose. Improper supplement of the oxygen and

glucose make the tumor cells into necrosis. GLUT1 and GLUT3 are signaling proteins increasing glucose transport and glycolysis to produce ATP. They generate the energy the tumor proliferation. As a growth promoting protein hormone, IGF2 encourages growth and mitogenic activity in order to extend the survival of tumor cells. Therefore, HIF1-alpha plays a controller of angiogenesis, energy production, and cell survival. Many of studies have pointed out relationship between HIF1-alpha and prognosis of malignancies (15). In the postoperative prognosis of OSCC, HIF1-alpha would play a certain role of disease recurrence even though tumor burden was almost totally removed. Few remnant tumor cells actively producing HIF1-alpha would have more chance to be survived and grow by the angiogenesis and energy generation positively regulated by the HIF1-alpha. The rapid growth would overcome the host immune defense mechanism and postoperative adjuvant treatment, eventually inducing recurrence.

Negative expression of CD40L in the cervical lymph nodes was an independent risk factor of relapse free survival with 3.5 times of risks. Additionally, with marginal significance, the

negative expression of CD40L was a risk factor of poor overall survival. CD40L is a protein primarily expressed on activated T cell and belongs to superfamily of type I TNF. It binds to CD40, co-stimulatory protein, of antigen presenting cells. Antigen presenting cell, such as dendritic cell, monocyte, and B-lymphocytes, expression CD40 provides the recognition of tumor antigens which is the first stage of host immune defense system. CD40L has been known protects circulating dendritic cells from apoptosis caused by antiapoptotic molecule Bcl2A (16). Additionally, CD40L promotes functional populations of immature dendritic cells. The CD40/CD40L interaction plays an important role on B-lymphocytes, promoting proliferation, differentiation, increase of co-stimulatory molecules, and elevation of antigen presentation. Finally, Interaction of CD40L and B-lymphocytes induce antitumor immunity. It is also known that activation of CD40 leads to stimulation of cytotoxic T-lymphocytes, memory T-lymphocytes, and natural killer cells in the experimental model (17, 18). Besides of activating various immune cells involving antitumor defense system, CD40L decreases the rate of cell proliferation by induction of spontaneous and Fas-induced apoptosis and also EGFr-

dependent inhibition of proliferation in the squamous cell carcinoma of head and neck (19). Both activation of host immune system and induction of apoptosis of tumor cell would irradiate the postoperative remnant tumor cell and suppress the tumor recurrence.

Positive expression of FAS showed closed relationship with relapse free and overall survival in univariate log rank. However, multivariate Cox regression failed to show the relationship between the positive expression of FAS and the relapse free survival. The different results of univariate and multivariate survival model were drawn from the close correlation between FAS and the TNM staging. FAS, which is a 250-270 kDa cytosolic protein, catalyzes the synthesis of palmitate from the condensation of malonyl-CoA and acetyl-CoA and plays an important role in energy homeostasis by converting excess carbon intake into fatty acids for storage. Since diet supplies most of fatty acids, FAS is expressed at low in normal human tissues. The cancer cell needs very long chain fatty acids, generated from the stearate and palmitate precursors and membrane phospholipids for cell division (20). FAS plays key role to generate the precursors and de novo

synthesis of the membrane phospholipid. Therefore, the over-expression of FAS might be associated with activities of cell division of the tumor. In fact, many literatures reported that over-expression of FAS in some malignancies, such as pancreatic ductal adenocarcinoma, renal cell carcinoma, and soft tissue sarcoma, is associated with poor clinical outcome by predicting increased risk of recurrence, metastasis, or shorter survival (21-24). But our study did not show the positive expression of FAS as a risk factor of recurrence OSCC but implied the close relationship between the positive expression of FAS and the overall survival.

Limitations of the study are the small number of primary tumor tissues and normal cervical lymph nodes. The large population of study would give more promising result of biomarkers predicting the postoperative prognosis.

CONCLUSION

To consider the tumor biology and host immune defense mechanism again the tumor, additional informations from the primary tumor and host normal lymph node could be used to supplement TNM staging. Since HIF1-alpha and CD40L shown the abilities to predict the relapse free and overall survival, they are used as the potential biomarkers of the tumor aggressiveness and host immune defense system with the TNM staging.

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Table 1. Clinicopathological characteristics of patients with oral squamous cell carcinoma.

	Number of patients (%)
Gender	
Male	45 (63.4%)
Female	24 (33.8%)
Age	
≤ 55	25 (36.2%)
> 55	44 (63.8%)
Primary site	
Oral tongue	40 (58.0%)
Buccal mucosa	13 (18.8%)
Floor of the mouth	8 (11.6%)
Retromolar trigone	7 (10.1%)
Alveolar ridge	1 (1.4%)
T stage	
T1	21 (30.4%)
T2	28 (40.6%)
T3	3 (4.3%)
T4	17 (24.7%)
TNM Staging	
I	18 (26.1%)
II	18 (26.1%)
III	10 (14.5%)
IV	23 (33.3%)
Lymph node metastasis (pN)	
N0	43 (62.3%)
N1	15 (21.7%)
N2	11 (16.0%)
Angiolymphatic invasion	
Yes	19 (27.5%)
No	50 (72.5%)
Perineural infiltration	
Yes	19 (27.5%)
No	50 (72.5%)
Extracapsular spread	
Yes	2 (3.6%)
No	54 (78.3%)
Recurrence	
Yes	19 (27.5%)
No	50 (72.5%)

*Pearson's chi-squared test

Table 2. Association between FAS expressions and clinicopathological characteristics of OSCC

	FAS expression		
	Negative	Positive	*p*-value*
Gender			
Male	22 (64.7%)	23 (65.7%)	0.930
Female	12 (35.5%)	12 (34.3%)	
Age			
≤ 55	16 (47.1%)	9 (25.7%)	0.065
> 55	18 (52.9%)	26 (74.9%)	
Primary site			
Oral tongue	19 (55.9%)	21 (60.0%)	0.814
Buccal mucosa	6 (17.6%)	7 (20.0%)	
Others	9 (26.5%)	7 (20.0%)	
T stage			
T1	15 (44.1%)	6 (17.1%)	0.119
T2	12 (35.3%)	16 (45.7%)	
T3	1 (2.9%)	2 (5.7%)	
T4	6 (17.6%)	11 (31.4%)	
TNM Staging			
I	12 (35.3%)	6 (17.1%)	**0.019**
II	7 (20.6%)	11 (31.41%)	
III	8 (23.5%)	2 (5.7%)	
IV	7 (20.6%)	16 (45.7%)	
Lymph node metastasis			
No	22 (64.7%)	21 (60.0%)	0.687
Yes	12 (35.3%)	14 (40.0%)	
Angiolymphatic invasion			
No	26 (78.8%)	23 (65.7%)	0.230
Yes	7 (21.2%)	12 (34.3%)	
Perineural infiltration			
No	23 (69.7%)	26 (74.3%)	0.673
Yes	10 (30.3%)	9 (25.7%)	
Extracapsular spread			
No	28 (96.6%)	25 (96.2%)	0.937
Yes	1 (3.4%)	1 (3.8%)	
Recurrence			
No	28 (82.4%)	22 (62.9%)	0.070
Yes	6 (17.6%)	13 (37.1%)	

*Pearson's chi-squared test

Table 3. Association between HIF1-alpha expressions and clinicopathological characteristics of OSCC

	HIF1-alpha expression		
	Negative	Positive	*p*-value*
Gender			
Male	18 (52.9%)	27 (77.1%)	**0.035**
Female	16 (47.1%)	8 (22.9%)	
Age			
≤ 55	11 (32.4%)	14 (40.0%)	0.509
> 55	23 (67.6%)	21 (60.0%)	
Primary site			
Oral tongue	21 (61.8%)	19 (54.3%)	0.559
Buccal mucosa	7 (20.6%)	6 (17.1%)	
Others	6 (17.6%)	10 (28.6%)	
T stage			
T1	8 (23.5%)	13 (37.1%)	0.253
T2	12 (35.3%)	16 (45.7%)	
T3	2 (5.9%)	1 (2.9%)	
T4	12 (35.3%)	5 (14.3%)	
TNM Staging			
I	8 (23.5%)	10 (28.6%)	0.098
II	5 (14.7%)	13 (37.1%)	
III	6 (17.6%)	4 (11.4%)	
IV	15 (44.1%)	8 (22.9%)	
Lymph node metastasis			
No	19 (55.9%)	24 (68.6%)	0.277
Yes	15 (44.1%)	11 (31.4%)	
Angiolymphatic invasion			
No	24 (72.7%)	25 (71.4%)	0.905
Yes	9 (27.3%)	10 (28.6%)	
Perineural infiltration			
No	22 (66.7%)	27 (77.1%)	0.336
Yes	11 (33.3%)	8 (22.9%)	
Extracapsular spread			
No	23 (92.0%)	30 (100%)	0.115
Yes	2 (8.0%)	0	
Recurrence			
No	28 (82.4%)	22 (62.9%)	0.070
Yes	6 (17.6%)	13 (37.1%)	

* Pearson's chi-squared test

Table 4. Association between Bcl2A expressions and clinicopathological characteristics of OSCC

	Bcl2A expression		
	Negative	Positive	*p*-value*
Gender			
Male	20 (60.6%)	25 (69.4%)	0.441
Female	13 (39.4%)	11 (30.6%)	
Age			
≤ 55	16 (48.5%)	9 (25.0%)	**0.043**
> 55	17 (51.5%)	27 (75.0%)	
Primary site			
Oral tongue	22 (69.7%)	17 (47.2%)	0.091
Buccal mucosa	3 (9.1%)	10 (27.8%)	
Others	7 (21.2%)	9 (25.0%)	
T stage			
T1	10 (30.3%)	11 (30.6%)	0.558
T2	15 (45.5%)	13 (36.1%)	
T3	2 (6.1%)	1 (2.8%)	
T4	6 (18.2%)	11 (30.6%)	
TNM Staging			
I	9 (27.3%)	9 (25.0%)	0.120
II	8 (24.2%)	10 (27.8%)	
III	8 (24.2%)	2 (5.6%)	
IV	8 (24.2%)	15 (41.7%)	
Lymph node metastasis			
No	22 (66.7%)	21 (58.3%)	0.475
Yes	11 (33.3%)	15 (41.7%)	
Angiolymphatic invasion			
No	27 (81.8%)	22 (62.9%)	0.082
Yes	6 (18.2%)	13 (37.1%)	
Perineural infiltration			
No	22 (66.7%)	27 (77.1%)	0.336
Yes	11 (33.3%)	8 (22.9%)	
Extracapsular spread			
No	28 (100.0%)	25 (92.6%)	0.142
Yes	0 (0.0%)	2 (7.4%)	
Recurrence			
No	27 (81.8%)	23 (63.9%)	0.096
Yes	6 (18.2%)	13 (36.1%)	

* Pearson's chi-squared test

Table 5. Association between MST4 expressions and clinicopathological characteristics of OSCC

