의학석사 학위논문

Expression of ERCC1, RRM1 and class III beta-tubulin in head and neck cancer and its clinical significance

두경부암에서 ERCC1, RRM1, class III beta-tubulin의 발현 및 임상적 의의

2013년 2월

서울대학교 대학원

의학과 이비인후과학 전공

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A thesis of the Master's degree

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Feb. 2013

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이 논문을 의학석사 학위논문으로 제출함

2012년 12월

서울대학교 대학원

의학과 이비인후과학 전공

김 희 진

김희진의 의학석사 학위논문을 인준함

2012년 12월

위 원 장	(인)
부위원장	(인)
위 원	(인)

Studies on roles of expression of ERCC1, RRM1 and class III beta-tubulin in head and neck cancer and its clinical significance

by
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A thesis submitted to the Department of Otorhinolaryngology in partial fulfillment of the requirement of the Degree of Master of Science in Otorhinolaryngology Head and Neck Surgery at the Seoul National University College of Medicine

Dec 2012

Approved by Thesis Committee:

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Abstract

Expression of ERCC1, RRM1 and class III beta-tubulin in head and neck cancer and its clinical significance

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Introduction: Management of head and neck cancer (HNCa) is very complicated, as surgery is limited by the anatomical extent of tumor and the desire to preserve essential organs. To achieve the optimal effects, additional therapeutic options based on molecular tumor profiling have been increasingly tried in HNCa. This study explored biomarker expression profiles of HNCa with the goal of developing therapeutic strategies.

Methods: Eighty three patients consisting of 74 cases of squamous cell carcinoma (SCC) and nine cases of undifferentiated carcinoma (UDC) were enrolled. Expression of three selected biomarkers – excision repair cross-complementation group 1 (ERCC1), class III ß-tubulin (TUBB3), and ribonucleotide reductase M1 (RRM1) – previously reported to be indicators of therapeutic responses in non-small cell lung cancer was investigated by immunohistochemistry (IHC) Human papilloma virus (HPV) status was examined by IHC for p16, in situ hybridization (ISH), and DNA chip analysis. Epstein-Barr virus (EBV) was detected by ISH.

Results: HPV was detected in 27 of 74 SCC cases, most frequently in the oropharynx (15 of 27 cases). EBV was detected in 11 cases including all nine UDC cases. Expression of the three biomarkers was relatively high throughout the primary location of HNCa, except for laryngeal SCC, which displayed low expression of ERCC1. High expression of ERCC1 was found in 64 of 70 cases of SCC (91.4 %), RRM1 in 56 of 70 cases of SCC (80.0%), and TUBB3 in 37 of 71 cases of SCC

(52.1%). Expression of TUBB3 was associated with histologic types. None of the nine cases of UDC expressed high levels of TUBB3 (*p=.001*). Also, high expression of TUBB3 was an independent predictor of better overall survival in SCC (*p=.020*). Additionally, positivity of p16 was related to longer disease free survival and overall survival.

Conclusions: Unlike lung carcinoma, expression of ERCC1 or RRM1 did not correlate with clinical parameters or HPV status. However, p16 and TUBB3 might be useful predictive biomarkers in HNCa.

Key words: Carcinoma, squamous cell of the head and neck • Alphapapillomavirus • ERCC1 protein, human • beta III-tubulin, protein, human • Tumor Markers, Biological

Student Number: 2011-21832

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LIST OF ABBREVIATIONS

HNCa: Head and neck cancer

HPV: Human papilloma virus

EBV: Epstein-Barr virus

TUBB3: class III beta-tubulin

CCRT: Concurrent chemoradiotherapy

DFS: Disease-free survival

OS: overall survival

CR: complete response

PR: partial response

SD: stable disease

PD: progressive disease

TMA: Tissue microarray blocks

IHC: Immune-histochemistry

INTRODUCTION

Head and neck cancer (HNCa) accounts for 3% of all cancers in the United States. Almost 50,000 new cases were diagnosed in the US in 2010[1]. In Korea, the incidence of HNCa is about 2% of all cancers, and it was ranked in the top 10 leading cancers among men in 2008[2]. The prognosis of HNCa is unpredictable, but is generally poor because over 50% of patients present with advanced disease status with regional lymph node or distant metastasis at the time of diagnosis.

Although surgery is the standard treatment for HNCa, this approach is often limited by the anatomical extent of the tumor and the desire to preserve essential organ function or external features. Therefore, radiotherapy and/or chemotherapy have been considered as alternatives to surgery in advanced HNCa. The need for additional therapeutic options like targeted therapy based on molecular tumor profiling is growing. Profiling also increases the understanding of HNCa and may provide new insights concerning therapy.

The molecular pathogenesis of HNCa is unclear. Because HNCa consists of a heterogeneous group of diseases occurring from the oral cavity to larynx, the characteristics of HNCa differ markedly regarding attributes like etiology, histology, growth rate, and aggressiveness[3,4]. For example, Human papilloma virus (HPV) and Epstein-Barr virus (EBV) are considered as causal factors and also important predictive biomarkers in a subset of HNCa[5]. p16 is often advocated as a surrogate marker of HPV based on the findings that HPV integration with transcription of viral oncoproteins induces overexpression of p16. However, the association of HPV, p16, or EBV shows preferential distribution according to organs or histology.

