Review

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Prognostic relevance of cell proliferation in head and neck tumors

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Cell proliferative activity has been extensively investigated in head and neck tumors. Ki67/MIB-1 immunostaining, tritiated thymidine or bromodeoxyuridine labeling indices, DNA S-phase fraction, proliferating cell nuclear antigen expression, potential doubling time and analysis of the nucleolar organizer region associated proteins (AgNORs) have shown significant correlation with prognosis in 4806 cases of tumors of the oral cavity, salivary glands, pharynx and larynx. However, this was not observed in 2968 other reported cases. Discrepancies may depend on various factors: the heterogeneity of the series, which include tumors from various anatomic sites and patients treated with different therapy, and the lack of standardization of methods for assessing cell proliferation. Furthermore, none of the methods currently applied can by themselves define the actual proliferative activity, as it depends both on the proportion of cells committed to the cycle (growth fraction) and the speed of the cell cycle. Indeed, the actual proliferative activity of a tumor could well be measured by the equation [*PA* = Ki67 or MIB-1 scores × AgNORs], as we did in pharyngeal carcinoma. Provided that large and homogeneous series are evaluated by standardized methods, cell proliferative activity can still be regarded as an inexpensive and reliable prognostic factor in head and neck tumors.
Key words: cell proliferative activity, head and neck tumors, prognosis

Introduction

Cell proliferation is regarded as one of the most important biological mechanisms in oncogenesis [1]. A survey of the results of a large number of studies has shown that proliferative activity is of high prognostic significance in several types of cancer [2]. A Medline-based search (PubMed of the National Library of Medicine, via Internet) up to July 2003 selected 6305 reports containing the terms 'proliferative activity and tumor'; 4373 reports containing the terms 'proliferative activity' and 'tumor diagnosis', and 1005 containing the terms 'proliferative activity' and 'tumor prognosis'.

Cell proliferation has also been extensively investigated in head and neck tumors: up to July 2003, 306 papers have been published on the proliferative activity in tumors of the oral cavity, salivary glands, pharynx and larynx.

The aim of this review is to discuss and summarize the results obtained so far as to the proliferative activity of head and neck tumors, in an effort to verify if cell proliferation can really provide useful prognostic information in these tumors or not.

Proliferation markers in tumor pathology

Proliferation markers can be classified into three main categories: (i) growth fraction markers; (ii) markers of specific phases of the cell cycle; and (iii) cell cycle time markers. The growth fraction, i.e. the proportion of the cells committed to the cycle, may be easily assessed by Ki-67 or MIB-1 antibodies, which identify an antigen expressed in G_1, S and G_2 phases of cycling cells [3, 4]. The M-phase can be evaluated by counting the mitotic figures: this is the oldest and, probably, the most popular way of assessing proliferation, even if strict morphological criteria for the recognition of mitotic figures are required [5]. S-phase fraction (SPF) can be assessed by incorporation techniques, such as the *in vivo* or *in vitro* incorporation with tritiated thymidine (TH3) or bromodeoxyuridine (BrdU), which can be regarded as the 'gold standard' marker of S-phase cells [1]. SPF can also be detected by static or flow cytometry (FCM) analysis of the DNA, or the immunohistochemical detection of proliferating cell nuclear antigen (PCNA/Cyclin), a nuclear protein involved in DNA synthesis [6]. The very reliable punctuated labeling of PCNA is identical to the labeling pattern obtained with BrdU [7] and is the method of choice of evaluating the S-phase index in histopathology [8]. Cell cycle time can be

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Table 1. Cell proliferative activity in oral cavity carcinoma