	MST4 expression		
	Negative	Positive	*p*-value*
Gender			
Male	23 (79.3%)	22 (55.0%)	**0.036**
Female	6 (20.7%)	18 (45.0%)	
Age			
≤ 55	8 (27.6%)	17 (42.5%)	0.203
> 55	21 (72.4%)	23 (57.5%)	
Primary site			
Oral tongue	16 (55.2%)	24 (60.0%)	0.915
Buccal mucosa	6 (20.7%)	7 (17.5%)	
Others	7 (24.1%)	9 (22.5%)	
T stage			
T1	8 (27.6%)	13 (32.5%)	0.539
T2	14 (48.3%)	14 (35.0%)	
T3	2 (6.9%)	1 (2.5%)	
T4	5 (17.2%)	12 (27.5%)	
TNM Staging			
I	8 (27.6%)	10 (25.0%)	0.444
II	10 (34.5%)	8 (20.0%)	
III	4 (13.8%)	6 (15.0%)	
IV	7 (24.1%)	16 (40.0%)	
Lymph node metastasis			
No	29 (69.0%)	23 (57.5%)	0.332
Yes	9 (31.0%)	17 (42.5%)	
Angiolymphatic invasion			
No	18 (64.3%)	31 (77.5%)	0.232
Yes	10 (35.7%)	9 (22.5%)	
Perineural infiltration			
No	20 (71.4%)	29 (72.5%)	0.923
Yes	8 (28.6%)	11 (27.5%)	
Extracapsular spread			
No	23 (100%)	30 (93.8%)	0.222
Yes	0	2 (6.3%)	
Recurrence			
No	20 (69.0%)	30 (75.0%)	0.580
Yes	9 (31.0%)	10 (25.0%)	

* Pearson's chi-squared test

Table 6. Association between ErbB3 expressions and clinicopathological characteristics of OSCC

	ErbB3 expression		
	Negative	Positive	*p*-value*
Gender			
Male	21 (65.6%)	24 (64.9%)	0.947
Female	11 (34.4%)	13 (35.1%)	
Age			
≤ 55	12 (37.5%)	13 (35.1%)	0.839
> 55	20 (62.5%)	24 (64.9%)	
Primary site			
Oral tongue	19 (59.4%)	21 (56.8%)	0.968
Buccal mucosa	6 (18.8%)	7 (18.9%)	
Others	7 (21.9%)	9 (24.3%)	
T stage			
T1	10 (31.3%)	11 (29.7%)	0.596
T2	15 (46.9%)	13 (35.1%)	
T3	1 (3.1%)	2 (5.4%)	
T4	6 (18.8%)	11 (29.7%)	
TNM Staging			
I	9 (28.1%)	9 (24.3%)	0.302
II	6 (18.8%)	12 (32.4%)	
III	7 (21.9%)	3 (8.1%)	
IV	10 (31.3%)	13 (35.1%)	
Lymph node metastasis			
No	20 (62.5%)	23 (62.2%)	0.977
Yes	12 (37.5%)	14 (37.8%)	
Angiolymphatic invasion			
No	25 (78.1%)	24 (66.7%)	0.293
Yes	7 (21.9%)	12 (33.3%)	
Perineural infiltration			
No	24 (75.0%)	25 (69.4%)	0.610
Yes	8 (25.0%)	11 (30.6%)	
Extracapsular spread			
No	26 (96.3%)	27 (96.4%)	0.979
Yes	1 (3.7%)	1 (3.6%)	
Recurrence			
No	27 (84.4%)	23 (62.2%)	**0.039**
Yes	5 (15.6%)	14 (37.8%)	

* Pearson's chi-squared test

Table 7. Association between STAG2 expressions and clinicopathological characteristics of OSCC

	STAG2 expression		
	Negative	Positive	*p*-value*
Gender			
Male	18 (66.7%)	21 (63.6%)	0.807
Female	9 (33.3%)	12 (36.4%)	
Age			
≤ 55	12 (44.4%)	10 (30.3%)	0.258
> 55	15 (55.6%)	23 (69.7%)	
Primary site			
Oral tongue	17 (63.0%)	19 (57.6%)	**0.028**
Buccal mucosa	1 (3.7%)	9 (27.3%)	
Others	9 (33.3%)	5 (15.2%)	
T stage			
T1	8 (29.6%)	6 (18.2%)	0.257
T2	11 (40.7%)	17 (51.5%)	
T3	0	3 (9.1%)	
T4	8 (29.6%)	7 (21.2%)	
TNM Staging			
I	7 (25.9%)	5 (15.2%)	0.523
II	7 (25.9%)	11 (33.3%)	
III	3 (11.1%)	7 (21.2%)	
IV	10 (37.0%)	10 (30.3%)	
Lymph node metastasis			
No	17 (63.0%)	19 (57.6%)	0.672
Yes	10 (37.0%)	14 (42.4%)	
Angiolymphatic invasion			
No	21 (77.8%)	21 (63.6%)	0.234
Yes	6 (22.2%)	12 (36.4%)	
Perineural infiltration			
No	20 (74.1%)	22 (66.7%)	0.533
Yes	7 (25.9%)	11 (33.3%)	
Extracapsular spread			
No	23 (92.0%)	23 (100%)	0.166
Yes	2 (8.0%)	0	
Recurrence			
No	17 (63.0%)	26 (78.8%)	0.176
Yes	10 (37.0%)	7 (21.2%)	

* Pearson's chi-squared test

Table 8. Association between CD40L expressions and clinicopathological characteristics of OSCC

	CD40L expression		
	Negative	Positive	*p*-value*
Gender			
Male	18 (62.1%)	21 (67.7%)	0.645
Female	11 (37.9%)	10 (32.3%)	
Age			
≤ 55	16 (55.2%)	6 (19.4%)	**0.004**
> 55	13 (44.8%)	25 (80.6%)	
Primary site			
Oral tongue	20 (69.0%)	16 (51.6%)	0.228
Buccal mucosa	5 (17.2%)	5 (16.1%)	
Others	4 (13.8%)	10 (32.3%)	
T stage			
T1	7 (24.1%)	7 (22.6%)	0.842
T2	13 (44.8%)	15 (48.4%)	
T3	2 (6.9%)	1 (3.2%)	
T4	7 (24.1%)	8 (25.8%)	
TNM Staging			
I	6 (20.7%)	6 (19.4%)	0.629
II	9 (31.0%)	9 (29.0%)	
III	3 (10.3%)	7 (22.6%)	
IV	11 (37.9%)	9 (29.0%)	
Lymph node metastasis			
No	17 (58.6%)	19 (61.3%)	0.833
Yes	12 (41.4%)	12 (38.7%)	
Angiolymphatic invasion			
No	20 (69.0%)	22 (71.0%)	0.866
Yes	9 (31.0%)	9 (29.0%)	
Perineural infiltration			
No	25 (86.2%)	17 (55.8%)	**0.008**
Yes	4 (13.8%)	14 (45.2%)	
Extracapsular spread			
No	20 (95.2%)	26 (96.3%)	0.856
Yes	1 (4.8%)	1 (3.6%)	
Recurrence			
No	17 (58.6%)	26 (83.9%)	**0.030**
Yes	12 (41.4%)	5 (16.1%)	

* Pearson's chi-squared test

Table 9. Association between CD80 expressions and clinicopathological characteristics of OSCC

	CD80 expression		
	Negative	Positive	*p*-value*
Gender			
Male	15 (60.0%)	24 (68.6%)	0.493
Female	10 (40.0%)	11 (31.4%)	
Age			
≤ 55	12 (48.0%)	10 (28.6%)	0.124
> 55	13 (52.0%)	25 (71.4%)	
Primary site			
Oral tongue	16 (64.0%)	20 (57.1%)	0.060
Buccal mucosa	1 (4.0%)	9 (25.7%)	
Others	8 (32.0%)	6 (17.1%)	
T stage			
T1	6 (26.0%)	8 (22.9%)	0.934
T2	12 (48.0%)	16 (45.7%)	
T3	1 (4.0%)	2 (5.7%)	
T4	6 (24.0%)	9 (25.8%)	
TNM Staging			
I	4 (16.0%)	8 (22.9%)	0.614
II	7 (28.0%)	11 (31.4%)	
III	6 (24.0%)	4 (11.4%)	
IV	8 (32.0%)	12 (34.3%)	
Lymph node metastasis			
No	11 (44.0%)	25 (71.4%)	**0.033**
Yes	14 (56.0%)	10 (28.6%)	
Angiolymphatic invasion			
No	18 (72.0%)	24 (68.6%)	0.775
Yes	7 (28.0%)	11 (31.4%)	
Perineural infiltration			
No	17 (68.0%)	25 (71.4%)	0.775
Yes	8 (32.0%)	10 (28.6%)	
Extracapsular spread			
No	19 (100%)	27 (93.1%)	0.242
Yes	0	2 (6.9%)	
Recurrence			
No	16 (64.0%)	27 (77.1%)	0.265
Yes	9 (36.0%)	8 (22.9%)	

* Pearson's chi-squared test

Table 10. Association between PTPRO expressions and clinicopathological characteristics of OSCC

	PTPRO expression		
	Negative	Positive	*p*-value*
Gender			
Male	13 (61.9%)	26 (66.7%)	0.712
Female	8 (38.1%)	13 (33.3%)	
Age			
≤ 55	9 (42.9%)	13 (33.3%)	0.465
> 55	12 (57.1%)	26 (66.7%)	
Primary site			
Oral tongue	13 (61.9%)	23 (59.0%)	0.125
Buccal mucosa	1 (4.8%)	9 (23.1%)	
Others	7 (33.3%)	7 (17.9%)	
T stage			
T1	6 (28.6%)	8 (20.5%)	0.555
T2	11 (52.4%)	17 (43.6%)	
T3	0	3 (7.7%)	
T4	4 (19.0%)	11 (28.2%)	
TNM Staging			
I	4 (19.0%)	8 (20.5%)	0.300
II	6 (28.6%)	12 (30.8%)	
III	6 (28.6%)	4 (10.3%)	
IV	5 (23.8%)	15 (38.5%)	
Lymph node metastasis			
No	11 (52.4%)	25 (64.1%)	0.377
Yes	10 (47.6%)	14 (35.9%)	
Angiolymphatic invasion			
No	16 (76.2%)	26 (66.7%)	0.443
Yes	5 (23.8%)	13 (33.3%)	
Perineural infiltration			
No	15 (71.4%)	27 (69.2%)	0.859
Yes	6 (28.6%)	12 (30.8%)	
Extracapsular spread			
No	16 (94.1%)	30 (96.8%)	0.660
Yes	1 (5.9%)	1 (3.2%)	
Recurrence			
No	16 (76.2%)	27 (69.2%)	0.568
Yes	5 (23.8%)	12 (30.8%)	

* Pearson's chi-squared test

Table 11. Relapse free survival for OSCC in primary tumor tissue array

	N	Relapse free survival rate (3 years)	*p*-value*
Total	69	72.9%	
TNM staging			
I/II/III	46	87.0%	
IV	23	42.1%	**<0.001**
FAS expression			
Negative	34	84.9%	
Positive	35	60.9%	**0.030**
HIF1-alpha expression			
Negative	34	81.6%	
Positive	35	65.4%	0.153
Bcl2A expression			
Negative	33	83.5%	
Positive	36	63.9%	0.112
MST4 expression			
Negative	29	71.4%	
Positive	40	74.1%	0.670
ErbB3 expression			
Negative	32	87.5%	
Positive	37	59.4%	**0.018**

*Univariate log rank test

Table 12. Relapse free survival for OSCC in lymph node tissue array

	N	Relapse free survival rate (3 years)	*p*-value*
Total	60	72.2%	
STAG2 expression			
Negative	27	66.7%	
Positive	33	77.0%	0.268
CD40L expression			
Negative	29	57.2%	
Positive	31	86.5%	**0.029**
CD80 expression			
Negative	25	62.9%	
Positive	35	79.0%	0.271
PTPRO expression			
Negative	21	81.0%	
Positive	39	67.1%	0.430

