J Oral Pathol Med (2005) 34: 329–33

www.blackwellmunksgaard.com/jopm

The high prognostic value of the histologic grade at the deep invasive front of tongue squamous cell carcinoma

H. Kurokawa[1], M. Zhang[2], S. Matsumoto[1], Y. Yamashita[1], T. Tomoyose[1], T. Tanaka[3], H. Fukuyama[2], T. Takahashi[1]

[1]*Second Department of Oral Maxillofacial Surgery,* [2]*Department of Oral Pathology and* [3]*Department of Surgery, Kyushu Dental College, Kitakyushu, Japan*

BACKGROUND: Although many histopathologic factors in squamous cell carcinoma of the tongue predict the prognosis, the major predictive factors have not been identified clearly. This study analyzed the prognostic value of the histologic grade at the deep invasive front of tongue squamous cell carcinoma.

METHODS: The clinicopathologic features of 124 consecutive patients seen between January 1985 and December 1999 with previously untreated squamous cell carcinoma of the tongue were reviewed. Their mean age was 58.5 years (range: 23–90) and the male–female ratio was 1.8: 1 (79 men and 45 women). There were 41, 40, 30, and 13 cases at stage I to stage IV, respectively. The clinicopathologic factors, especially the histologic grade at the deep invasive front (invasive front grade, IFG), were analyzed to determine factors predicting prognosis.

RESULTS: The 5-year disease-free survival rate of the patients treated with curative aim only was 66.7%. Clinicopathologic factors significantly associated with the prognosis were T classification, tumor size, stage classification, tumor depth, macroscopic appearance, cervical lymph node metastasis (nodal metastasis), microvascular invasion, and IFG. In a multivariate analysis, patients with tumor depth ≥4 mm, IFG ≥8 points, and nodal metastasis had a reduced disease-free survival and IFG ≥11 points had a predictive value for nodal metastasis (odds ratio: 7.34; $P = 0.0019$).

CONCLUSION: This study found that a high IFG malignancy score had a high prognostic value for squamous cell carcinoma of the tongue.

J Oral Pathol Med (2005) **34**: 329–33

Keywords: deep invasive front; histologic grade; oral squamous cell carcinoma; prognostic factor; tongue carcinoma

Correspondence: Hideo Kurokawa, Second Department of Oral and Maxillofacial Surgery, Kyushu Dental College, 2-6-1 Manazuru, Kokura Kita-Ku, Kitakyusyu City 803-8580, Japan. Tel: +81-93-582-1131, Fax: +81-93-592-3056, E-mail: kuro@kyu-dent.ac.jp
Accepted for publication April 6, 2004

Introduction

Patients with oral squamous cell carcinoma can die from failure to control the primary lesion or regional lymph node metastases (1–4). An analysis of the prognostic factors is important for predicting the prognosis and achieving a high survival rate in these patients (4–8). Several reports have examined the usefulness of clinical and pathologic factors related to the prognosis of oral squamous cell carcinoma (1–12). These factors have only slightly significant effects on prognosis and have not been well described. Furthermore, few studies have applied multivariate analysis to the clinicopathologic factors associated with cervical lymph node metastasis and prognosis to squamous cell carcinoma of the tongue (4, 11, 13–15). This study is a retrospective review of the clinical and pathologic data on tongue carcinomas, and sought to determine the factors predicting cervical lymph node metastasis and prognosis.

Patients and methods

We reviewed the medical records of 147 patients who were treated for squamous cell carcinoma of the tongue at the Second Department of Oral and Maxillofacial Surgery of Kyushu Dental College between 1985 and 1999. We excluded 23 patients with disseminated disease, other serious illnesses or poor general condition, which made treatment with curative intent impossible. Our study focuses on the remaining 124 patients from whom biopsy specimens with proven tumor infiltration into the connective tissue of sufficient quantity for malignancy grading were obtained. All the patients underwent partial glossectomy, hemiglossectomy, or total glossectomy with immediate reconstruction using skin graft, myocutaneous flaps, or free flaps. No patients received pre-operative treatment. Classical or modified radical neck dissection was performed in all patients with clinically positive neck nodes.

The patients consisted of 79 men (63.7%) and 45 women (36.3%). Their mean age was 58.5 years (range:

23–90). There were 41, 40, 30, and 13 cases at stages I–IV, respectively. The macroscopic appearances were classified as exophytic (superficial, papillomatous, and granulomatous) or endophytic (ulcerative and erosive). Tumor size was expressed as the maximum tumor diameter.