Recently, several new predictive biomarkers for chemo-sensitivity and patient survival have been identified, most predominantly in non-small cell lung cancer[9, 12,13]. The most

representative of these biomarkers are the excision repair cross-complementation group 1 (ERCC1), class III ß-tubulin (TUBB3), and ribonucleotide reductase M1 (RRM1). ERCC1 plays an important role in repairing DNA damage caused by platinum agents and may therefore be useful in predicting which patients will benefit from platinum-based therapy[6]. The isotype composition of ß-tubulins has been related to taxane-based chemotherapy responsiveness[7, 8]. The RRM1 gene encodes a regulatory subunit of the enzyme ribonucleotide reductase that is the key molecular target of gemcitabine[9-11].

Because platinum and taxane are also major chemotherapeutic agents in head and neck squamous cell carcinoma (HNSCC), it would be very reliable if these biomarkers could be related to the therapeutic outcome of HNSCC. In contrast to lung cancer, little is known about the significance of these proteins in HNSCC. Therefore, we aimed to explore the expression of these relevant biomarkers and also investigated its clinical significance in HNCa.

MATERIALS & METHODS

Patients and study design

Eighty three patients diagnosed with HNCa occurring in the nasopharynx, oral cavity, oropharynx, hypopharynx, and larynx at Boramae Medical Center between January 2008 and March 2012 were enrolled. Clinical information including demographic data, clinical stage, treatment methods, treatment response, recurrence, and follow-up data were obtained from electronic medical records. The demographic data and clinicopathologic characteristics are summarized in Table 1. The median age of the patients was 62.9 years (range 22-87 years) and 92.9% were male. The neoplastic lesions consisted of 74 SCC and nine undifferentiated carcinoma (UDC) (Table 1, Figure 2). Tumor stage ranged from I-IV according to the

American Joint Committee on Cancer staging[14]. Concurrent chemoradiotherapy (CCRT) was used as a neoadjuvant or adjuvant therapy to surgery, and was also used as a primary therapy when a tumor was unresectable and displayed good response to the therapy. A tumor with extra-capsular extension, resection margin insufficiency, lymph node metastasis, and involvement of adjacent structure (pT3 or T4), CCRT was considered for adjuvant therapy. In some cases, three cycles of induction chemotherapy were tried before CCRT was started. After 2-3 months of CCRT, patients were reevaluated through an imaging study to detect remnant tumor and the remaining lesion was removed by salvage surgery. During CCRT, 35 mg/m^2 of cisplatin per week was given to all patients during radiotherapy; the treatment was done seven or eight times. Radiotherapy was performed 5 days a week and in total involved 7020 cGy in 39 fractions for gross tumor volume, 5940 cGy in 33 fractions for high risk clinical target volume, and 4500-5040 cGy in 25-28 fractions for low risk clinical target volume.

Treatment outcome analysis

Tumor response was assessed 3 months after the completion of the treatment. Evaluation of tumor response consisted of clinical examination, nasopharyngolaryngoscopy, computed tomography (CT) and magnetic resonance imaging (MRI) of the primary site and the neck. Recurrence was defined as local if they were within the zone of the primary tumor and as regional if they occurred elsewhere including neck lymph nodes. The data regarding patient demographics, dose intensity of chemotherapy, response to treatment, disease-free survival (DFS), and overall survival (OS) were obtained by medical record review. Tumor responses were assessed radiologically at base-line and after every two treatment cycles. Designations of complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were based on the standardized response definitions established by the World

Health Organization[15]. Failure of treatment was defined as the state of PR, SD, or PD on completion of the definite primary treatment. The response rate was defined as total number of CR and PR patients divided by total number of evaluable patients. OS was calculated as the time between the beginning of chemo-therapy and death or censored at last follow-up.

Pathologic review and construction of tissue microarray blocks

Every cancer specimen was taken before the start of primary treatment, and all cases were reviewed by the experienced pathologist (KJE). Tissue microarray blocks (TMA) were constructed by transferring each 0.2 cm core from the most representative area on the mother block to a new paraffin block containing 40-50 cases of HNCa (Superbiochips Laboratories, City, Korea). Triplicates of each TMA were used for immunohistochemistry (IHC) as described subsequently

IHC and interpretation

A known standard protocol using a Benchmark XT automated immunostainer (Ventana, Tuscon, AZ, USA) was used to perform IHC on formalin embedded, paraffin embedded tissue sections. After deparaffinization, heat-induced antigen retrieval was done using citrate buffer (CC1 protocol; Ventana). Reactivity was detected using the Ultra-View detection kit (Ventana). The antibodies used were class III beta-tubulin (TUBB3; Covance, City, NJ, USA), ERCC1 (GeneTex, City, TX, USA), RRM1 (Proteintech, City, IL, USA), and P16 (Dako, City, CA, USA). Sections of normal tonsil tissues were included as external positive controls and stromal cells surrounding the tumor area served as internal positive controls[16]. Assessment of IHC was done with a semiquantitative grading method (H-score system); the percentage of positive tumors was calculated for each specimen and a proportion score was assigned (0 if 0%, 0.1 if 1% to 9%, 0.5 if 10% to 49%, and 1.0 if 50% or more). The median value of the H

score was chosen as the cutoff point for separating low and high levels of ERCC1, TUBB3, and RRM1 expression. Tumors with a H score exceeding 1.0 were deemed to be in the high expression group of ERCC1, TUBB3, and RRM1[13] (Figure 1).