*Univariate log rank test

Table 13. Multivariate analysis of relapse free survival for OSCC

	p-value*	HR	95% CI
TNM stage IV	**<0.001**	**6.990**	**2.361 – 20.70**
FAS	0.082	1.005	0.999 – 1.011
HIF1-alpha	**0.006**	**1.020**	**1.006 – 1.034**
Bcl2A	0.936	1.000	0.993 – 1.006
ErbB3	0.621	1.003	0.991 – 1.015
CD40L	**0.020**	**0.978**	**0.960 – 0.996**

*Multivariate Cox regression

Table 14. Disease free survival for OSCC in primary tumor tissue array

	N	Disease free survival rate (3 years)	*p*-value*
Total	69	75.9%	
TNM staging			
I/II/III	46	91.3%	
IV	23	42.1%	**<0.001**
FAS expression			
Negative	34	84.9%	
Positive	35	66.9%	**0.050**
HIF1-alpha expression			
Negative	34	84.8%	
Positive	35	67.9%	0.186
Bcl2A expression			
Negative	33	86.8%	
Positive	36	66.4%	**0.049**
MST4 expression			
Negative	29	78.4%	
Positive	40	74.1%	0.647
ErbB3 expression			
Negative	32	90.5%	
Positive	37	62.4%	**0.006**

*Univariate log rank test

Table 15. Disease free survival for OSCC in lymph node tissue array

	N	Disease free survival rate (3 years)	*p*-value*
Total	60	75.6%	
STAG2 expression			
Negative	27	70.0%	
Positive	33	80.4%	0.374
CD40L expression			
Negative	29	60.8%	
Positive	31	89.7%	**0.011**
CD80 expression			
Negative	25	70.8%	
Positive	35	79.0%	0.485
PTPRO expression			
Negative	21	81.0%	
Positive	39	72.4%	0.479

*Univariate log rank test

Table 16. Multivariate analysis of disease free survival for OSCC

	p-value*	HR	95% CI (lower – upper)
TNM stage IV	**<0.001**	**12.381**	**3.437 – 44.60**
FAS	0.084	1.006	0.999 – 1.012
HIF1-alpha	**0.007**	**1.024**	**1.007 – 1.042**
Bcl2A	0.973	1.000	0.993 – 1.007
ErbB3	0.578	1.004	0.999 – 1.018
CD40L	**0.022**	**0.973**	**0.950 – 0.996**

*Multivariate Cox regression

Table 17. Overall survival for OSCC in primary tumor tissue array

	N	Overall survival rate (3 years)	*p*-value*
Total	69	71.3%	
TNM staging			
I/II/III	46	85.9%	
IV	23	41.6%	**<0.001**
FAS expression			
Negative	34	80.9%	
Positive	35	61.9%	**0.007**
HIF1-alpha expression			
Negative	34	79.1%	
Positive	35	63.9%	0.148
Bcl2A expression			
Negative	33	84.2%	
Positive	36	60.4%	**0.035**
MST4 expression			
Negative	29	74.7%	
Positive	40	68.7%	0.337
ErbB3 expression			
Negative	32	90.5%	
Positive	37	54.4%	**0.003**

*Univariate log rank test

Table 18. Overall survival for OSCC in lymph node tissue array

	N	Overall survival rate (3 years)	*p*-value*
Total	60	70.4%	
STAG2 expression			
Negative	27	73.9%	
Positive	33	67.4%	0.549
CD40L expression			
Negative	29	61.5%	
Positive	31	79.1%	0.184
CD80 expression			
Negative	25	71.8%	
Positive	35	69.3%	0.734
PTPRO expression			
Negative	21	85.7%	
Positive	39	61.9%	0.181

*Univariate log rank test

Table 19. Multivariate analysis of overall survival for OSCC

	p-value*	HR	95% CI (lower – upper)
TNM stage IV	**0.002**	**5.101**	**1.857 – 14.01**
FAS	0.157	1.004	0.998 – 1.010
HIF1-alpha	**0.017**	**1.014**	**1.003 – 1.026**
Bcl2A	0.699	1.001	0.996 – 1.007
ErbB3	0.905	1.001	0.991 – 1.011
CD40L	**0.034**	**0.984**	**0.969 – 0.999**
PTPRO	0.244	1.005	0.997 – 1.014

*Multivariate Cox regression

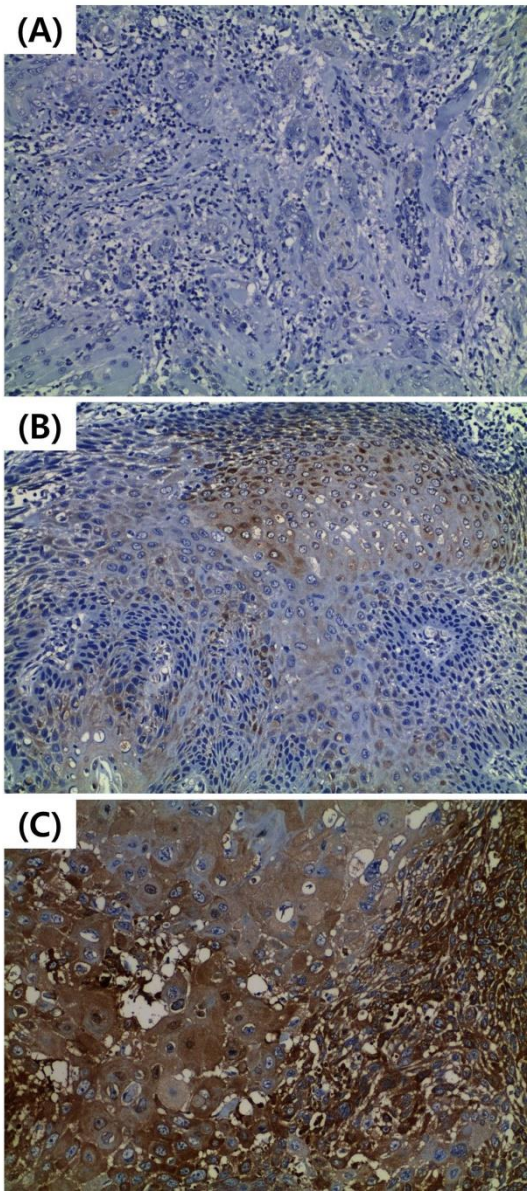


Figure 1. The staining extent of FAS immunohistochemistry in the primary tumors (x200 magnification). A. H score 0 (100% of no staining) B. H score 60 (50% of no staining, 40% of weak staining, and 10% of moderate staining) C. H score 240 (20% of weak staining, 20% of moderate staining, and 60% of strong staining)

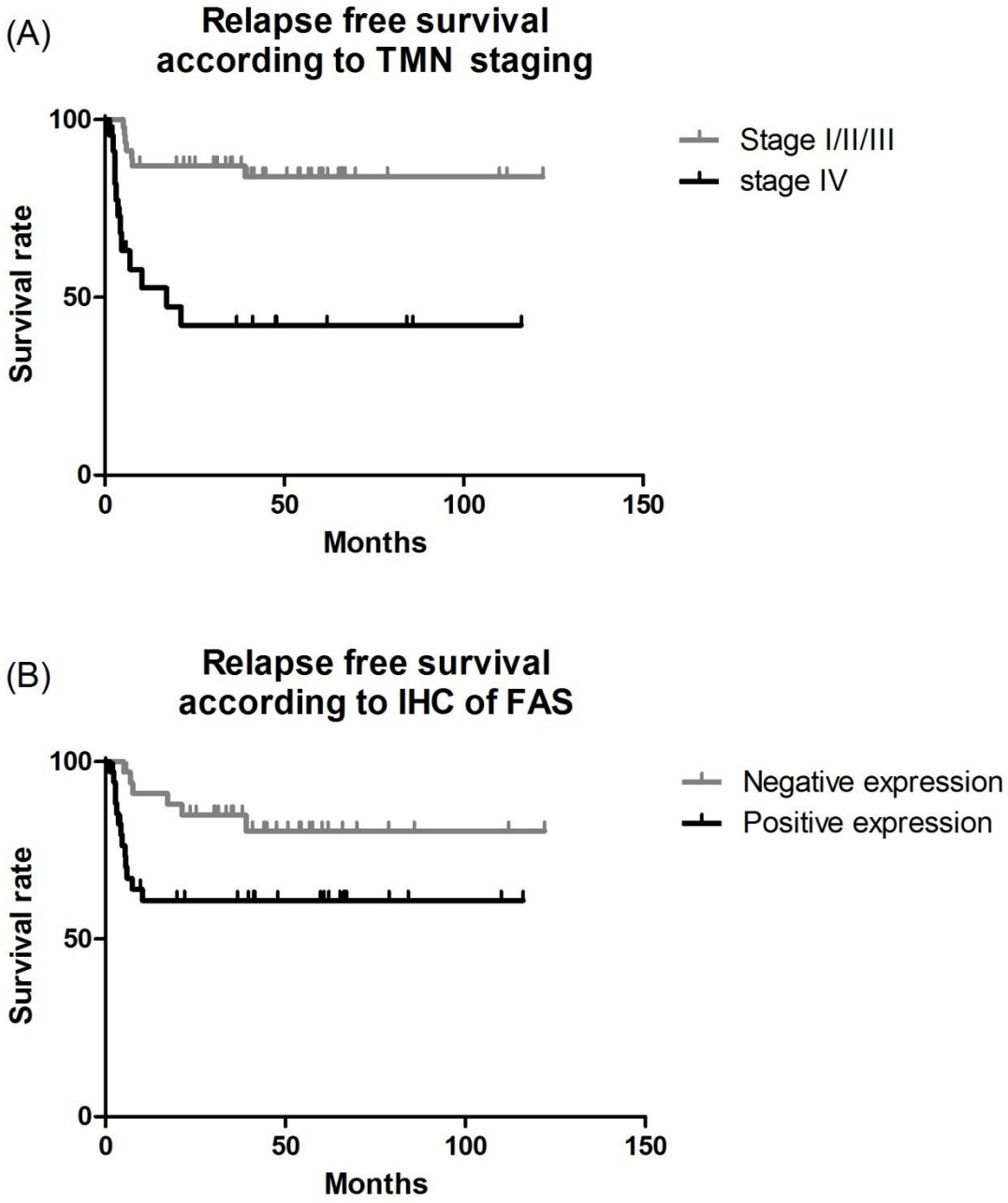


Figure 2. Relapse free survival curves. A. TMN staging. B. FAS. C. HIF1-alpha. D. Bcl2A. E. MST4. F. ErbB3. G. STAG2. H. CD40L. I. CD80. J. PTPRO