Histopathologic evaluation

Paraffin-embedded specimens were retrieved from the files of the Oral Pathology Department. Serial sections were cut 4–6 μm thick. Alternate sections were stained with hematoxylin and eosin (H & E). The initial biopsy for histologic diagnosis was undergone so that the excised specimen included a portion that was as deep as possible in terms of the tumor-host border, and contained a representative portion to determine the histologic grade of the malignancy.

The histologic grade of the malignancy was determined using the grading system of Anneroth et al. (16) (Anneroth's malignancy). The histologic malignancy grade of the deep invasive front area (invasive front grade, IFG) was determined using the method of Bryne et al. (17). For each tumor, the degree of keratinization, nuclear polymorphism, pattern of invasion, and host response (degree of leukocyte infiltration) were graded and given scores between 1 and 4 (Table 1). Tumor depth was measured from the surface of the normal mucosa to the deepest portion of the tumor (4). Microvascular invasion was also recorded. Two pathologists and two oral surgeons, who were blind to the clinical data, reviewed all the pathologic specimens. The histologic assessments were judged by agreement of three or more reviewers.

Statistical analysis

All the data were tabulated, and statistical tests were performed using the STATVIEW software package (SAS Institute, Cary, NC, USA). The correlation between Anneroth's malignancy and IFG was evaluated using the Spearman rank correlation test. The correlation between clinicopathologic factors and disease-free survival was analyzed using the log-rank test. Survival curves were plotted using the Kaplan–Meier method (18). The prognostic significance of clinicopathologic factors on disease-free survival was assessed using Cox's multivariate proportional hazards regression analysis. The prognostic significance of clinicopathologic factors in cervical lymph node metastasis (nodal metastasis) was assessed using Fisher's exact test, univariate and multivariate logistic regression analyses. The results were considered significant when the $P < 0.05$.

Results

The mean total score of histologic malignancy was 13.2 ± 3.3 points for Anneroth's malignancy and 9.5 ± 2.7 points for IFG. A linear correlation between Anneroth's malignancy and IFG was observed ($P = 0.771$, $R^2 = 0.596$, $P < 0.0001$; Fig. 1). After the selection of cut-off levels, single parameter analysis by means of log-rank test revealed significant results for total malignancy scores. Figure 2 shows the disease-free survival for IFG. Patients with <8 points had significantly better prognoses than did those with more than 8 points.

Univariate analysis of disease-free survival

Forty of the 124 patients died because of failure to control the primary site (25 patients), regional lymph node metastasis (13 patients), or distant metastasis (two patients). The disease-free survival rate at 5 years for the patients was 66.7%. Disease-free survival was correlated with T classification ($P < 0.0001$), tumor size ($P < 0.0001$), Stage classification ($P < 0.0001$), tumor depth ($P < 0.0001$), macroscopic appearance ($P = 0.0051$), nodal metastasis ($P < 0.0001$), microvascular invasion ($P = 0.0024$), and IFG ($P < 0.0001$). Disease-free survival was not correlated with late cervical lymph node metastasis only (Table 2).

Cox's proportional hazard regression analysis of disease-free survival

As with the multivariate analysis of the clinicopathologic factors in disease-free survival, the clinicopathologic factors were divided into five broad categories: tumor size (T-classification and Stage classification), tumor depth, cervical lymph node metastasis, macroscopic appearance, and IFG (Anneroth's malignancy and microvascular invasion). Cox's proportional

Table 1 Histological malignancy grading system by Bryne et al. (17) used in the present study

	Score			
Morphologic feature	*1*	*2*	*3*	*4*
Degree of keratinization	Highly keratinized (>50% of the cells)	Moderately keratinized (20–50% of the cells)	Minimal keratinization (5–20% of the cells)	No keratinization (0–5% of the cells)
Nuclear polymorphism	Little nuclear polymorphism (>75% mature cells)	Moderately abundant nuclear polymorphism (50–70% mature cells)	Abundant nuclear polymorphism (25–50%a mature cells)	Extreme nuclear polymorphism (0–25% mature cells)
Pattern of invasion	Pushing, well delineated infiltrating borders	Infiltrating, solid cords, bands and/or strands	Small groups or cords of infiltrating cells (n > 15)	Marked and widespread cellular dissociation in small groups and/or in single cells (n > 15)
Host response (infiltration of leukocytes)	Marked	Moderate	Slight	None