In situ hybridization of HPV and EBV

In situ hybridization (ISH) for HPV and EBV was performed using the Automated Staining System (Ventana) according to the manufacturer's instructions. Inform HPV III probe sets (Ventana) capable of identifying 13 types of high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66) and 13 types of low-risk HPV (6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73 and 81) were used. EBV ISH was done with a fluorescein-conjugated EBV oligonucleotide probe targeting EBV-encoded small RNAs (EBER; Novocastra, City, UK). Both HPV and EBV positive signals were visualized as strong black spots at the tumor cell nuclei.

HPV genotyping by DNA chip analysis

HPV genotyping was carried out using a microarray system that includes probes of 43 types of HPV (Goodgene, Seoul, Korea) as previously described[17]. DNA was extracted from the paraffin blocks using QIAamp DNA FFPE kit (Qiagen, Valencia, CA, USA).

Statistical analyses

The baseline characteristics of the HNCa patient groups were compared using Fisher's exact test for discrete variables and the Mann-Whitney U-test for continuous variables. DFS rates and OS rates were estimated using the Kaplan-Meier method. The prognostic values were studied with the use of a Cox model stratified according to center and adjusted for significant prognostic factors for survival (sex and the stage of disease) and factors associated with HPV, p16, ERCC1, TUBB3, RRM1 (age, sex, T staging, and N staging). All reported *p*-values are

two sided and $p < .05$ was considered significant.

RESULTS

Characteristics of the patients

Table 1 summarizes the general characteristics of patients. The most common sites were the oropharynx (27 out of 83, 32.5%) and larynx (25 out of 83, 30.1%), followed by nasopharynx (12, 14.5%), oral cavity (12, 14.5%), and hypopharynx (7, 8.4%). All UDC cases involved the nasopharynx. Because some patients were lost to follow-up after biopsy, T and N categories were assigned to 81 patients. The information of T, N, and M categories was combined to assign an overall stage as I, III, III, and IV in 79 patients. Fifteen patients (18.5%) were stage I, 13 (16.0%) were stage II, 17 (21.0%) were stage III, and 34 (42.0%) were stage IV HNCa. Lymph node metastasis was found in 49 (60.5 %) cases; among them, only one patient had distant metastasis at the time of diagnosis. Of 83 patients, 68 (81.9%) finished their primary treatment completely. As a primary treatment of HNCa, 33 patients underwent surgery. Twenty-nine patients received CCRT with cisplatin and six were treated with radiotherapy only. As a result, primary treatment failed in 11 of 68 patients (16.2%). During a median follow-up of 24.2 months, 25 patients (36.8%) recurred locally or regionally, 13 (19.1%) developed distant metastasis, and14 patients (20.6%) died.

Detection of HPV, EBV, p16, ERCC1, RRM1, and TUBB3 in HNCa

Results of IHC in HNCa are presented shown in Table 2. HNSCC lesions included the three cases in the nasopharynx, 12 in the oral cavity, 27 in the oropharynx, seven in the hypopharynx, and 24 in the larynx. HPV was found in 27 of 82 cases (32.9%). Twenty three

cases harboring high-risk HPV consisted of 15 cases with HPV type 16, four cases with type 18, and four others. Among the low-risk HPV infected cases, two cases involved HPV type 6 and two others the sentence is incomplete. p16 positivity almost always coincided with HPV. However, seven cases showed p16 positivity but no detection of HPV, either by ISH or chip analysis. EBV was detected in 11 of 83 cases (13.3%) and most (nine of 11) were undifferentiated carcinoma. HPV and p16 positivity is known to be very prevalent in oropharyngeal cancers. Presently, all the EBV positive cases with the exception of one involved the nasopharynx, and every undifferentiated carcinoma involved the nasopharynx. High expression of ERCC1 was detected in 64 of 70 cases (91.4 %), but there was no significant difference regarding ERCC1 expression between histologic types; 56 of 61 in SCC, and eight of nine in UDC (p=.577). Regarding anatomic location, ERCC1 was highly expressed regardless of tumor location and the low expression group involved the larynx only. RRM1 was identified in 56 of 70 (80.0%) cases at high levels, with no significant differences evident between histologic types; 51 of 62 in SCC, and five of eight in UDC (p=.193). On the other hand, expression of TUBB3 revealed significant differences regarding histologic types; 37 of 62 (59.7%) SCC revealed high expression but none of the nine cases of UDC (p=.001) did. Expression of TUBB3 and RRM1 was high throughout all the locations of HNCa, except the nasopharyx (p=.085 and p=.008, respectively).

Correlation of protein expression and clinical parameters

There were no differences in outcome of primary treatment, presence of recurrence or distant metastasis, and survival rate between groups of different treatment modality. Association of HPV or p16 positivity was significantly high in the CCRT group (p=.026 and p=.027, respectively). Otherwise, expression of ERCC1, TUBB3 and RRM1 seemed to have no significant differences in the treatment modality. Infection of HPV and positivity for p16

were significantly associated with lower T stages (p=.040 and p=.008, respectively). However, there were no differences in the expressions of ERCC1, RRM1, TUBB3 between T stages or N stages. Other biomarkers did not affect any clinical parameters, such as outcome of primary treatment, presence of recurrence, distant metastasis, and survival rate (Table 3).

DFS and OS according to protein expression

Survival analysis was performed in 61 patients who completed primary treatment and had a sufficient follow-up period. The 2-year OS rate was 87.64%, the 2-year DFS rate was 54.97%, and the median follow-up was 33.6 months in our study group. Figures 3 and 4 showed the Kaplan-Meier estimates of the probability of OS and DFS.

