대한두경부종양학회지
제 28 권 제 2 호 2012

Expression of VEGF, p53, Apaf-1 and Caspase-9 in Head and Neck Squamous Cell Carcinoma

Sung Hak Lee, MD, Yeo Ju Kang, MD, Ui Ju Jo, MD,
Youn Soo Lee, MD, PhD, Chang Suk Kang, MD, PhD,

Department of Pathology, Seoul St. Mary's Hospital, The Catholic University of Korea, College of Medicine, Seoul, Korea

두경부 편평세포암에서 VEGF, p53, Apaf-1 and Caspase-9의 발현

가톨릭대학교 의과대학 서울성모병원 병리과학교실
이성학 · 강여주 · 조의주 · 이연수 · 강창석

= 국문초록 =

배 경

편평상피암은 두경부의 악성종양 중 가장 흔하며, 임상적인 경과가 불량하다. 따라서 나쁜 예후를 가지는 환자군을 조기에 선별하여 더 적극적인 치료의 시행을 결정짓는 표지자의 필요성이 대두된다. 우리는 일련의 두경부 편평세포암 검체에서 몇몇 분자 표지자의 예후적 유용성을 평가하고자 하였다.

방 법

23예의 두경부 편평세포암 검체를 대상으로 VEGF, p53, Apaf-1, caspase-9의 발현과 몇몇 임상병리학적 지표들간의 연관성을 면역조직화학염색을 통해 조사하였다.

결 과

환자군은 남성이 더 많았으며 평균연령은 63.7세였다. 1기가 5예, 2기가 2예, 3기가 8예, 4기가 8예였다. 평균생존기간은 37.3개월이었다. VEGF단백 발현은 종양의 크기와 통계적으로 유의한 연관성을 보였다. 이와 더불어 VEGF 단백 발현은 병기, 그리고 림프관 침습과 연관적인 경향성을 보였다. 그러나 VEGF단백 발현과 생존기간과는 관련성이 없었다. 또한, Apaf-1과 caspase-9의 단백발현은 다른 임상지표, 환자의 생존기간과는 관련이 없었다.

결 론

VEGF단백 발현은 두경부 편평세포암 환자에서 나쁜 임상경과를 예측할 수 있게 하는 표지자로서의 역할을 할 수 있다. 또한 본 연구는 두경부 편평세포암에서 별로 연구되지 않은 Apaf-1과 caspase-9의 발현상태를 밝힌 점에서 의의가 있다.

중심 단어 : 편평세포암 · 두경부암 · Vascular endothelial growth factor · 면역조직화학염색.

Introduction

Head and neck squamous cell carcinoma(HNSCC) is the sixth most common malignancy characterized by an aggressive growth pattern, high-degree of local invasiveness, and a relatively high rate of local regional recurrence.[1,2] More than 90% of tumors in the head and neck are squamous cell carcinomas.[3] Despite improvements in the diagnosis and treatment of this malignancy, the survival rate has remained unchanged over the last 30 years and the most reliable prognostic factor is still the status of lymph node metastasis at the time of diagnosis.[4,5] Thus, there is a need for better markers that can

Received : September 25, 2012 / **Revised** : October 24, 2012
Accepted : October 29, 2012
교신저자 : 이연수, 137-701 서울 서초구 반포동 505
가톨릭대학교 의과대학 서울성모병원 병리과학교실
전화 : (02) 2258-1626 · 전송 : (02) 2258-1628
E-mail : lys9908@catholic.ac.kr

identify patients with poor prognosis and guide the use of more aggressive treatment modalities in early stages of the disease.

Angiogenesis is considered to be a very important biological factor in many neoplasms and crucial both for the growth of a tumor and for the occurrence of distant metastasis.[6] Among the factors in relation to tumor angiogenesis, vascular endothelial growth factor(VEGF) is a leading molecular marker. VEGF is involved in the proliferation, differentiation, and migration of vascular endothelial cells and enhances endothelial cell survival by avoiding apoptosis.[7,8] Various studies reported upregulation of VEGF in different malignancies ; however, their roles in HNSCC carcinogenesis are not clear.[9]