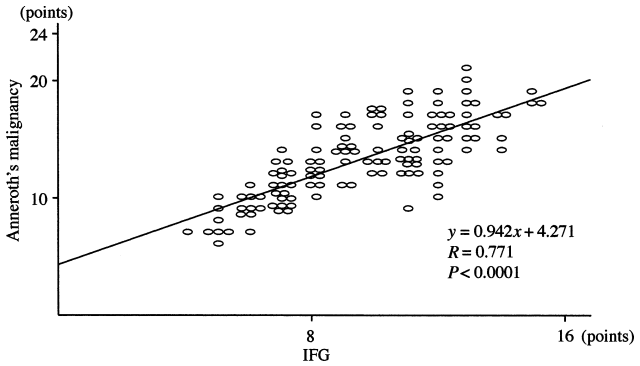

Figure 1 Correlation between Anneroth's malignancy and invasive front grade (IFG).

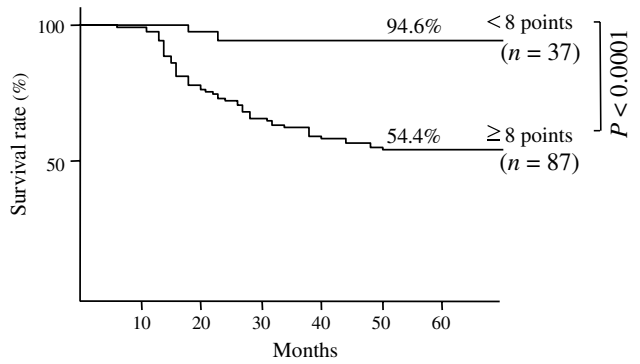

Figure 2 Disease-free survival curves in relation to total invasive front grading score.

Table 2 Univariate analysis of disease-free survival

Factors	*Hazards ratio*	*95% CI*	*P-value*
T classification	2.79	1.99–3.91	< 0.0001
Tumor size			
< 30 mm vs. ≥30 mm	6.10	1.31–11.49	< 0.0001
Stage classification	13.70	6.21–30.30	< 0.0001
Tumor depth			
< 4 mm vs. ≥4 mm	9.75	3.47–27.44	< 0.0001
Macroscopic appearance			
Exophitic vs. endophytic	3.87	1.50–9.99	0.0051
Nodal metastasis			
Absent vs. present	5.45	2.89–10.27	< 0.0001
Late nodal metastasis			
Absent vs. present	1.98	0.913–4.31	0.0835
Microvascular invasion			
Absent vs. present	2.64	1.41–4.93	0.0024
Anneroth's malignancy			
< 13 points vs. ≥13 points	4.21	1.49–10.34	0.0002
IFG			
< 8 points vs. ≥8 points	10.44	2.52–43.32	< 0.0001

hazards regression analysis of these five categories demonstrated that tumor depth ≥4 mm ($P = 0.0360$), nodal metastasis ($P = 0.0211$), and IFG ≥8 points ($P = 0.0035$) had predictive values (Table 3).

Table 3 Cox's multivariate proportional hazards regression analysis of disease-free survival

Factors	*Hazards ratio*	*95% CI*	*P-value*
Tumor size			
< 30 mm vs. ≥30 mm	1.35	0.90–2.04	0.1490
Tumor depth			
< 4 mm vs. ≥4 mm	3.26	1.08–9.82	0.0360
Nodal metastasis			
Absent vs. present	2.41	1.14–5.09	0.0211
Macroscopic appearance			
Exophitic vs. endophytic	1.50	0.56–4.06	0.4229
IFG			
< 8 points vs. ≥8 points	12.64	1.28–24.8	< 0.0035

Table 4 Logistic regression analysis in cervical lymph node metastasis

	Univariate analysis		*Multivariate analysis*	
Factors	*Odds ratio*	*P-value*	*Odds ratio*	*P-value*
Tumor size				
< 30 mm vs. ≥30 mm	4.19	0.0011	2.95	0.176
Macroscopic appearance				
Exophitic vs. endophytic	7.27	0.0003	3.22	0.076
Tumor depth				
< 4 mm vs. ≥4 mm	4.55	0.0010	2.88	0.360
IFG				
< 11 points vs. ≥11 points	3.19	0.0066	7.34	0.0019

Logistic regression analysis of clinicopathologic factors correlated with nodal metastasis

Four clinicopathologic factors that correlated with nodal metastasis were selected: tumor size, macroscopic appearance, tumor depth, and IFG. A comparison between nodal metastasis and total malignancy scores was tested under and above 11 points by mean of Fisher's exact test. If the cut-off level was set at 11 points, a significant difference was seen with number of patients with nodal metastasis. The multivariate logistic regression analysis of the four factors showed a correlation between nodal metastasis and IFG ≥11 points ($P = 0.0019$; Table 4).