Univariate analysis revealed that sex and tumor location were important factors affecting the prolongation of DFS (Table 4). Female patients (N=5) and patients with oral cavity or hypopharynx cancer had the worse prognosis (p=.002 and p=.0063, respectively). The five female patients were younger than the male patients (mean age 63.05 vs 57.80, p=.082). Four involved the oral cavity and the remaining female had oropharyngeal cancer. The four females with oral cavity cancer recurred after successful primary treatment with a mean DFS of 9 months; most were p16 and HPV negative.

Expression of ERCC1 or RRM1 did not show any significant correlation with patient survival. The OS rate was significantly lower in patients with high TUBB3 compared with those with low TUBB3 (p=.033). Low TUBB3 expression was associated with longer survival, but without statistical significance (Figure 3). DFS and OS results were similar. Only low TUBB3 expression tended to have longer DFS than that of high expression group, but without significance (p=.075) (Figure 4).

Multivariate analysis showed that positivity of p16 was a predictive factor of DFS and OS (Table 5). Moreover, high TUBB3 expression was also an independent predictor of the

prolongation of OS (Hazard Ratio 31.077; 95% Confidence Interval, 1.702-567.514) (p=.020).

DISCUSSION

This study investigated the expressions of ERCC1, TUBB3, and RRM1 in HNCa, and analyzed their correlation and clinical significance with various clinicopathologic parameters as well as viral status. Expression of these markers varied according to anatomic locations of tumor and histologic subtypes. Confirming prior reports, oropharyngeal tumors showed the highest HPV infection and p16 expression. HPV positivity was 34.1% in all head and neck tissues, of which 55.6% was exclusively in the oropharynx. Previous studies demonstrated that incidence of HPV positivity in the tonsillar SCC subgroup of oropharyngeal SCC was about 50% in Korean [18], which similar to that of Westerners[19-21].

A recent study on HPV and p16 of tonsillar SCC in a Korean population revealed 80% and 68% positivity of p16 and HPV, respectively[22]. Among the 18 cases of tonsillar SCC in our study, nine (50%) were HPV positive and 12 (66.7%) were p16 positive, coinciding with previous results. Interestingly, four cases were positive for p16 but negative for HPV. There are several explanations for this finding. One possible explanation is that the HPV detection method of HPV was not sensitive enough, especially HPV ISH[18]. The other and more rational explanation is that the over-expression of p16 is induced by other mechanisms independent of HPV. Although many cases of p16 positivity also harbored HPV, only p16 expression was associated with better OS and DFS, not HPV status. Our finding supports the idea that prognostic benefits of HPV positivity in HNCa results from the strong tumor suppressive effects of p16[22].

Several related studies also focused on biomarker expression with the aim of correlating biomarker expression with tumor location for HNCa[23]. However, the present study is the first to comprehensively analyze the expressions of ERCC, RRM1, and TUBB3, along with important clinicopathologic parameters such as anatomic location and HPV. The expressions of these biomarkers significantly differed according to tumor location, which suggest that the pathogenesis or therapeutic efficacy might be different between the primary origins, although the histology is the same. For example, RRM1 was not expressed in SCC of the oral cavity but was highly expressed in SCC of the hypopharynx. Moreover, expression of TUBB3 was associated with histologic type: no UDC samples showed high TUBB3 expression, while displaying exuberant expression of ERCC1 and RRM1. These findings suggest that anatomic location and histologic types should be considered in the selection of a chemotherapeutic regimen. Paclitaxel has been used for neoadjuvant therapy before concomitant chemoradiotherapy for treatment of nasopharyngeal UDC. Some studies reported that paclitaxel was very effective for nasopharyngeal UDC, and might be correlated to biomarker distribution[24]. The role of ERCC1, RRM1, or TUBB3 as a predictor of treatment response or as a prognostic indicator has been explored in other tumors, most frequently in non-small cell lung cancer[9,12,13,26]. In one study, a low level of expression of ERCC1 by tumor cells was associated with longer survival after adjuvant treatment with cisplatin-based chemotherapy in non-small cell lung cancer[13]. However, the role of these markers in HNCa has not yet been fully explored. In early stage laryngeal cancer, reduced ERCC 1 expression is related to radioresistance[25]. In our study, ERCC1 did not influence patient outcome. However, there were only five cases with low ERCC1 levels, and all were advanced stage (T4) laryngeal SCC. Primary treatment failed in four of the five cases, three patients experienced recurrences, and two patients died of SCC. These results suggest that loss of ERCC1 might

be related to the aggressive behavior of laryngeal SCC. Further studies should be conducted to confirm this suggestion. Regarding RRM1, there have been few studies about RRM1 expression of HNSCC, and preclinical studies have shown that high levels or specific mutations on RRM1 are associated with gemcitabine resistance[6, 28-31]. However, our results failed to show any significant differences of RRM1 expression on patient outcome. Although there were limitations due to the small number of cases and heterogeneous treatment modalities, the results lend credence to the view that the role of RRM1 in determining chemo-sensitivity might not be very crucial in HNSCC. To date, gemcitabine is not the first-line chemotherapeutic agent in HNSCC.