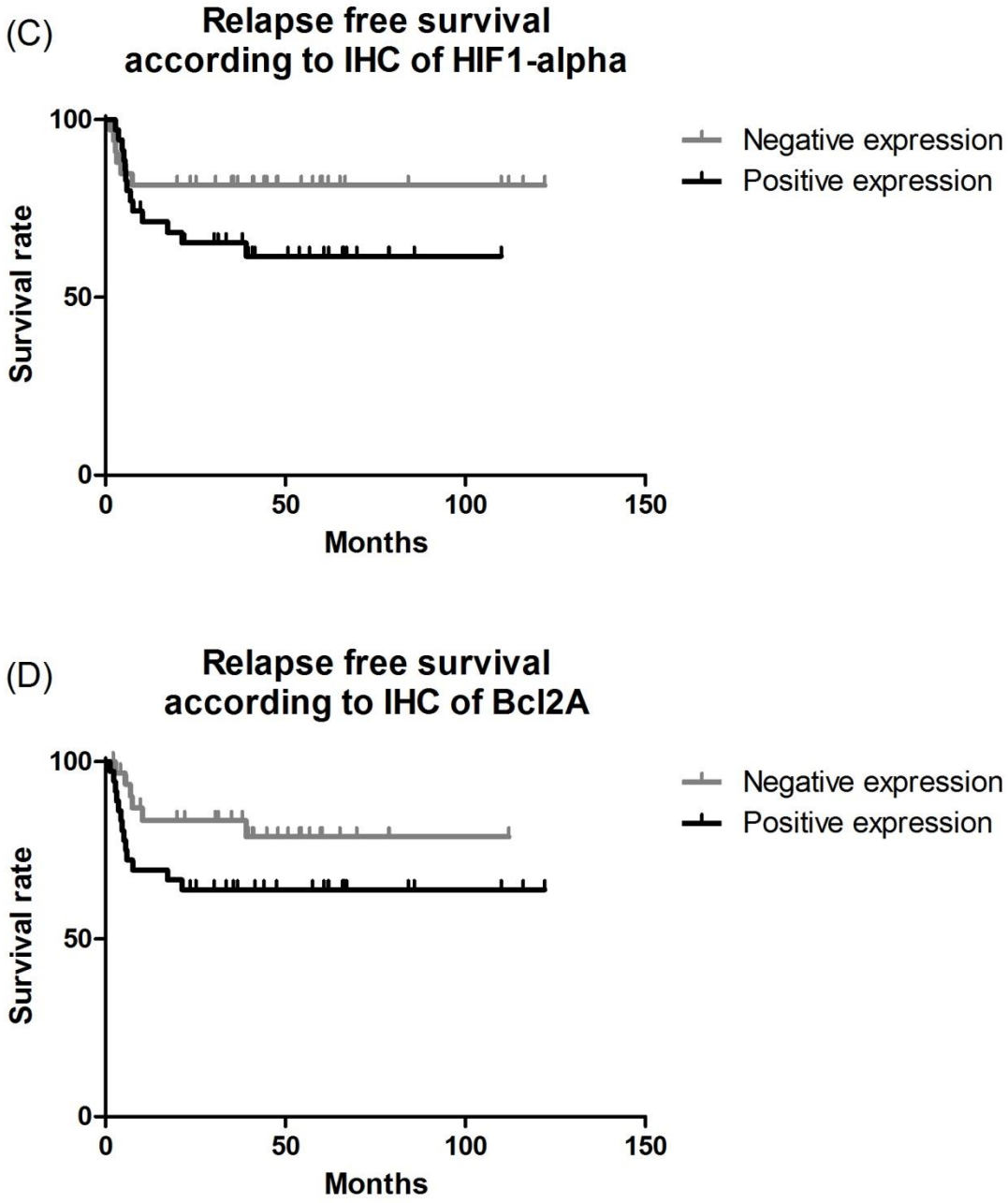
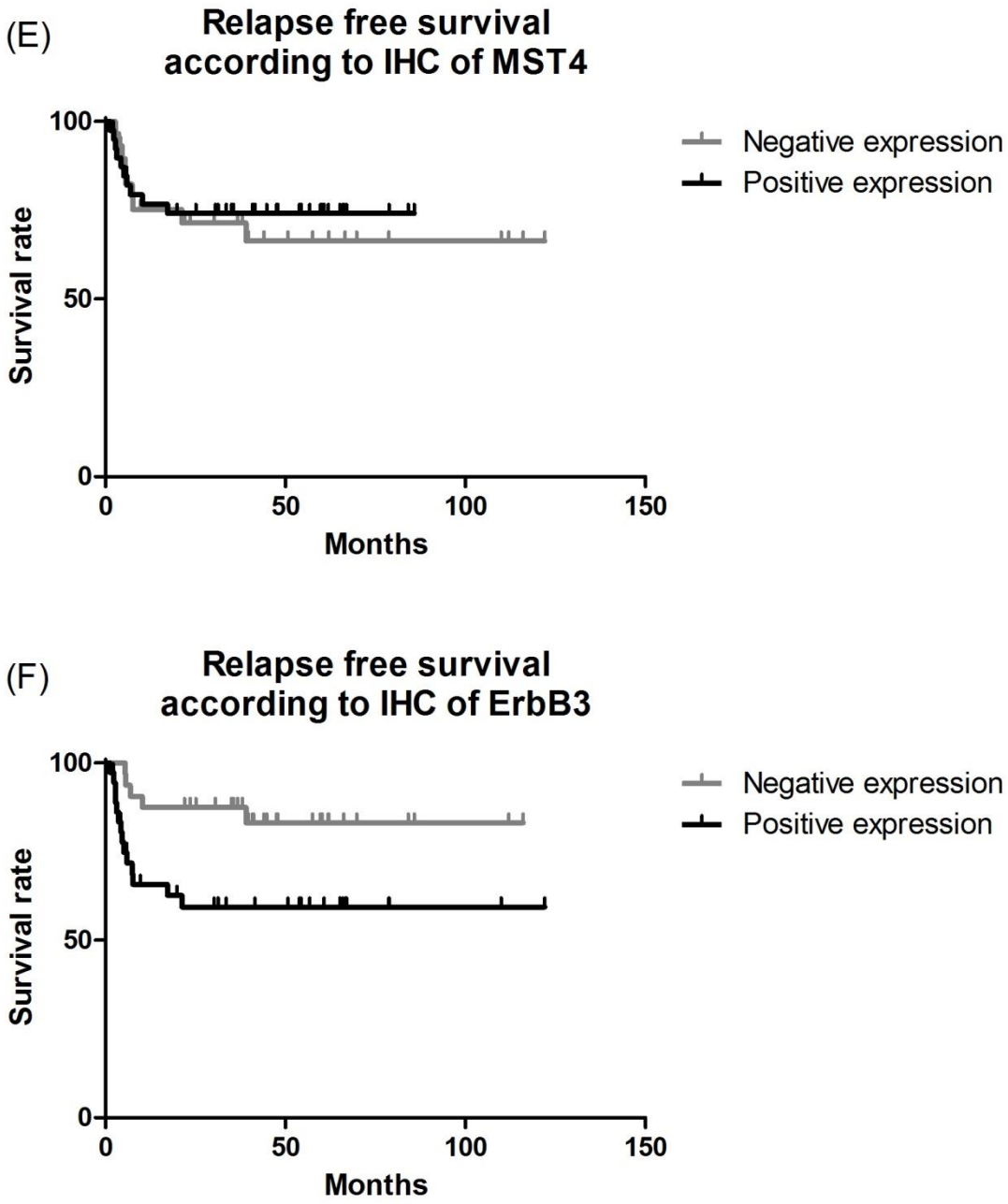


Figure 2. Relapse free survival curves. A. TMN staging. B. FAS. C. HIF1-alpha. D. Bcl2A. E. MST4. F. ErbB3. G. STAG2. H. CD40L. I. CD80. J. PTPRO



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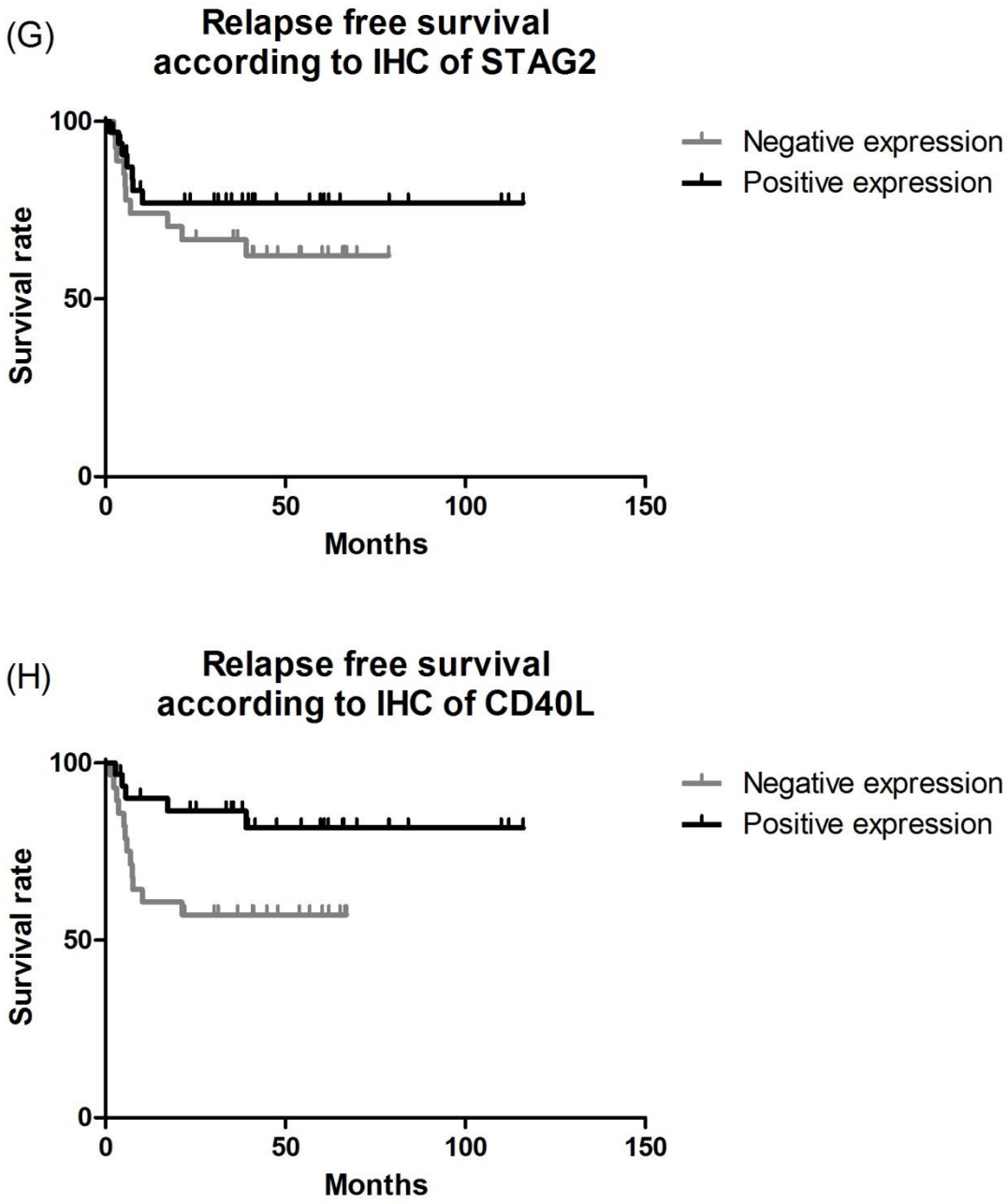