Discussion

Numerous studies (8, 10, 12, 15, 16, 19–23) have examined the histologic grading of malignancy in squamous cell carcinoma in the head and neck region since Broder's classification system (24) based on the proportion of differentiated cells to undifferentiated or anaplastic cells was first published. Subsequently, a two-factor grading system involving the degree of cellular differentiation and growth in depth was introduced and has generally been accepted. Broder's classification (24) of squamous cell carcinoma based on the differentiation or maturation of the tumor cell population alone remains of limited value when evaluating both the prognosis and choice of treatment. Jacobsson et al. (25) extended these two factors to four parameters for each and applied the resulting microscopic multifactorial

principle to glottic carcinoma. They found a correlation between the sum of eight parameters and the prognosis. Eneroth and Moeberger, Willen et al. (26), Lund et al. (27), and Crissman et al. (28) subsequently used this system. Anneroth et al. (16) reported that the histopathologic grading system, which is a modification of the method recommended by Jacobsson et al. (25), proved to be of high, independent prognostic value in oral squamous cell carcinoma. Recently, tumor differentiation, mode of invasion, mitotic activity, nuclear polymorphism, microvascular invasion, lympho-plasmacytic infiltration, and histologic grade of the malignancy have all been reported to affect overall survival (5–8, 11, 13–15). However, these factors reflect the characteristics of the entire tumor, and have only a slightly significant effect on prognosis. Generally, most tumors consist of heterogenous cell populations with variable biologic behavior, and tumor behavior is dependent on a complex interrelationship between tumor and host (29–34). Recent evidence suggests that cells present at the invasive tumor front of a carcinoma have different molecular characteristics than those in superficial areas of the tumor, making the invasive tumor front the most important area of the tumor for prognostication (18, 35–37). Bryne et al. (17) first described a multiple histologic grading system for the invasive tumor front area in head and neck tumors, based on the pattern of invasion, degree of keratinization, nuclear polymorphism, and host response. They reported a strong correlation between the total malignancy grade and prognosis in glottic carcinoma. However, few studies have used multivariate analysis to identify the predictive value of the histologic malignancy scores of the invasive tumor front in associated with the prognosis and survival rates in oral squamous cell carcinoma (23, 38, 39). This study analyzed the prognostic value of the histologic grade of malignancy in the deep invasive front in tongue squamous cell carcinoma. Bryne et al. (17) and Kearsley et al. (40) observed strong correlation between the total malignancy grade of several pathologic parameters and prognosis in oral squamous cell carcinoma. In our study, the univariate analysis showed that the 5-year survival rates differed significantly between each strata of clinicopathologic factor: T classification, tumor size, Stage classification, tumor depth, macroscopic appearance, cervical lymph node metastasis, microvascular invasion, and IFG. Cox's multivariate analysis showed that tumor depth ≥4 mm, cervical lymph node metastasis, and IFG ≥8 points were all significant independent prognostic factors for squamous cell carcinoma of the tongue.

Generally, cervical lymph node metastasis in oral squamous cell carcinoma patients indicates a poor prognosis (1, 2, 4, 12, 40–42). The various histopathologic factors reported as reliable parameters for determining regional metastasis are not available preoperatively and so cannot be used to decide on regional treatment (4). In our univariate analysis, tumor size, macroscopic appearance, tumor depth, and IFG all differed significantly between patients with and without cervical lymph node metastasis. Moreover, our multivariate analysis identified IFG $^{3}11$ points as having a powerful predictive value for cervical lymph node metastasis.

We concluded that tumor depth ≥4 mm, IFG ≥8 points, and cervical lymph node metastasis decreased the disease-free survival and IFG ≥11 points had predictive value for cervical lymph node metastasis in squamous cell carcinoma of the tongue. Therefore, the malignancy IFG provides useful prognostic information when selecting the most appropriate treatment modalities.