Apoptosis, a process of programmed cell death, is essential in the cell growth for the maintenance of homeostasis.[10] p53 tumor suppressor gene plays a critical role in apoptosis, cell cycle control, DNA damage response, and maintaining the integrity of genome.[11] Mutation of p53 tumor suppressor gene is the most frequent genetic alteration identified in malignant tumors.[11] Mutated p53 is also commonly seen in HNSCC. However, despite the fact that the presence of p53 mutation and its effect on prognosis in HNSCC has been widely investigated in the various literature, no consensus exists about its clinical role in establishing important parameters such as treatment responses or prognostic value.[12]

A central component of the apoptotic apparatus is a family of cystein-containing, aspartate-specific proteases termed caspases.[13] Two regulatory pathways of the caspase cascades have been well studied so far.[13] The assembly of a death-inducing signaling complex(DISC) at the Fas receptor(Fas) plays a major role in the first pathway. The release of cytochrome c(Cyt c) from the mitochondria triggers the second pathway, which subsequently causes apoptosis by activation of Caspase-9 and Caspase-3. Apoptotic protease-activating factor 1(Apaf-1) has been shown to participate as an adaptor molecule in the sequential activation of Caspase-9 and Caspase-3.[13] Kuwahara et al.,[14] suggest that Caspase-9-dependent apoptosis plays an important role in cisplatin-induced HNSCC apoptosis.

In this study, we investigated the expression of VEGF, p53, Apaf-1 and caspase-9 protein in HNSCC patients, in relation to histopathological parameters and clinical outcome.

Materials and Methods

1. Patients and clinicopathologic features

Histologic sections of HNSCC resected from 2000 through 2010 were retrieved from the archives of the Departments of Hospital Pathology at Yeouido St. Mary's Hospital. We selected the radical resection cases only and excluded the cases with follow-up loss or having uncertain survival data during that period. Twenty-three cases were enrolled for this study. The study cases are composed of thirteen laryngeal carcinomas, one hypopharyngeal carcinomas and nine nasal cavity carcinomas of various grades and stages. A hypopharyngeal carcinoma was classified as laryngeal carcinoma category. We excluded the oropharyngeal and tongue cancers due to elimination of heterogeneity in the HNSCC. Clinicopathological information was obtained for all cases, including sex, age, tumor location, clinical stage, TNM classification, tumor differentiation, growth pattern, resection margin involvement, lymphatic, vascular and perineural invasion and overall survival. The study was performed after approval of the local institutional review board(IRB).

2. Immunohistochemistry and Staining Interpretation

Tissue sections(4μm thickness) were prepared from formalin-fixed, paraffin-embedded tissues and mounted on 3-aminopropylmethoxysilane-coated slides. Immunohistochemical staining for VEGF(Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1 : 150), p53(DO-7, Ventana Medical Systems Inc. Tucson, Arizona, USA, dilution 1 : 200), Apaf-1(5E11, Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1 : 100) and caspase-9(LAP6, Diagnostic BioSystems Inc. Pleasanton, CA, USA, dilution 1 : 100) were used. 3, 3-Diaminobenzidine was used as the chromogen and the sections were counter-stained with hematoxylin. Evaluation of the immunohistochemical staining was carried out independently by two pathologists.

p53, Apaf-1 and caspase-9 expression was categorized as negative(0, <10%), focally positive(1+, 10−50%), diffusely positive(2+, >50%). For the assessment VEGF protein expression, the staining was also scored semiquantitatively as follows : 0(no staining at all and weakly positive) ; 1+(moderately positive) ; and 2+(strongly positive).

3. Statistical analysis

Data were analyzed using the statistical software, SPSS(version 13.0 ; SPSS, Chicago, Illinois) for Windows. Statistical analysis was performed for comparison of various histopathologic parameters with VEGF, p53, Apaf-1 and caspase-9. An analysis of the correlation among the immunohistochemical markers was also conducted. Categorical variables were analyzed using χ^2 or Fisher exact test and a P value of <0.05 was considered significant.

An analysis of the correlation between the immunohistochemical markers and survival was conducted. Overall survival(OS) was defined as the period between the time of surgery and death. Survival times of patients still alive were censored

with the date of the last follow-up. Kaplan-Meier estimation was used for survival analysis, and survival curves were compared using the log-rank test.