One study reported that in HNSCC, TUBB3, but not ERCC1, is a prognostic and predictive marker for chemotherapy[16], which is consistent with our results. It is quite predictable that high the expression of TUBB3 may induce chemoresistance to anti-microtubule agents, thus the prognostic value of TUBB3 is partly attributable to the lack of response to docetaxel, which is a part of the standard induction regimen in HNCa. However, in a large-scale study with 265 patients, low expression of TUBB3 was associated with better DFS and OS, regardless of docetaxel treatment[27]. The collective previous and present results suggest a more potential or multidirectional role of TUBB3 involved in patient outcome in HNCa. Further supporting evidence of TUBB3 as a universal prognostic indicator independent of therapeutic modalities has been reported[33-35].

CONCLUSION

ERCC, RRM1, and TUBB3 were differentially expressed according to the anatomical localization and histologic type. Despite the heterogeneity of the treatment modality, this

study suggests that p16 positivity and low TUBB3 expression positively contributes to favorable prognosis in HNCa.

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Table 1. General characteristics of all patients

		No. of patients	%
Gender	Male	77 (mean age, range	92.8
	Female	6 (mean age, range	7.2
Pathology	Squamous cell carcinoma	74	89.2
	Undifferentiated carcinoma	9 (all nasopharynx)	10.8
Organ	nasopharynx	12	14.5
	oral cavity	12	14.5
	oropharynx	27	32.5
	hypopharynx	7	8.4
	larynx	25	30.1
T staging	T1,2	53	66.3
	T3,4	27	33.7
N staging	N0	30	37.5
	N1	17	21.3
	N2,3	33	41.2
Stage	I,II	28	35.4
	III,IV	51	64.6
Treatment	Surgery	33	48.5
	CCRT	29	42.6
	Radiotherapy	6	8.9

Table 2. The expression of HPV, EBV, p16, ERCC1, RRM1, and TUBB3 in head and neck cancer according to pathology and involved organs.

		Squamous cell carcinoma (*N=74*)						Undifferentiated carcinoma(*N=9*)	*p-value*
		NPhx	OC	OPhx	HPhx	Lx	*p-value*		
HPV	positive	2	1	15	3	5	*0.009	1	0.259
	negative	1	11	12	4	19		8	
p16	positive	1	3	17	2	3	*0.002	0	0.051
	negative	2	9	10	5	21		9	
EBV	positive	1	0	1	0	0	0.114	9	* 0.000
	negative	2	12	26	7	24		0	
ERCC1	high	2	9	20	7	18	0.099	8	0.577
	low	0	0	0	0	5		1	
TUBB3	high	0	8	11	5	13	0.085	0	*0.001
	low	3	2	12	2	6		9	
RRM1	high	0	10	18	5	18	*0.008	5	0.193
	low	3	0	3	2	3		3	

Nphx, nasopharynx; OC, oral cavity; Ophx, oropharynx; Hphx, hypopharynx; Lx, larynx

* *p-value* < *.005,* Fischer's exact test

Table 3. Correlation of protein expression and clinical parameters in the HNSCC with completed primary treatment

		HPV		p16		ERCC1		TUBB3		RRM1	
		+	-	+	-	high	low	high	low	high	low
T staging	T1,2	15	22	14	23	24	10	19	13	20	9
	T3,4	6	16	7	15	2	2	10	8	8	4
N staging	N0	*5	*20	*4	*21	19	1	11	10	13	8
	N1	*4	*8	*4	*8	7	0	7	3	5	1
	N2,3	*12	*10	*13	*9	8	3	11	8	11	3
Outcome of primary treatment	success	6	5	4	7	37	4	23	17	32	7
	fail	15	33	16	32	9	1	7	3	8	2
Recurrence	No	15	21	15	21	28	2	14	16	25	5
	recur	6	17	5	18	18	3	16	4	15	4
Distant metastasis	No	17	27	17	27	34	4	21	15	29	6
	yes	3	10	3	10	10	1	8	5	9	3
Survival	alive	17	29	18	28	35	3	*20	*19	33	6
	death	4	9	2	11	11	2	*10	*1	7	3

* *p-value* $< .005$, Fischer's exact test

Table 4. Factors associated with disease free survival (DFS)

		No. of patients	DFS		OS	
			Median DFS (months)	*p-value*	Mean survival (months)	*p-value*
Age	<60	32	34.40	0.271	48.95	0.426
	≥60	29	53.87		67.10	
Sex	Male	56	52.04	*0.002	62.69	0.559
	Female	5	10.60		33.00	
Organ	Nphx	3	45.00	*0.063	49.00	0.461
	OC	11	20.12		48.73	
	Ophx	21	44.23		49.72	
	Hphx	4	17.75		40.75	
	Lx	22	53.87		71.22	
T stage	T1,2	40	14.50	0.857	64.57	0.720
	T3,4	21	13.40		57.47	
N stage	N0	27	20.00	0.270	68.70	0.072
	N1	12	11.86		67.27	
	N2,3	22	10.44		43.20	
HPV	Positive	21	42.40	0.214	69.67	0.759
	Negative	38	44.91		57.43	
P16	Positive	20	44.91	0.111	52.28	0.275
	Negative	39	42.86		57.53	
ERCC1	High	46	49.42	0.589	56.61	0.874
	Low	5	32.20		68.00	
TUBB3	High	30	44.58	0.075	56.21	0.096
	Low	20	46.58		54.29	
RRM1	High	40	47.46	0.984	63.54	0.486
	Low	9	39.49		46.30	

Table 5. Hazard ratios for disease-free survival and overall survival.