References

1. Cunningham MJ, Johnon JT, Myers EN, Schramm VL Jr, Thearle PB. Cervical lymph node metastasis after local excision of eraly squamous cell carcinoma of the oral cavity. *Am J Surg* 1986; **152**: 361–6.
2. Spiro RH, Huvos AG, Wong GY, Spiro JD, Gnecco CA, Strong EW. Predictive value of tumor thickness in squamous cell carcinoma confined to the tongue and floor of the mouth. *Am J Surg* 1986; **152**: 345–50.
3. Nyman J, Mercke C, Lindstroem J. Prognostic factors for local control and survival of cancer of the oral tongue: a retrospective analysis of 230 cases in Western Sweden. *Acta Oncologica* 1993; **32**: 667–73.
4. Kurokawa H, Yamashita Y, Takeda S, Zhang M, Fukuyama H, Takahashi T. Risk factor for late cervical lymph node metastases in patients with stage I or II carcinoma of the toungue. *Head Neck* 2002; **24**: 731–6.
5. Nathanson A, Agren K, Bjoerklund A. Evaluation of some prognostic factors in small squamous cell carcinoma of the mobile tongue. *Head Neck Surg* 1989; **11**: 387–92.
6. Tytor M, Olofsson J. Prognostic factors in oral cavity carcinoma. *Acta Otolaryngol* 1992; **112**: 75–8.
7. Overholt SM, Eicher SA, Wolf P, Weber RS. Prognostic factors affecting outcome in lower gingival carcinoma. *Laryngoscope* 1996; **106**: 1335–9.
8. Kurokawa H, Yamashita Y, Murata T, et al. Histological grading of malignancy correlates with regional lymph node metastasis and survival of patients with oral squamous cell carcinoma. *Fukuoka Acta Med* 1989; **89**: 225–31.
9. Yamamoto E, Miyakawa A, Kohama G. Mode of invasion and lymph node metastasis in squamous cell carcinoma of the oral cavity. *Head Neck Surg* 1984; **6**: 938–47.
10. Kurokawa H, Yamashita Y, Ishibashi H, Kajiyama M. Study of histological grading of malignancy in oral squamous cell carcinoma: correlate with prognosis. *Jpn J Oral Maxillofac Surg* 1996; **42**: 1–7.
11. Asakage T, Yokose T, Mukai K, et al. Tumor thickness predicts cervical metastasis in patients with stage I/II carcinoma of the toungue. *Cancer* 1998; **82**: 1443–8.
12. Kurokawa H, Yamashita Y, Murata T. Clinicopathological evaluation of prognostic factors of oral squamous cell carcinoma patients with stage I and II. *J Kyushu Dent Soc* 1998; **52**: 399–404.
13. Morton RP, Ferguson CM, Lambie NK, Whitlock ML. Tumor thickness in eraly tongue cancer. *Arch Otolaryngol Head Neck Surg* 1994; **120**: 717–20.
14. Fukano H, Matsumura H, Hasegawa Y, Nakamura S. Depth of invasion as a predictive factor for cervical lymph node metastasis in tongue carcinoma. *Head Neck* 1997; **19**: 205–10.