Results

1. Patients and clinicopathologic features

Sixteen(69.6%) of the study subjects were male and seven (30.4%) were female. The age range of subjects was between 22 and 88 years(mean ; 63.7 years). Five cases(21.7%) had stage I disease, two(8.7%) stage II, eight(34.8%) stage III and eight (34.8%) stage IV. For the primary tumor(T), there were six (26.1%) T1, two(8.7%) T2, seven(30.4%) T3 and eight (34.8%) T4. For the regional lymph nodes(N), twenty(87.0%) were N0, two(8.7%) N1 and one(4.3%) N2. There were no distant metastasis(M) cases. Twelve cases(52.3%) showed well differentiated carcinomas, ten(43.5%) moderately differentiated carcinomas and one(4.3%) poorly differentiated carcinoma.

Survival times were calculated from the date of surgery. The mean survival was 37.3 months ; the longest recorded survival was 108 months. These data are summarized in Table 1.

2. Immunohistochemistry and statistical analysis

Immunohistologic characteristics of various molecular markers and clinicopathologic factors are shown in Table 2. Fourteen(60.9%) of subjects had expression of TP53. Of the positive cases, five cases(21.7%) showed focal positivity and nine (39.1%) showed diffuse immunoreactivity(Fig. 1). Twenty-two cases(95.7%) had immunoreaction of Apaf-1. Two(8.7%) showed focal reactivity and twenty(87.0%) showed diffuse reactivity. All cases were immunoreactive for caspase-9. Fourteen (60.9%) had focal positivity and nine(39.1%) had diffuse positivity. For VEGF expression, five cases(21.7%) showed negative or weak reactivity, eight(34.8%) moderate and ten(43.5%)

Table 1. Clinicopathologic characteristics of 23 patients with clinical stages

Characteristic	All	Stage I	Stage II	Stage III	Stage IV
No. of patients	23	5(21.7%)	2(8.7%)	8(34.8%)	8(34.8%)
Mean age(years)	63.7	54.2	79.5	63.6	65.9
Sex					
Male	16(69.6%)	4	1	6	5
Female	7(30.4%)	1	1	2	3
Tumor location					
Nasal cavity	9(39.1%)	2	1	3	3
Larynx	14(60.9%)	3	1	5	5
Clinicopathologic parameters					
Primary tumor					
T1-T2	8(34.8%)	5	2	1	0
T3-T4	15(65.2%)	0	0	7	8
LN metastasis					
N0	20(87.0%)	5	2	5	8
N1-N2	3(13.0%)	0	0	3	0
Diatant metastasis					
M0	23(100%)	5	2	8	8
M1	0(0%)	0	0	0	0
Tumor differentiation					
WD	12(52.3%)	2	2	4	4
MD-PD	11(47.7%)	3	0	4	4
Tumor border					
E	8(34.8%)	3	1	3	1
E & I-I	15(65.2%)	2	1	5	7
Resection margin involvement					
RM present	9(39.1%)	1	1	3	4
Lymphatic invasion	3(13.0%)	0	0	1	2
Vascular invasion	2(8.7%)	0	0	0	2
Perineural invasion	2(8.7%)	0	0	1	1
SD(months)	37.3(2−108)	57.4(2−108)	16.0(5−27)	34.4(8−64)	33.0(8−82)

WD : well differentiated, MD : moderately differentiated, PD : poorly differentiated, E : Expanding growth, E & I : Expanding & infiltrative growth, I : Infiltrative growth, RM : resection margin, SD : Survival duration

Table 2. Relationship between the clinicopathologic parameters and immunoexpression of molecular markers

	P53	Apaf-1	Caspase-9	VEGF
Immunoexpression				
0	9(39.1%)	1(4.3%)	0(0%)	5(21.7%)
1+	5(21.7%)	2(8.7%)	14(60.9%)	8(34.8%)
2+	9(39.1%)	20(87.0%)	9(39.1%)	10(43.5%)
p-value				
Stage	0.363	0.209	0.657	0.089
T	0.162	1.000	0.643	0.019
N	0.253	1.000	1.000	0.560
M	N/A	N/A	N/A	N/A
Differentiation	0.214	1.000	0.214	1.000
Growth	1.000	0.269	1.000	0.379
Resection margin	0.383	0.538	0.383	1.000
Lymphatic invasion	1.000	1.000	0.538	0.068
Vascular invasion	1.000	1.000	0.142	0.178
Perineural invasion	1.000	1.000	1.000	0.178
Survival duration	0.976	N/A	0.748	0.984