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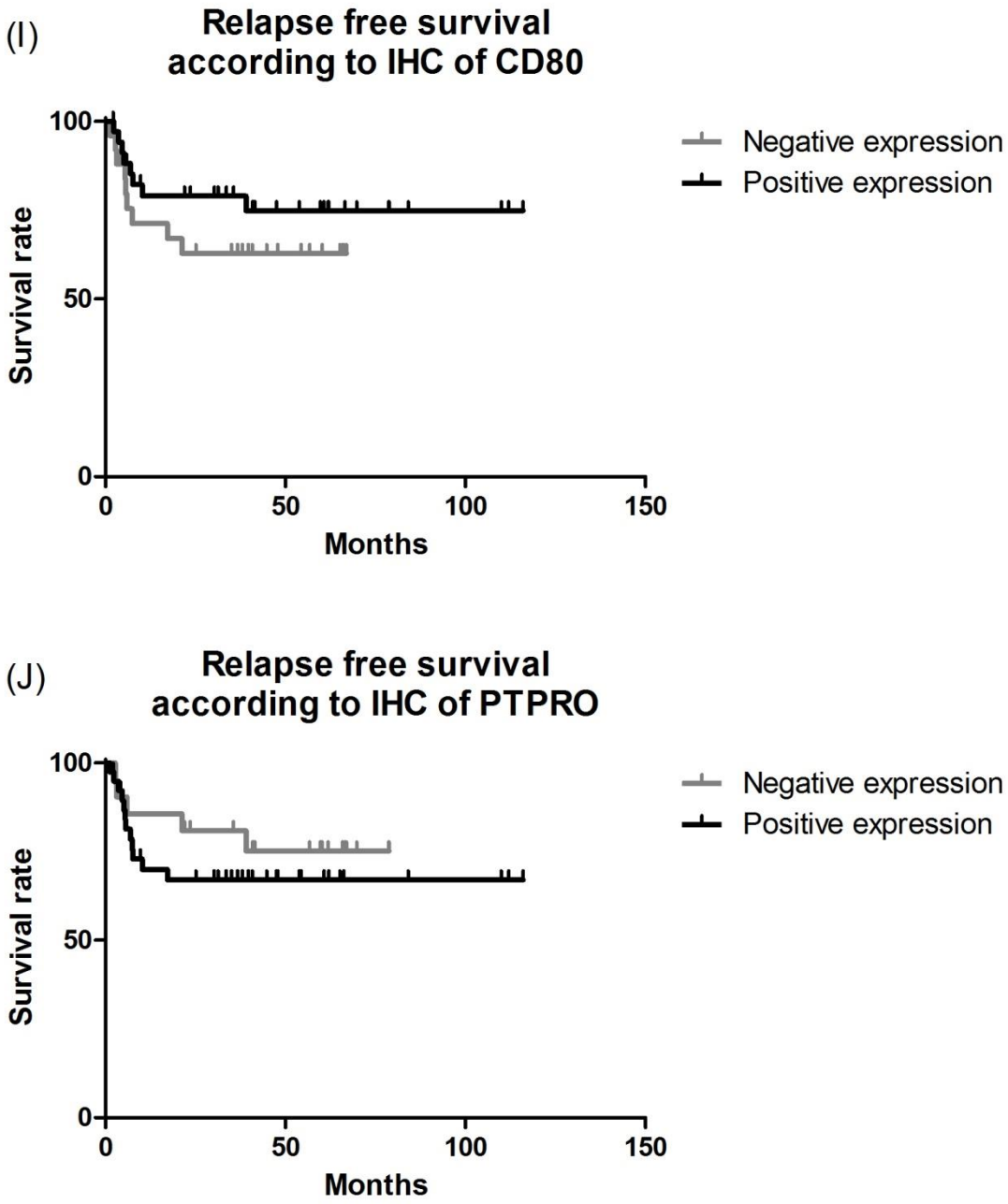

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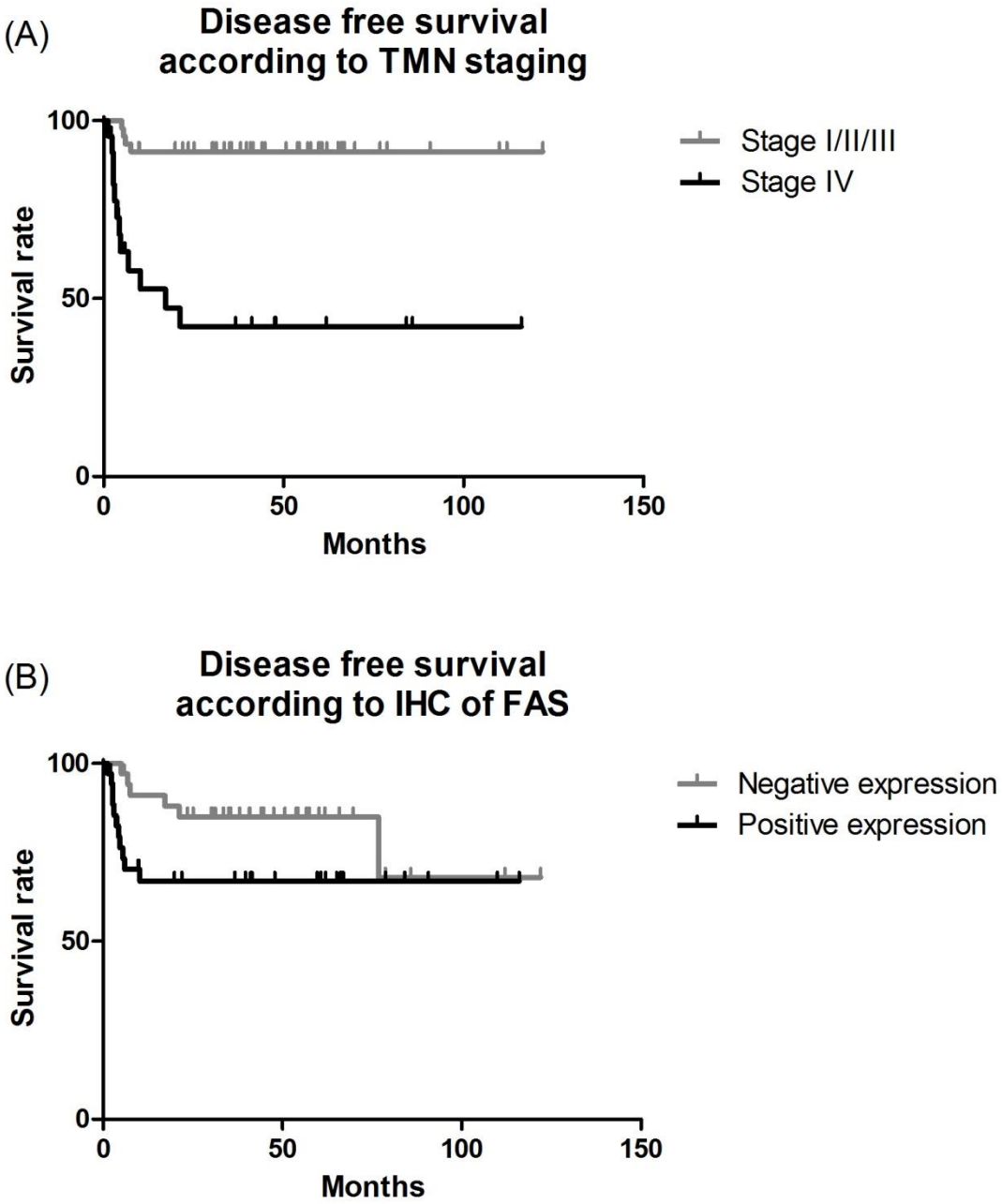

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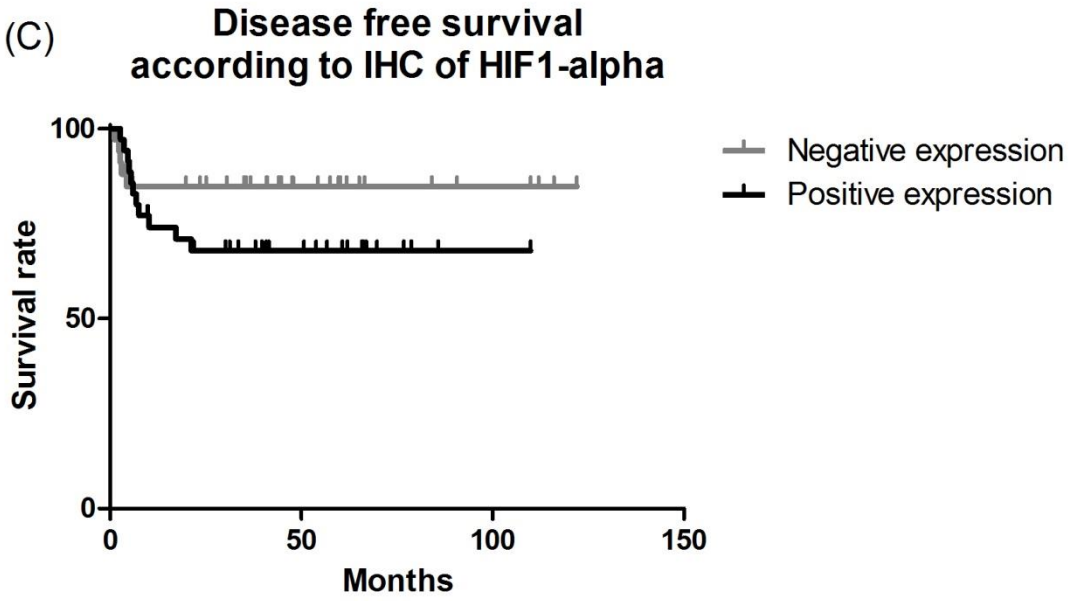