15. Yamashita Y, Kurokawa H. Histological grading of malignancy in squamous cell carcinoma of the tongue related to the clinical eavaluation. *Asian J Oral Maxillofac Surg* 2000; **12**: 161–7.
16. Anneroth G, Batsakis J, Lunna M. Review of the literaure and a recommended system of malignancy grading in oral ssquamous cell carcinoma. *Scand J Dent Res* 1987; **95**: 229–49.
17. Bryne M, Jenssen N, Boysen M. Histological grading in the deep invasive front of T1 and T2 glottic squamous cell carcinomas has high prognostic value. *Virchows Arch* 1995; **427**: 277–81.
18. Kaplan E, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–81.
19. Holm LS, Lundqist PG, Silfversward C, Sobin A. Histological grading of malignancy in squamous cell carcinoma of the toungue. *Acta Otolaryngol* 1982; **94**: 185–92.
20. Rasgon BM, Cruz RM, Hilsinger RL, Sawicki JE. Relation of lymph node metastasis to histopathologic appearance in oral cavity and oropharyngeal carcinoma: a case series and literature review. *Laryngoscope* 1989; **99**: 1103–9.
21. Osaki T, Hirota J, Yoneda K, Yamamoto T, Ueta E. Clinical and histopathologic characteristics of tongue and gingiva carcinomas with occult and clinically evident cervical lymph node metastasis. *Int J Oral Maxillofac Surg* 1996; **25**: 274–8.
22. Gluckman JL, Pavelic ZP, Welkoborsky HJ. Prognostic indicators for squamous cell carcinoma of the oral cavity: a clinicopathologic correlation. *Laryngoscope* 1997; **107**: 1239–44.
23. Kurokawa H, Yamashita Y, Matsumoto S, et al. Estimation of invasive front grading and correlation with effect of preoperative chemotherapy in oral squamous cell carcinoma. *Asian J Oral Maxillofac Surg* 2003; **15**: 186–93.
24. Broders AC. Carcinoma of the mouth:types and degrees of malignancy. *Am J Roentgenol Red Ther Nucl Med* 1927; **17**: 90–3.
25. Jacobsson PA, Eneroth GM, Killander D, Moeberger G, Martensson B. Histologic classification and grading of malignancy in carcinoma of the larynx (a pilot study). *Acta Radiol Ther Phys Biol* 1973; **12**: 1–8.
26. Willen R, Nathanson A, Moeberger G, Anneroth G. Squamous cell carcinoma of the gingiva. Histological classification and grading of malignancy. *Zcta Otolaryngol* 1975; **79**: 146–54.
27. Lund C, Sogaard H, Elbrond O, Jorgensen K, Andersen AP. Epidermoid carcinoma of the tongue. Histologic grading in the clinical evaluation. *Acta Radiol Ther Phys Biol* 1975; **14**: 513–21.
28. Crissman JD, Gluckman J, Whiteley J, Quenelle D. Squamous cell carcinoma of the floor of the mouth. *Head Neck Surg* 1980; **3**: 2–11.
29. Yamamoto E, Kohama G, Sunakawa H, Iwai M, Hiratsuka H. Mode of invasion, bleomycin sensitivity and clinical course in squamous cell carcinoma of the oral cavity. *Cancer* 1983; **51**: 2175–80.
30. Odell EW, Jani P, Sherrif M, et al. The prognostic value of individual histologic grading parameters in small lingual squamous cell carcinoma. The importance of the pattern of invasion. *Cancer* 1994; **74**: 789–94.
31. Bundgaard T, Bentzen SM, Wildt J, Sorensen FB, Sogaard H, Nielsen JE. Histopathologic, stereologic, epidemiologic, and clinical parameters in the prognostic evaluation of squamous cell carcinoma ot he oral cavity. *Head Neck* 1996; **18**: 142–52.
32. Jacob R, Welkoborsky HJ, Mann WJ. Heterogeneity of squamous cell carcinomas of the head and neck – analysis of tumor biologic factors and proliferation rates. *Laryngoscope* 1996; **106**: 1170–5.
33. Kurokawa H, Yamashita Y, Takeda S, Miura K, Murata T, Kajiyama M. The expression of proliferating cell nuclear antigen (PCNA) and p53 protein correlate with prognosis of patients with oral squamous cell carcinoma. *Fukuoka Acta Med* 1999; **90**: 6–13.
34. Kurokawa H, Yakashita Y, Takeda S. Estimation of tumor necrosis factor and manganese superoxide dismutase in oral squamous cell carcinoma – available methods for predicting the prognosis and monitoring the treatment. *Asian J Oral Maxillofac Surg* 2000; **12**: 141–8.
35. Bryne M, Koppang HS, Lilleng R, Stene T, Bang G, Dabelsteen E. New malignancy grading is a better prognostic indicator than Broder's grading in oral squamous cell carcinoma. *J Oral Pathol Med* 1989; **18**: 432–7.
36. Bryne M. Prognostic value of various molecular and cellular feartures in oral squamous cell carcinoma. *J Oral Pathol Med* 1991; **20**: 413–20.
37. Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margines of oral squamous cell carcinomas has high prognostic value. *J Pathol* 1992; **166**: 375–81.
38. Piffko J, Bankfalvi A, Oefner D. Prognostic value of histological factors (malignancy grading and AgNOR content) assessed at the invasive front of oral squamous cell carcinoma. *Br J Cancer* 1997; **75**: 1543–6.
39. Bankfalvi A, Piffko J. Prognostic and predictive factors in oral cancer: the role of the invasive tumor front. *J Oral Pathol Med* 2000; **29**: 291–8.
40. Kearsley JH, Bryson G, Battistutta D, Collins RJ. Prognostic importance of cellular DNA content in head and neck squamous cell cancer. *Int Cancer* 1991; **47**: 31–7.
41. Teichgraeber JF, Clairmont AA. The incidence of occult metastases for cancer of oral tongue and floor of the mouth: treatment rationale. *Head Neck Surg* 1984; **7**: 15–21.
42. Fakih AR, Rao RS, Borges AM, Patel AR. Elective versus therapeutic neck dissection in early carcinoma of the toungue. *Am J Surg* 1989; **158**: 309–13.