Parameters considered statistically significant. N/A : not available

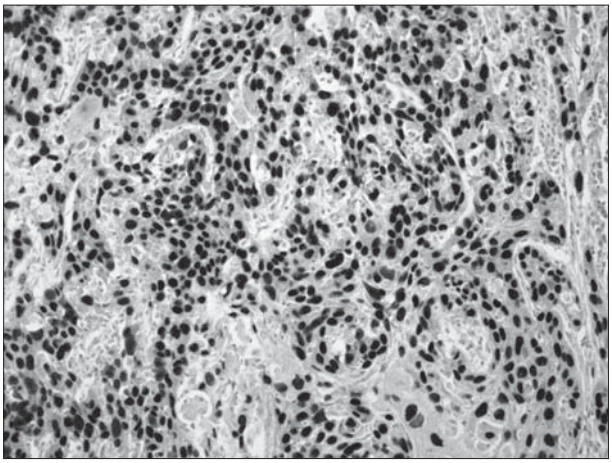

Fig. 1. Immunohistochemical finding for TP53 proteins shows strong nuclear reaction in neoplastic cells.

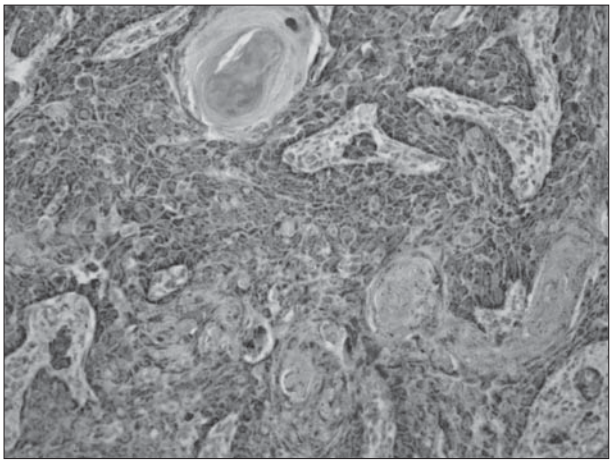

Fig. 2. Majority of tumor cells within this section of HNSCC exhibit a positive cytoplasmic immunohistochemical staining for VEGF.

strong immunopositivity(Fig. 2).

For the expression of TP53, Caspase-9 and Apaf-1, we did not find any correlation between the clinicopathologic parameters including survival and the status of TP53 expression. The expression of VEGF protein was statistically significant for the primary tumor(T) of HNSCC. In addition to this finding, there was trend for correlations of VEGF immunoreactivity with stage and lymphatic invasion. We also assessed the relationships of immunoexpression among molecular markers(Table 3). However, there were no correlations among immunohistochemical markers. In addition, we assessed the influence of VEGF protein expression on overall survival without taking into account post-surgical treatment, but no correlation with VEGF protein expression was found(Fig. 3).

Table 3. Relationship of immunoexpression among molecular markers

p-value	P53	Apaf-1	Caspase-9	VEGF
P53	N/A	0.538	1.000	0.669
Apaf-1	0.538	N/A	0.253	1.000
Caspase-9	1.000	0.253	N/A	0.669
VEGF	0.669	1.000	0.669	N/A

Discussion

Angiogenesis is a crucial step for the growth, invasion and metastasis of tumors.[15] VEGF is a primary angiogenic agent that serves to increase vascular permeability and enhance endothelial cell proliferation, migration and differentiation.[16] A considerable proliferation in the vascularity occurs during the transition from normal mucosa, through dysplasia, to invasive