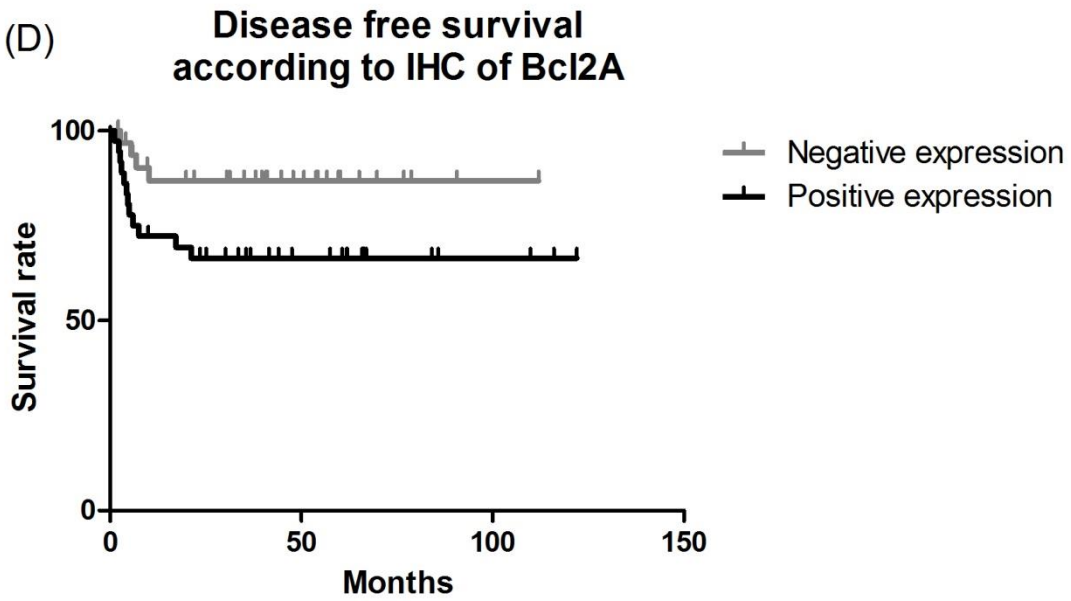

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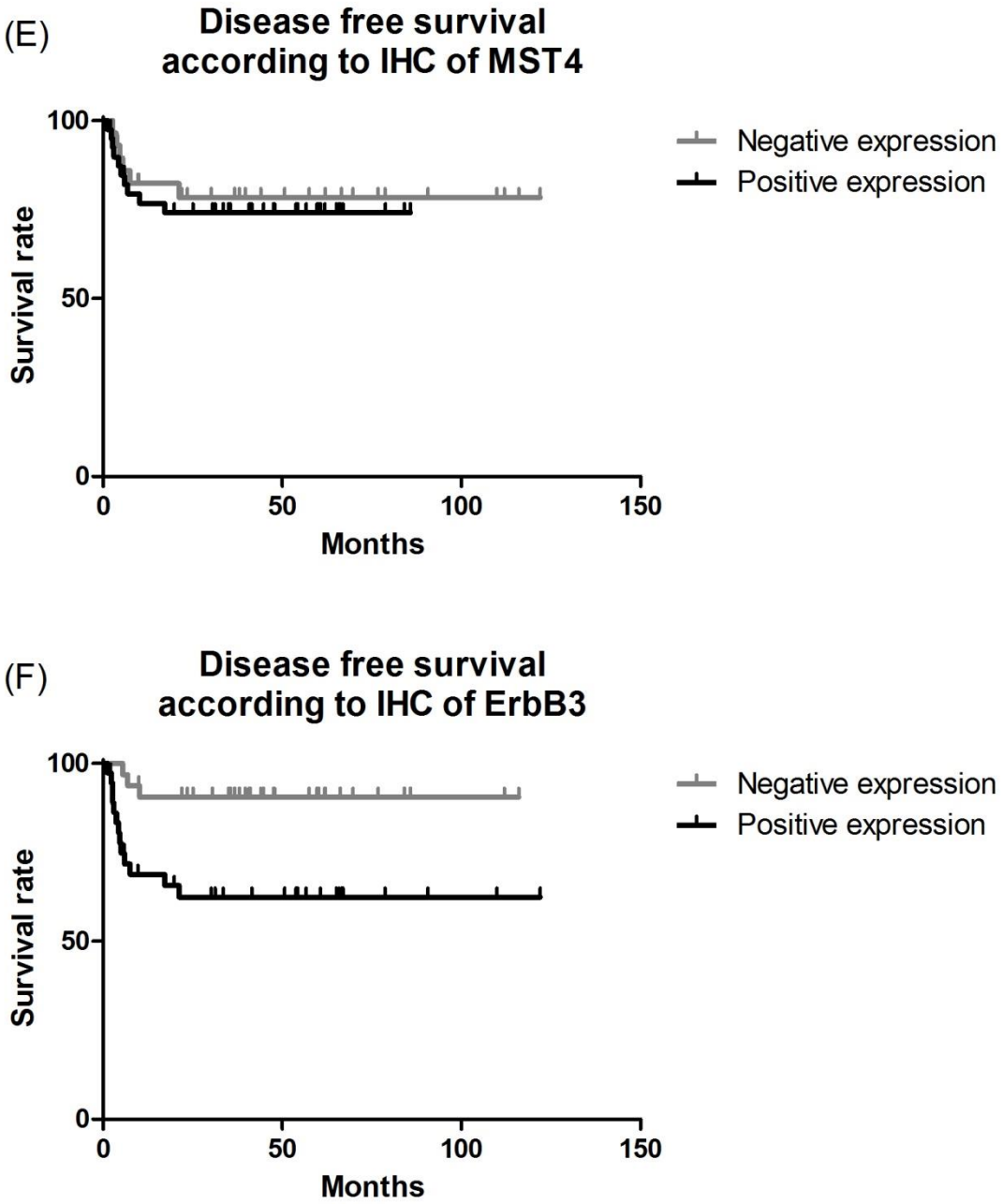

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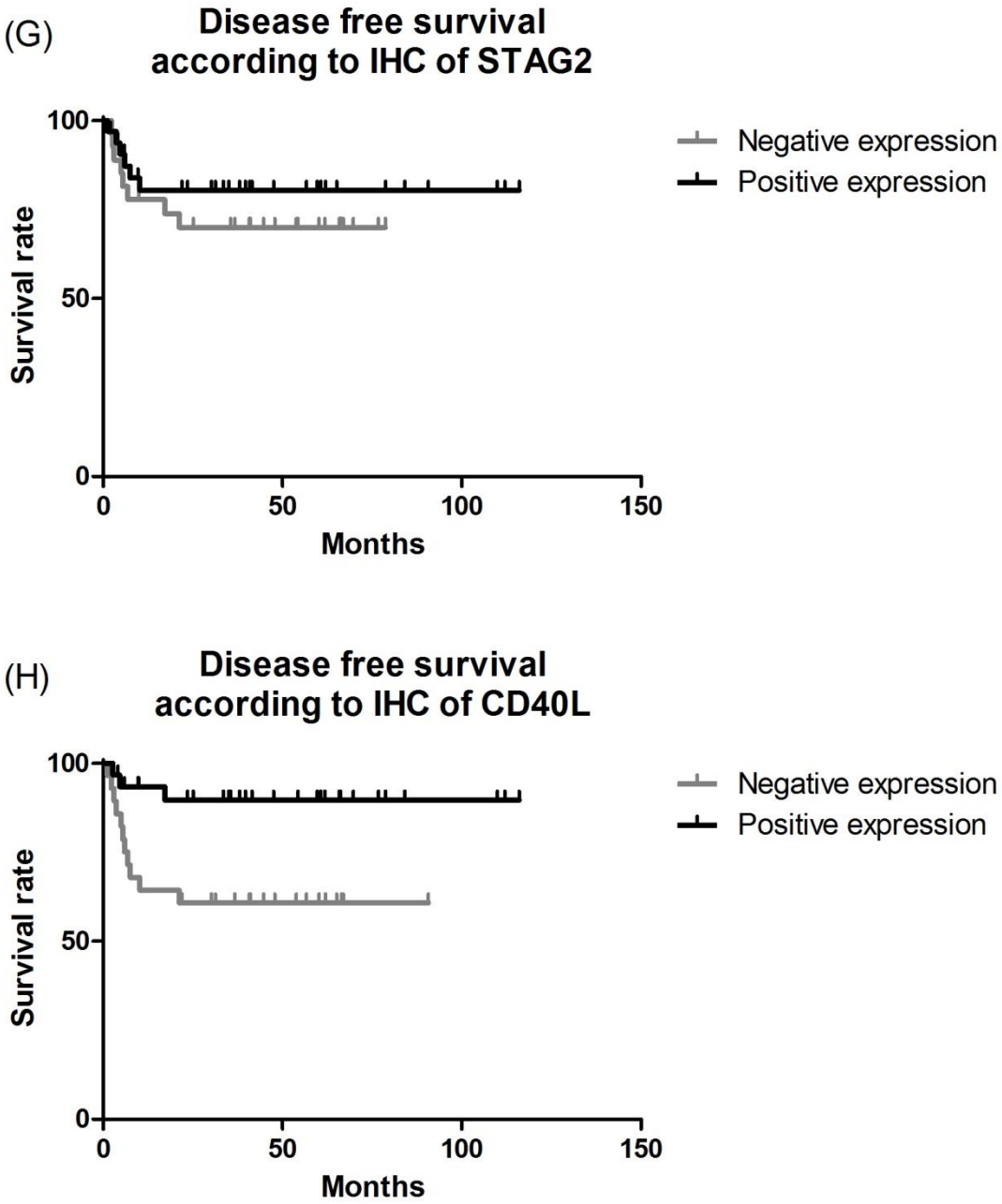

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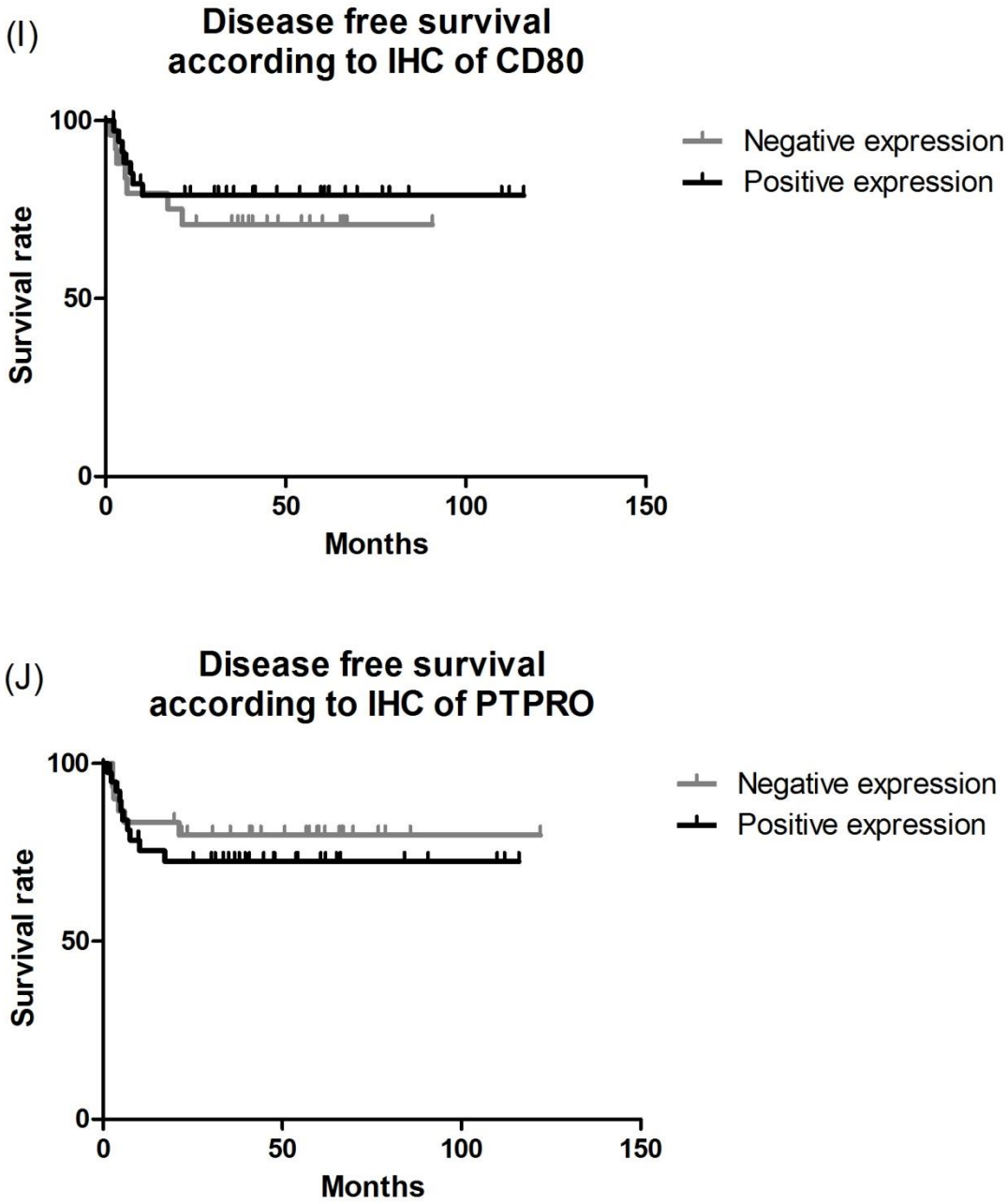