	Disease-free survival (DFS)		Overall survival (OS)	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
HPV	0.123 (0.007 -2.143)	0.151	0.483(0.067-3.481)	0.470
p16	0.016 (0.001-0.280)	*0.005	0.040(0.003-0.570)	*0.018
ERCC1	0.174 (0.010-3.100)	0.234	0.545(0.053-5.655)	0.611
TUBB3	2.842 (0.292-27.655)	0.368	31.077(1.702-567.514)	*0.020
RRM1	10.955 (0.787-152.460)	0.075	0.039(0.001-1.256)	0.067

Figure 1. Representative immunohistochemical results in head and neck squamous cell carcinoma. (A) Low expression group of ERCC1 (H-score <1); (B) high expression group of ERCC1 (H-score ≥1); (C) low expression group of TUBB3 (H-score <1); (D) high expression group of TUBB3 (H-score ≥1); (E) low expression group of RRM1 (H-score <1); and (F) high expression group of RRM1 (H-score ≥1).

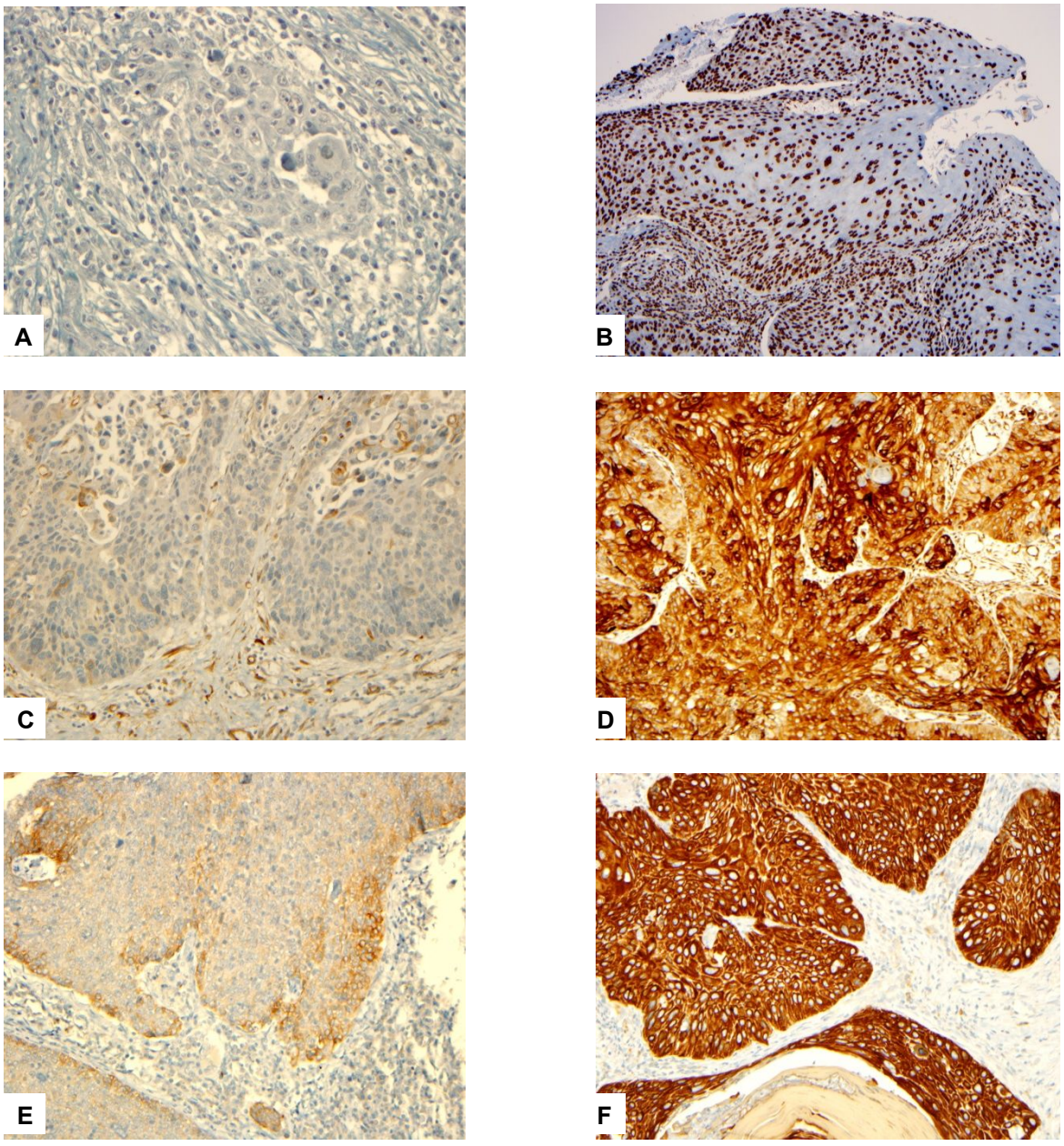


Figure 2. Characteristics of the enrolled head and neck cancer patients

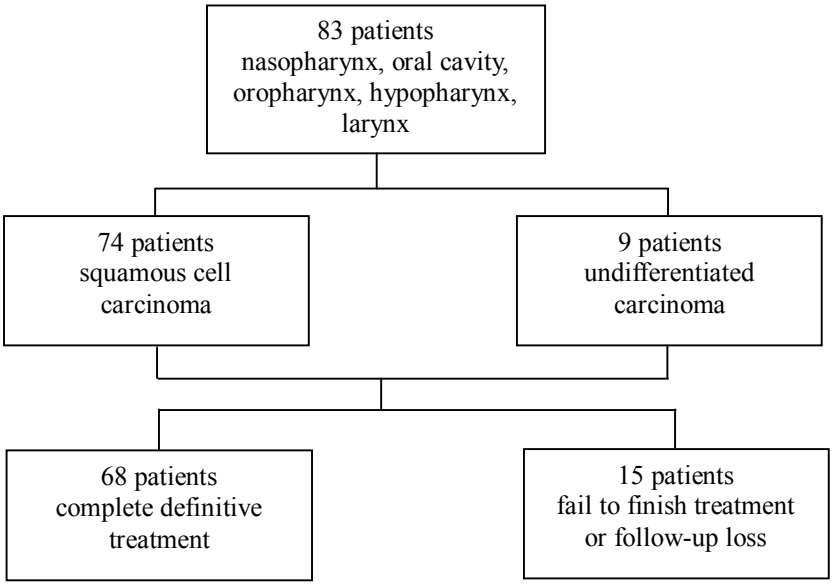
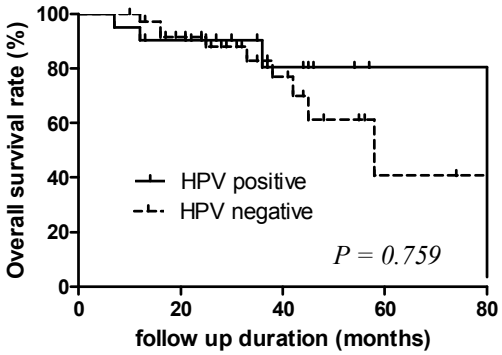
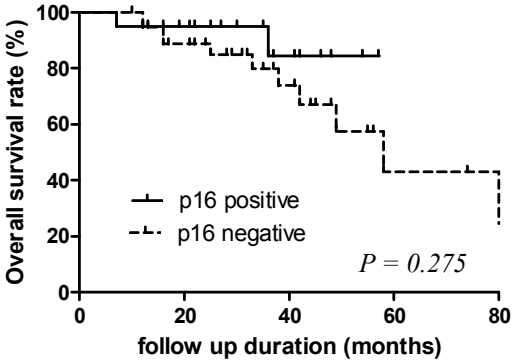


Figure 3. Overall survival according to protein expression in head and neck squamous cell carcinoma.

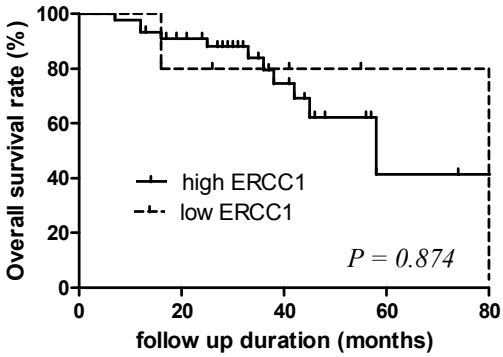
HPV



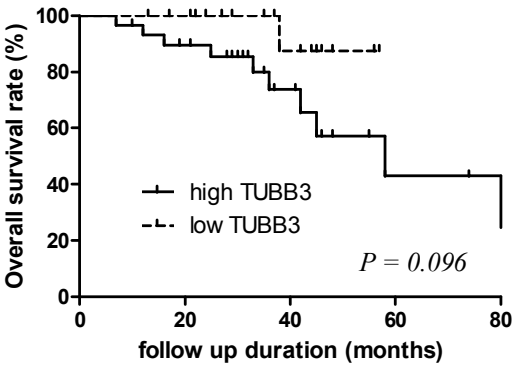
B. p16



C. ERCC1



D. TUBB3



E. RRM1

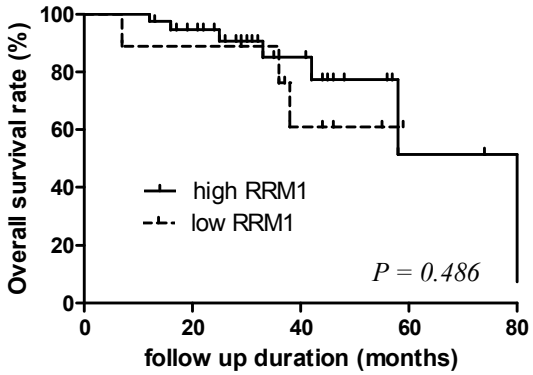
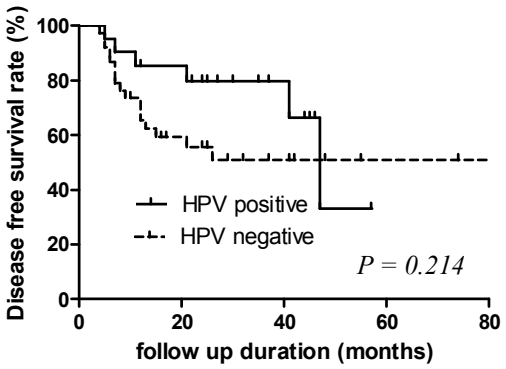
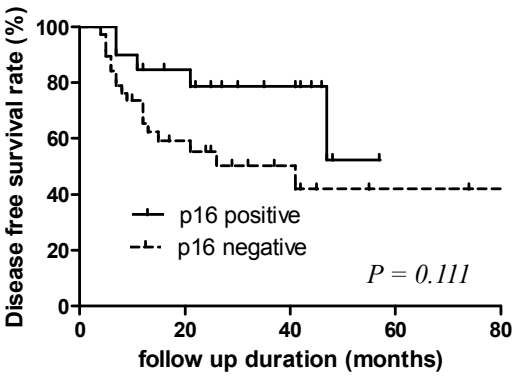


Figure 4. Disease free survival according to protein expression in head and neck squamous cell carcinoma.