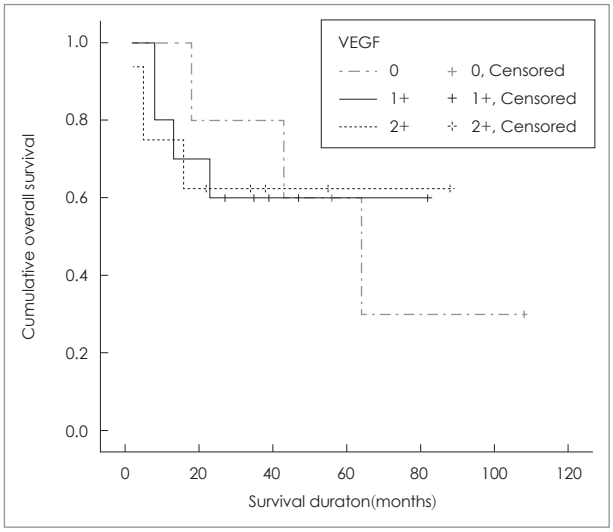

Fig. 3. Kaplan-Meier analyses of the influence of VEGF immunoexpression on overall survival of HNSCC. Patients whose tumors with VEGF protein expression(1t and 2t) show no correlation of improved overall survival than patients whose tumors with VEGF immunonegativity(0)(p=0.984, by the log-rank test).

HNSCC. In addition, tumor progression from early to advanced carcinomas is also closely associated to vascularity.[16] According to Sauter et al.,[9] advanced lesions(stage III and IV squamous cell carcinomas) showed a significant increase in VEGF expression as compared with early lesions(dysplasias to early invasive squamous cell carcinomas) and normal mucosa. Moreover, the meta-analysis by Kyzas et al.,[17] showed that VEGF expression by immunohistochemistry was associated with worse overall survival in patients with HNSCC and they revealed trends for modest correlations of VEGF expression with higher clinical stage and poor differentiation. In our study, there was significant correlation between VEGF expression and primary tumor(T), but we revealed no correlation with tumor differentiation. Meanwhile, in our study, there was trend for correlations of VEGF overexpression with stage and lymphatic invasion. The capability of VEGF to increase vascular permeability in lymphatics may provide a possible explanation for this association.[7] Moreover, several studies have also revealed that VEGF expression was a more significant prognostic factor for overall survival outcome than standard clinicopathologic predictors such as TNM stage.[16,18] Despite a significant correlation between VEGF expression and primary tumor(T) and trend for correlations between VEGF overexpression and stage and lymphatic invasion, however, our study found no correlation with VEGF protein expression and overall survival. This can be attributed to relatively small case numbers enrolled in this study, which is one of limitations of the current study. The prognostic value of VEGF in patients with HNSCC was also examined in the context of other proposed molecular markers, and several studies in the meta-analysis addressed a significant association of VEGF with other key molecular markers, such as cyclooxygenase-2, TP53, and Ki-67 labeling index.[17] However, in the present study, we found no correlations among molecular markers.

Among the most common genetic events that occur in the genesis of tumors, including HNSCC are mutations in the p53 tumor suppressor gene. The level of p53 positive cells as determined with immunostaining has prognostic value for disease-free survival in some studies.[19,20] In HNSCC, the prognostic role of p53 mutations is still unclear due to contradictory reports. In the meta-analysis by Tandon et al.,[12] the studies for oropharynx indicated that p53 overexpression showed a survival advantage ; in contrast the studies for oral cavity indicated that p53 overexpression was correlated with poor survival rate. Meanwhile, Dijkema et al.,[21] showed no relationship between p53 protein expression and prognosis, regardless of treatment modality. The problem of heterogeneity across studies in study-level and patient-level characteristics might have an effect on these discrepancies, making it difficult to ascertain a clear picture. In the present study, we did not find any correlation between the clinicopathologic parameters including survival and the status of TP53 expression. These conflicting results may be explained due to the limitations of immunohistochemistry as a detection method for protein expression status. Alteration of p53 protein function can be caused by a number of mutational and non-mutational mechanisms, which may not be detected by immunohistochemistry assay.[21] In addition, there may be the differences in the demographic characteristics of groups studied. Further studies are required in which p53 expression is investigated in a more standardized and biologically informative manner to establish the prognostic relevance of the molecular marker.