Parameter	Reference	No. of cases	Response
Prognostic value			
Ki67/MIB-1 immunostaining	Girod et al. [41]	216	Survival
	Matsumoto et al. [42]	20	N
	Sittel et al. [34]	56	DFS
	Xie et al. [43][a]	85	DFS
PCNA immunostaining	Storkel et al. [44][a]	100	Grade, survival[b]
	Alcalde et al. [45]	33	T, N
	Gasparini et al. [36][a]	73	OS[b], DFS
	Tsai and Jin [46]	38	T
	Daniele et al. [47]	54	N
	Welkoborsky et al. [48][a]	42	OS[b], DFS
	Sakurai et al. [49]	36	T, N, OS
TH3/BrdU-LI/T_{pot}	Chauvel et al. [50][a]	87	OS[b]
	Hemmer [51]	33	T, N
	Mukhopadhyay et al. [52]	27	Grade, T, N
	Veneroni et al. [14][a]	69	OS[b]
	Corvò et al. [33]	35	DFS
DNA-SPF	Gomez et al. [53][a]	38	OS[b]
	Oya and Ikemura [54][a]	79	N[b]
AgNORs	Sano et al. [55]	39	OS
	Hirsch et al. [56]	66	Stage
	Teixeira et al. [57][a]	43	DFS[b]
	Chatterjee et al. [58]	39	Grade, T
	Piffko et al. [15][a]	80	OS[b], DFS[b]
	Piffko et al. [59][a]	94	Metastasis[b],OS[b]
	Xie et al. [60][a]	51	OS[b], DFS[b]
	Xie et al. [61][a]	80	DFS[b]
Total		1613	
No prognostic value			
Ki67/MIB-1 immunostaining	Roland et al. [23][a]	79	OS, N
	Valente et al. [28][a]	31	DFS
	Gonzalez-Moles et al. [62]	74	OS
	Piffko et al. [63][a]	100	OS
	Stoll et al. [64]	107	OS, DFS
	Bettendorf and Herrmann [65]	329	T, N, OS
	Carinci et al. [66][a]	25	OS
	Okamoto et al. [67]	59	N
PCNA immunostaining	Sommer and Olofsson [68]	64	T, N, DFS, OS
	Sittel et al. [34]	56	DFS
TH3/BrdU-LI/T_{pot}	Cooke et al. [22]	105	T, N, OS
	Costa et al. [35][a]	100	DFS
DNA-SPF	Mohr et al. [20]	142	OS
Total		1271	

[a]Studies with multivariate analysis.

[b]Studies in which the proliferative activity has prognostic significance in multivariate analysis.

DFS, disease-free survival; N, N stage; OS, overall survival; T, T stage.

evaluated by the potential doubling time (T_{pot}), a procedure that requires *in vivo* intravenous BrdU infusion and bivariate FCM [9], or by the quantification of the argyrophilic proteins associated with the nucleolar organizer regions (AgNORs), loops of DNA which transcribe to ribosomal RNA [10]. AgNOR proteins can be easily detected on routinely fixed and paraffin embedded tissues [11]. The AgNOR quantity is strictly related to the rapidity of cell proliferation: the higher the AgNOR quantity, the shorter the doubling time [12, 13].

Oral cavity carcinoma

A large number of papers, reporting a total of 1613 cases, have shown the prognostic value of cell proliferation in squamous cell carcinoma (SCC) of the oral cavity. In general, a high proliferative activity is associated with poor prognosis, even if one series of 69 oral/oropharyngeal SCC patients, who were treated with surgery alone, reported a high TH3-LI (where LI is labeling index) associated with a better prognosis [14]. Almost 30% of the cases (492 of 1613) were investigated using AgNOR analysis, even if this method of assessing cell proliferation is not extensively applied to tumor pathology. Of particular interest are the results obtained by Piffko et al. [15] in 80 SCC patients, where the quantity of AgNORs at the invasive front of the tumor was reported to be the most significant independent prognostic factor. This finding not only provides a functional background to the well-established clinical relevance of the histopathological tumor front grading [16], but also suggests the use of an aggressive surgical approach for those patients with a high AgNOR quantity. However, in a large number of papers, reporting a total 1271 cases, cell proliferative activity in oral SCC lacks any prognostic value. Literature reports are summarized in Table 1.