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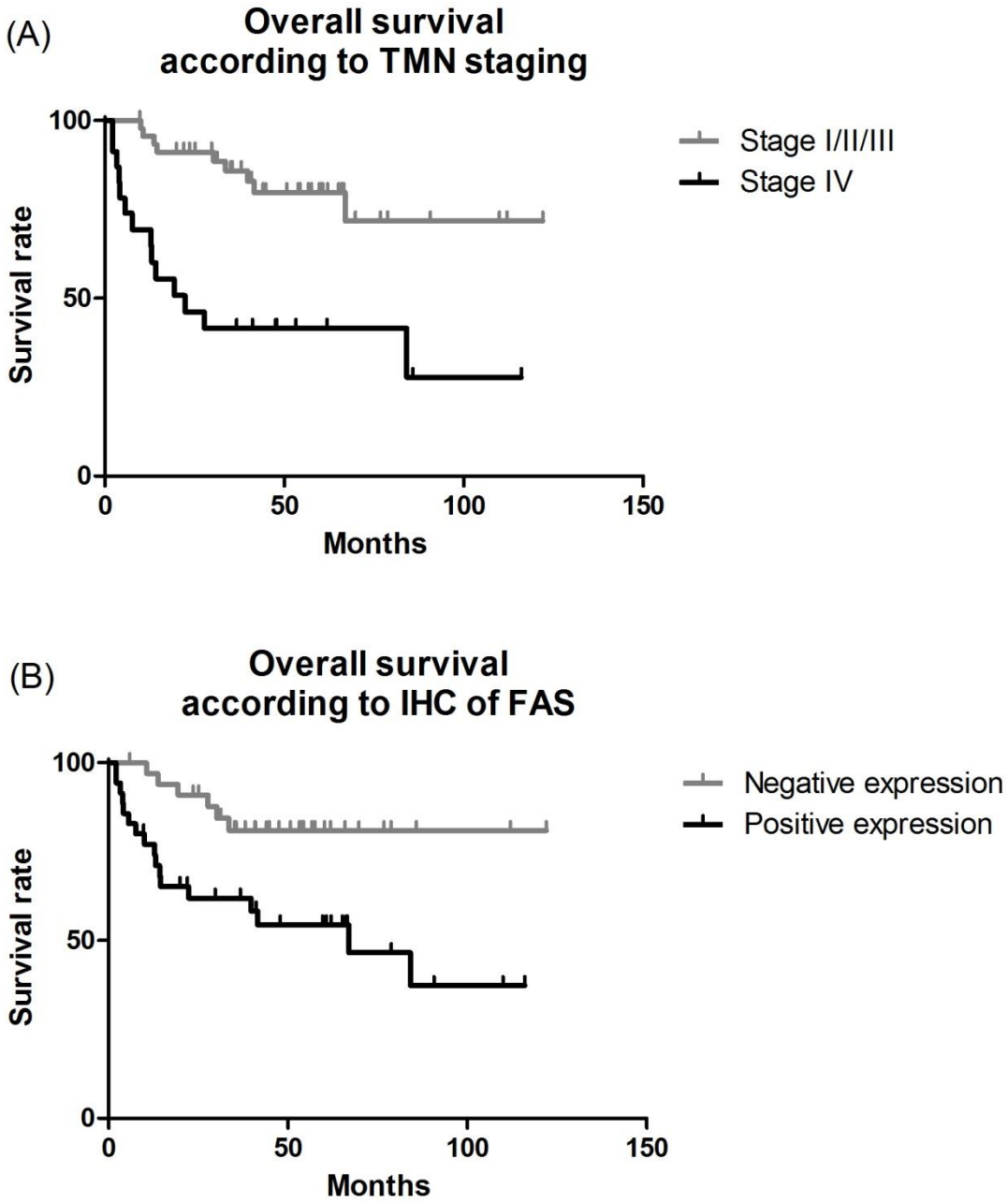
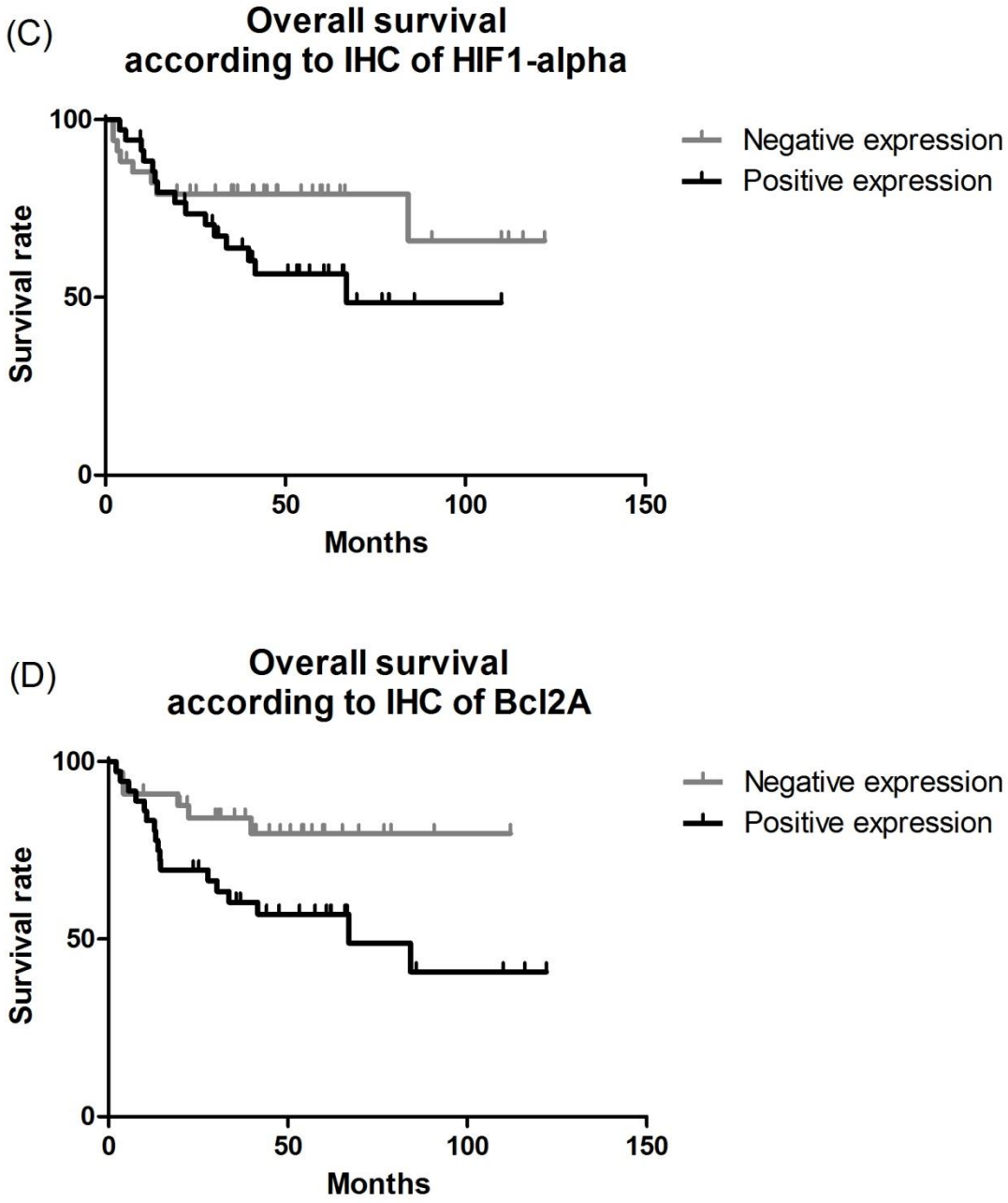


Figure 4. Overall survival curves. A. TMN staging. B. FAS. C. HIF1-alpha. D. Bcl2A. E. MST4. F. ErbB3. G. STAG2. H. CD40L. I. CD80. J. PTPRO



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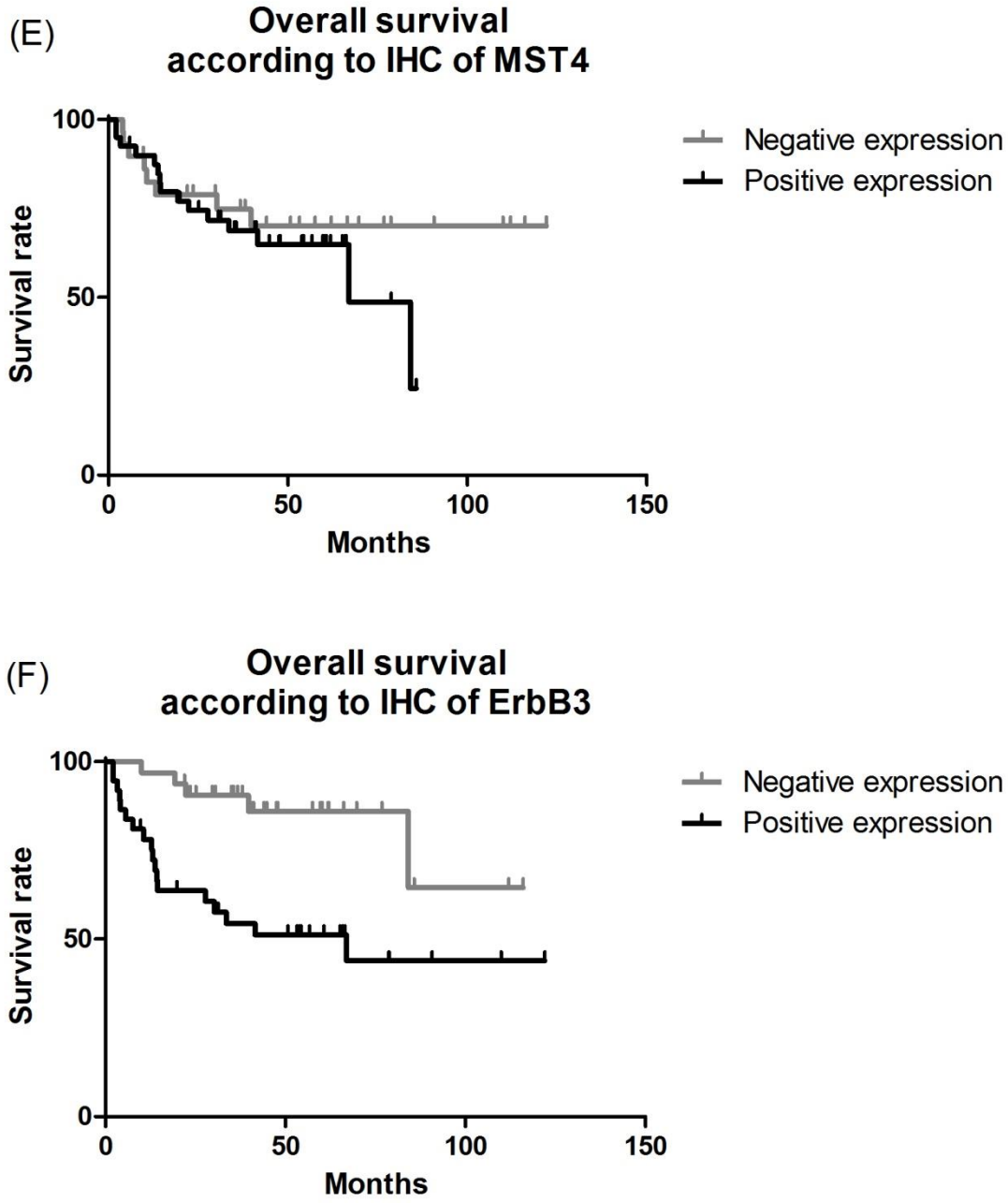