Caspases play an important role in apoptotic signaling pathway.[22] Among caspases, caspases 8, 9 and 10 are considered as an initiator, whereas caspases 3, 6 and 7 are considered as an effector.[13] Apaf-1 has been shown to participate as an adaptor molecule, which binds to Casp-9 and causes its activation, and then activated Casp-9 in turn activates Casp-3.[23] The present study is one of the very few studies in which Caspase-9 and Apaf-1 protein expression are investigated in relation to other molecular markers and prognosis in HNSCC. In the study by Oh et al.,[24] both caspase-6 and -9 were highly expressed in the esophageal squamous cell carcinoma(ESCC), irrespective of the pathological characteristics. On the other hand, these pro-apoptotic molecules are negatively or weakly expressed in normal esophageal mucosa. These results suggest that changes of expression of these proteins might be involved in the development of ESCC. However, they found no

relationship between clinicopathological parameters including survival, with expression of caspase-6 and caspase-9. Likewise, in the present study, the expressions of Caspase-9 and Apaf-1 protein were noted in the greater part of cases. Our study also revealed no correlation between Caspase-9 and Apaf-1 expression with other parameters and patient survival. Meanwhile, caspase-9-dependent apoptosis is shown to play an important role in cisplatin-induced HNSCC apoptosis.[14] Unfortunately, we did not have sufficient clinical informations about adjuvant treatment modalities including chemotherapy or radiotherapy. Thus, we could not analyze the data in this aspect.

There are several studies with respect to the potential role of caspase-9 and Apaf-1 in malignant tumors. Deficiency of caspase-9 and Apaf-1 confers resistance to apoptosis, promotes tumor cell survival and increases tumor aggressiveness in a variety of tumors.[25,26] On the contrary, Papay et al., showed that elevated expression of caspase-9 is significantly associated with the metastatic potential of primary non-small cell lung carcinoma.[27] In addition, the study by Strater et al., found that, in colon carcinomas, expression of caspase-9 is significantly associated with poor survival. Also, caspase-9 may be an independent prognosticator in colon carcinoma.[28] Taken together, those previous studies suggest that caspase-9 might not just act as an inducer of apoptosis but have a more complex function in the pathogenesis of malignant tumors by several mechanisms including alternative splicing.[29] Further studies are required for identifying the clinical significance of caspase-9 and Apaf-1 in conjunction with other apoptosis regulators in HNSCC.

In conclusion, high expression of VEGF was concerned with T stage, indicating that VEGF expression can be molecular marker for prediction of the aggressive behaviors of HNSCC although this result awaits confirmation in a larger study.