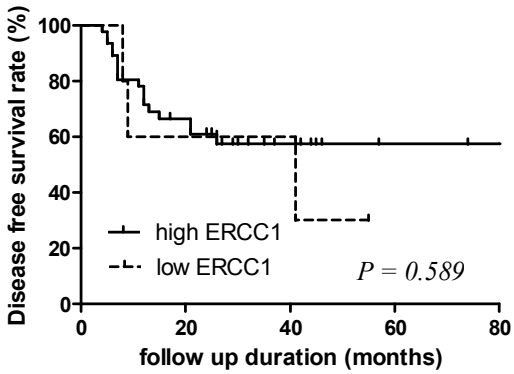
A. HPV



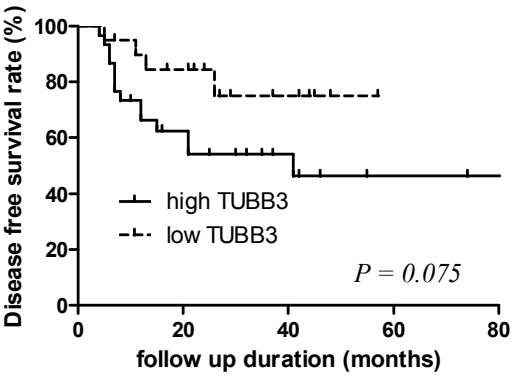
B. p16



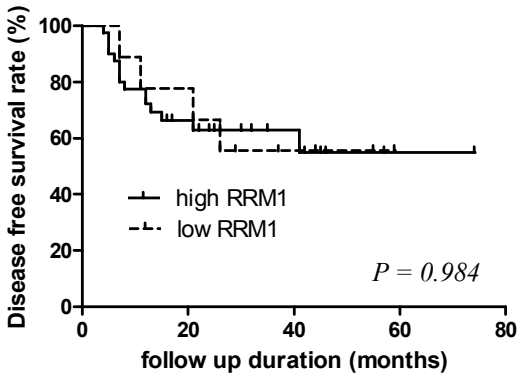
C. ERCC1



D. TUBB3



E. RRM1



국문초록

서론: 두경부암의 치료는 매우 복잡하며 특히 수술은 종양의 해부학적 범위와 기관을 보존하기 위한 욕구 때문에 제한되기도 한다. 궁극적인 효과를 얻기 위해 두경부 암에서 분자종양의 분석에 기반을 둔 추가적인 치료의 역할이 증가하고 있다. 본 연구는 치료 계획을 발전시키기 위해 두경부 종양에서 생체 마커의 발현과 임상적 의미를 분석하고자 하였다.

방법: 총 83명의 환자 중 74례가 편평세포상피암을 가지고 있었고 9례가 미분화 암을 가지고 있었다. Tissue microarray block(TMA)를 이용하여 두경부 암 조직에 대해 이전 비소세포성 폐암의 치료 반응의 표식자로 보고된 바 있는 excision repair cross-complementation group 1 (ERCC1), class III ß-tubulin (TUBB3), and ribonucleotide reductase M1 (RRM1) 등과 같은 세 가지의 생체마커를 사용하였다. HPV 감염 여부는 in situ hybridization (ISH)와 DNA chip 분석을 통해 알아보았고, EBV 감염 여부는 ISH를 통해 알아보았다.

결과: HPV 는 구인두에서 가장 빈번하게 발생하여 74 례 중 27 례에서 발견되었다. EBV 는 9 례의 미분화암 모두를 포함하여 총 11 례에서 발현되었다. 세 개의 생체마커의 발현은 두경부암의 여러 위치에 상대적으로 높게 발현되었으나, 후두의 편평세포암에서 ERCC1 이 낮게 발현되었다. ERCC1 은 70 명 중 64 명 (91.4%)에서 높게 발현되었고, RRM1 은 70 명 중 56 명 (80.0%)에서 높게 발현되었다. TUBB3 는 조직학적 분류에 따라 발현 정도가 유의하게 차이가 났는데, 미분화암에서는 9 명 중 아무에게도 높게 발현되지 않았다 (*p=.001)*. 다변량 분석 상에서, p16 의 양성은 무질병 생존률과 전반적인 생존률 모두의 예측 인자가 되었다. TUBB3 의 높은 발현 여부는 전반적인

생존률 (HR 31.077; 95% CI, 1.702 – 567.514) (*p=.020*)을 연장시키는데 독립접인 예측 인자로 나타났다.

결론: 두경부암에서 생체마커의 발현은 해부학적 위치 및 조직학적 특성과 관련하여 차이를 보였다. 두경부암에서 p16과 TUBB3의 발현 정도가 유용한 예측 인자로 사용될 수 있을 것으로 기대한다.

주요어 : 두경부의 편평세포암종, 알파 유두종 바이러스, 인간의 ERCC1 단백질, 인간의 beta III-tubulin 단백질, 생물학적 종양 마커

학 번 : 2011-21832