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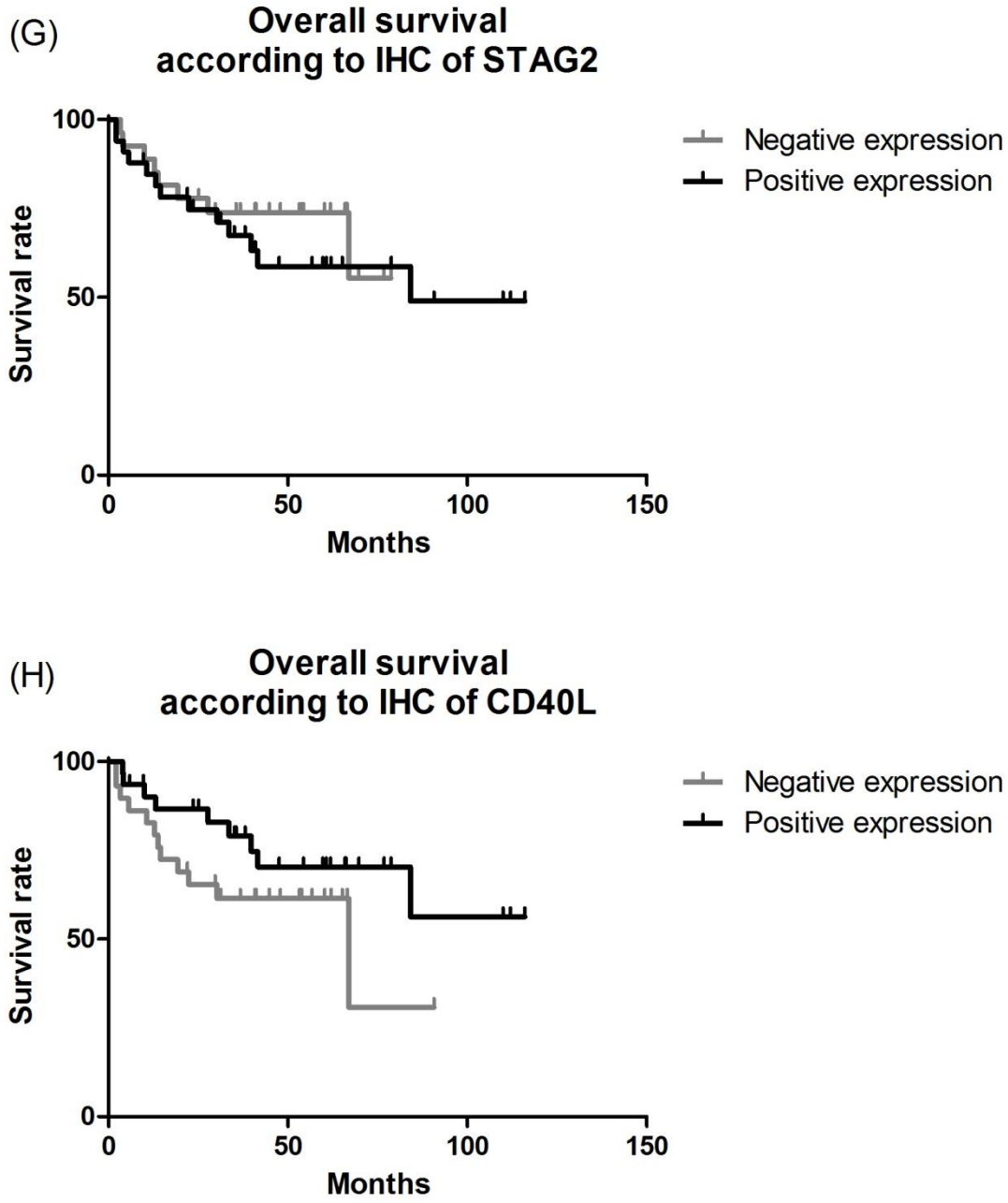

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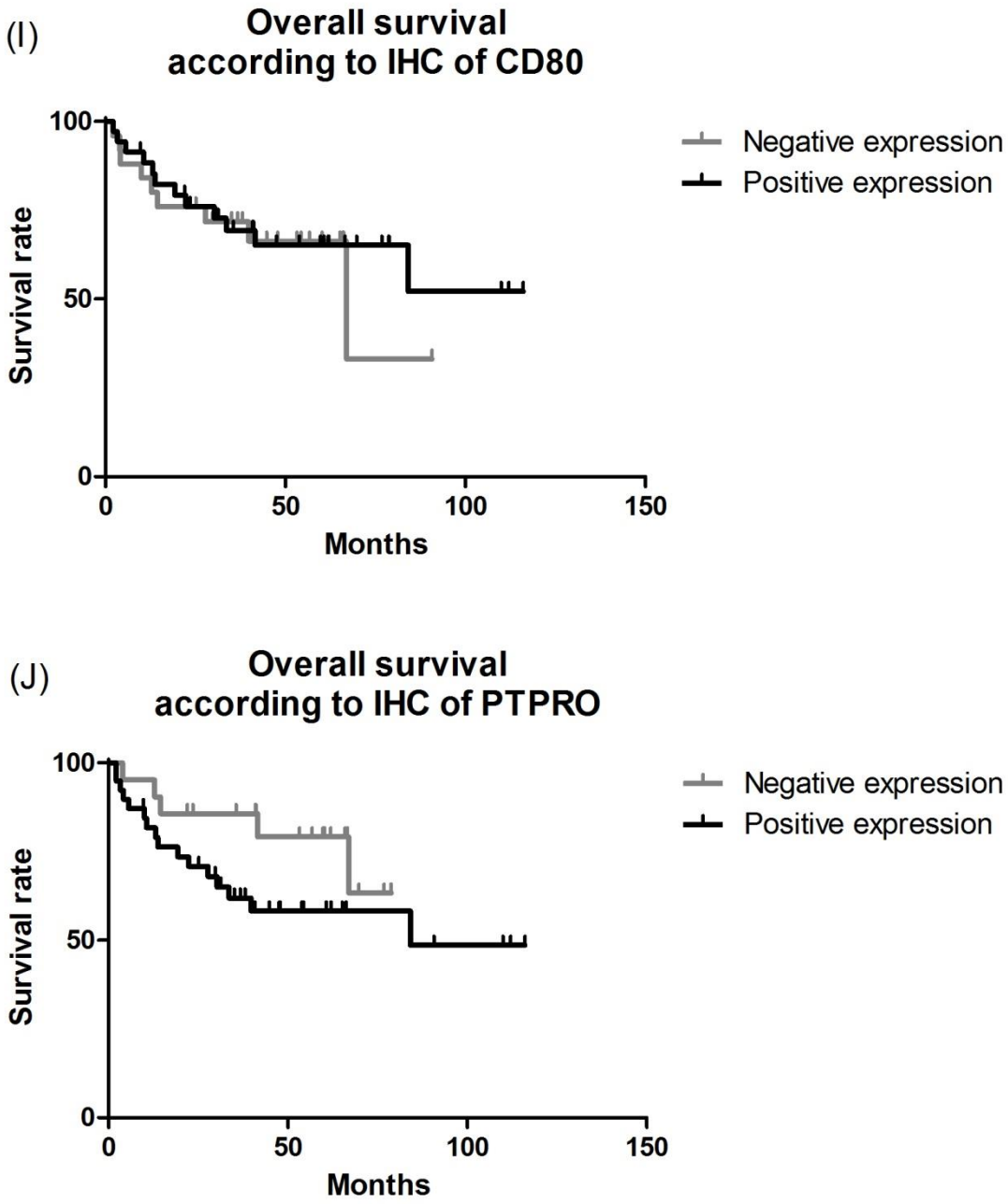

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요약(국문초록)

서론: 구강 상피세포암은 세계적으로 발병률이 높은 악성종양 중 하나이다. 악성종양의 치료법이 발달했음에도 불구하고 지난 수 세기 동안 구강 상피세포암의 5년 생존율은 50% 수준을 유지하고 있다. 종양에 대한 TNM 분류법이 예후예측에 주로 사용되고 있으나 이 분류법은 종양이 가지는 생물학적 특성이나 숙주의 종양 방어 면역 작용을 반영하지 못한다. mRNA Microarray를 사용한 종양세포의 생물학적 특징과 숙주 방어 면역 체계에 대한 이전 연구에서 예후와 관련이 깊을 것으로 보이는 9가지 생체지표를 찾았다. 그 중 5가지는 종양세포에서 발현되는 유전자이고 나머지 4개는 면역체계에서 발현되는 유전자이다. 본 연구에서는 면역조직화학염색법을 통해 생체지표의 예후 예측 정도를 확인해보고자 한다.

방법: 2003 년부터 2011 년까지 수술을 받은 모든 구강 상피세포암 환자를 대상으로 하였다. 총 69 명의 종양조직과 60 명의 종양조직이 침범이 없는 정상 경부임파선으로 Tissue Microarray 를 구축하였다. 9 개의 생체지표 중 FAS, HIF1-alpha, Bcl2A, MST4, ErbB3 는 종양세포에 염색을 시행하였으며, STAG2, CD40L, CD80, PTPRO 는 임파선에 염색을 시행하였다. 면역조직화학염색은 염색의 범위와 강도를 고려한 반정량적 점수계산법(H 점수)에 따라 등급을 나누었다. 면역조직화학염색 결과를 바탕으로 생체지표들을 임

상병리학적 소견과 임상결과로 무재발, 무질병, 전체 생존율을 비교해 분석하였다.

결과: 총 69 개의 케이스 중, 혀가 구강 상피세포암의 가장 다빈도 발생한 부위였다. 종양의 TNM 분류 중에서는 제 4 기(33.3%)가 가장 흔했으며 그 다음으로는 제 1 기(26.1%), 제 2 기(26.1%), 제 3 기(14.5%)가 있었다. 성공적인 종양절제에도 불구하고 수술 후 종양 재발률은 27.5%였다. TNM 제 4 기가 나쁜 생존률의 높은 위험요인으로 나타났다 ($p < 0.001$). 9 가지의 생체지표들 중에서 FAS 만이 TNM 분류와 강한 양의 상관관계를 나타내었다 ($p = 0.019$). 종양세포에서 FAS, HIF1-alpha, ErbB3 의 양성발현이 종양의 재발과 유의성을 보였다 ($p = 0.070$, $p = 0.070$, $p = 0.039$). 임파선에서 CD40L 의 음성발현은 종양의 재발과 유의한 관계를 보였다 ($p = 0.030$). 다변수 Cox 분석결과 종양세포에서 TNM 4 기, HIF1-alpha 의 H 점수 증가와 임파선에서 CD40L 의 H 점수 감소가 각각 독립적으로 무재발생존률에서 나쁜 예후를 보였다($p < 0.001$, HR=6.990, 95% CI 2.361-20.70 for TNM 4 기, $p = 0.006$, HR=1.020, 95% CI 1.006-1.034 for HIF1-alpha; $p = 0.020$, HR=0.978, 95% CI 0.960-0.996 for CD40L). 전체생존률에서는 HIF1-alpha 의 H 점수 증가, CD40L 의 H 점수 감소가 TNM 분류와 각각 독립적으로 나쁜 예후인자들로 나타났다. ($p =$

0.008, HR=1.024, 95% CI 1.007−1.042 for HIF1−alpha; p = 0.022, HR=0.973, 95% CI 0.950−7.996 for CD40L)

결론: 본 연구에서는 종양세포와 종양에 대한 숙주 방어 면역에서 각각 HIF1−alpha와 CD40L의 발현 정도가 구강 상피세포암의 수술 후 예후를 예측하는 생체지표로 사용될 수 있음을 확인하였다.

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주요어 : 구강, 상피세포암, 예후, 조직면역화학염색, 조직어레이

학 번 : 2011−23747