References

1) Kaomongkolgit R, Chaisomboon N, Pavasant P. *Apoptotic effect of alpha-mangostin on head and neck squamous carcinoma cells. Arch Oral Biol. 2011;56:483-490.*
2) Zbaren P, Lehmann W. *Frequency and sites of distant metastases in head and neck squamous cell carcinoma. An analysis of 101 cases at autopsy. Arch Otolaryngol Head Neck Surg. 1987; 113:762-764.*
3) Koh JY, Cho NP, Kong G, Lee JD, Yoon K. *p53 mutations and human papillomavirus DNA in oral squamous cell carcinoma: Correlation with apoptosis. Br J Cancer. 1998;78:354-359.*
4) Neville BW, Day TA. *Oral cancer and precancerous lesions. CA Cancer J Clin. 2002;52:195-215.*
5) Kowalski LP, Medina JE. *Nodal metastases: Predictive factors. Otolaryngol Clin North Am. 1998;31:621-637.*
6) Hanahan D, Weinberg RA. *The hallmarks of cancer. Cell. 2000; 100:57-70.*
7) Breslin JW, Pappas PJ, Cerveira JJ, Hobson RW 2nd, Duran WN. *VEGF increases endothelial permeability by separate signaling pathways involving ERK-1/2 and nitric oxide. Am J Physiol Heart Circ Physiol. 2003;284:H92-H100.*
8) Gupta K, Kshirsagar S, Li W, Gui L, Ramakrishnan S, Gupta P, et al. *VEGF prevents apoptosis of human microvascular endothelial cells via opposing effects on MAPK/ERK and SAPK/JNK signaling. Exp Cell Res. 1999;247:495-504.*
9) Sauter ER, Nesbit M, Watson JC, Klein-Szanto A, Litwin S, Herlyn M. *Vascular endothelial growth factor is a marker of tumor invasion and metastasis in squamous cell carcinomas of the head and neck. Clin Cancer Res. 1999;5:775-782.*
10) Danial NN, Korsmeyer SJ. *Cell death: critical control points. Cell. 2004;116:205-219.*
11) Hollstein M, Sidransky D, Vogelstein B, Harris CC. *p53 mutations in human cancers. Science. 1991;253:49-53.*
12) Tandon S, Tudur-Smith C, Riley RD, Boyd MT, Jones TM. *A systematic review of p53 as a prognostic factor of survival in squamous cell carcinoma of the four main anatomical subsites of the head and neck. Cancer Epidemiol Biomarkers Prev. 2010; 19:574-587.*
13) Cohen GM. *Caspases: The executioners of apoptosis. Biochem J. 1997;326(Pt 1):1-16.*
14) Kuwahara D, Tsutsumi K, Kobayashi T, Hasunuma T, Nishioka K. *Caspase-9 regulates cisplatin-induced apoptosis in human head and neck squamous cell carcinoma cells. Cancer Lett. 2000; 148:65-71.*
15) Sarkis SA, Abdullah BH, Abdul Majeed BA, Talabani NG. *Immunohistochemical expression of epidermal growth factor receptor(EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and lymphangiogenesis. Head Neck Oncol. 2010;2:13.*
16) Johnstone S, Logan RM. *The role of vascular endothelial growth factor(VEGF) in oral dysplasia and oral squamous cell carcinoma. Oral Oncol. 2006;42:337-342.*
17) Kyzas PA, Cunha IW, Ioannidis JP. *Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: A meta-analysis. Clin Cancer Res. 2005;11:1434-1440.*
18) Smith BD, Smith GL, Carter D, DiGiovanna MP, Kasowitz KM, Sasaki CT, et al. *Molecular marker expression in oral and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2001;127:780-785.*
19) Cruz I, Napier SS, van der Waal I, Snijders PJ, Walboomers JM, Lamey PJ, et al. *Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. J Clin Pathol. 2002;55:98-104.*
20) Tabor MP, van Houten VM, Kummer JA, Vosjan MJ, Vlasblom R, Snow GB, et al. *Discordance of genetic alterations between*

primary head and neck tumors and corresponding metastases associated with mutational status of the TP53 gene. Genes Chromosomes Cancer. 2002;33:168-177.

21) Dijkema IM, Struikmans H, Dullens HF, Kal HB, van der Tweel I, Battermann JJ. *Influence of p53 and bcl-2 on proliferative activity and treatment outcome in head and neck cancer patients. Oral Oncol. 2000;36:54-60.*
22) Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. *Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Molecular Cell. 1998;1:949-957.*
23) Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al. *Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997;91:479-489.*
24) Oh JE, Kim MS, Kang MR, Kim YR, Kim HS, Yoo NJ, et al. *Expression of apoptosis-related proteins caspase-6, caspase-9, FLIP and BNIP3 in oesophageal squamous cell carcinomas. Pathology. 2010;42:492-493.*
25) Soengas MS, Alarcon RM, Yoshida H, Giaccia AJ, Hakem R, Mak TW, et al. *Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. Science. 1999;284:156-159.*
26) Zlobec I, Minoo P, Baker K, Haegert D, Khetani K, Tornillo L, et al. *Loss of APAF-1 expression is associated with tumour progression and adverse prognosis in colorectal cancer. European Journal of Cancer. 2007;43:1101-1107.*
27) Papay J, Krenacs T, Moldvay J, Stelkovics E, Furak J, Molnar B, et al. *Immunophenotypic profiling of nonsmall cell lung cancer progression using the tissue microarray approach. Appl Immunohistochem Mol Morphol. 2007;15:19-30.*
28) Strater J, Herter I, Merkel G, Hinz U, Weitz J, Moller P. *Expression and prognostic significance of APAF-1, caspase-8 and caspase-9 in stage II/III colon carcinoma: Caspase-8 and caspase-9 is associated with poor prognosis. Int J Cancer. 2010;127:873-880.*
29) Wang P, Shi T, Ma D. *Cloning of a novel human caspase-9 splice variant containing only the CARD domain. Life Sci. 2006;79:934-940.*