Curiously, we noted no paper reporting a lack of prognostic significance for AgNOR analysis. This might well imply that

Table 2. Cell proliferative activity in salivary gland tumors

Parameter	Reference	No. of cases	Response
Prognostic value			
Ki67/MIB-1 immunostaining	Skalova et al. [69]	33	DFS, OS, grade
	Skalova et al. [70]	30	Recurrence
	Hellquist et al. [71]	32	OS
	Nordgard et al. [72][a]	44	DFS[b]
	Yin et al. [73]	71	OS, grade
	Okabe et al. [74][a]	31	OS, grade
	Xin and Paulino [75]	18	Grade, metastasis
PCNA immunostaining	Batsakis [40][a]	43	OS[b]
	Cho et al. [76]	30	OS, DFS
DNA-SPF	Greiner et al. [77]	45	OS
	Hicks et al. [78][a]	48	OS[b]
	Franzen et al. [79][a]	51	DFS[b]
	Pinto et al. [80]	26	Grade
	Junquera et al. [81]	44	T, DFS
AgNORs	Chomette et al. [82]	17	OS, DFS
	Chomette et al. [83]	31	OS, DFS
	Vuhahula et al. [84]	34	Metastasis
	Xie et al. [85][a]	37	DFS[b]
Total		665	
No prognostic value			
PCNA immunostaining	Felix et al. [86][a]	30	OS
DNA-SPF	Timon et al. [87][a]	45	Recurrence, N, OS
	Felix et al. [86][a]	30	OS
AgNORs	Fonseca and Soares [88]	18	Metastasis, OS
	Timon et al. [87][a]	45	Recurrence, N, OS
Total		168	

[a]Studies with multivariate analysis.

[b]Studies in which the proliferative activity has prognostic significance in multivariate analysis.

DFS, disease-free survival; N, N stage; OS, overall survival; T, T stage.

the rapidity of cell proliferation, as assessed by AgNOR quantification, is probably the kinetic parameter which correlates better with the outcome of oral cavity SCC.

Salivary gland tumors

A lot of papers, reporting a total of 665 cases, have shown that high proliferative activity is associated with a worse prognosis in salivary gland tumors of various histological type. In particular, high AgNOR counts were indicators of poor overall survival (OS) and/or disease-free survival (DFS) in 119 cases from four different series. Three authors, however, reporting a total of 168 cases, did not confirm the prognostic value of cell proliferation in salivary gland tumors. A summary of the reports in the literature is shown in Table 2.

Pharyngeal carcinoma

To the best of our knowledge, literature reports few papers that have investigated the cell proliferative activity of pharyngeal carcinoma. Eight, for a total of 451 cases, show an association between cell proliferation and prognosis in pharyngeal carcinoma. However, five other authors, for a total of 317 cases, did not confirm the prognostic value of cell proliferation. The results of the literature are shown in Table 3.

Laryngeal carcinoma

Cell proliferation evaluation has been used most extensively in the investigation of laryngeal carcinomas, and a large number of publications, reporting a total of 2077 cases, have shown its prognostic value. In general, high proliferative activity is associated with a worse prognosis, even if high Ki67 scores were associated with a favorable response to radiotherapy in one series of 36 T2 laryngeal cancers [17], and a low DNA-SPF was found to predict recurrence in 23 glottic carcinomas that achieved complete local remission after curative radiotherapy [18]. However, a few studies, reporting a total of 1212 cases, failed to demonstrate the prognostic value of cell proliferative activity in laryngeal carcinoma. Table 4 shows a summary of the literature reports.

Personal findings

A total of 94 cases of pharyngeal carcinoma were retrospectively investigated in our laboratory. Patient age ranged from 29 to 87 years (mean, 60.1 years) of which 68 were male and 26 female. Forty-two carcinomas were from the oro-rhinopharynx and 52 from hypopharynx-pyriform sinus. All cases were squamous cell carcinoma; according to UICC [19], 34 were grade 2 and 60 grade 3; 12 were stage T1, 25 T2, 36 T3 and 21 T4; 43 were N0, 18 N1, 5 N2 and 28 N3. Out of 42 oro-rhinopharyngeal carcinomas, 10 (23.8%) were stages 1–2 and 32 (76.2%) stages 3–4. Out of 52 hypopharynx-pyriform sinus carcinomas, seven (13.5%) were stages 1–2 and 45 (86.5%) stages 3–4. After diagnosis, all patients underwent only external ^{60}Co radiotherapy. A minimum follow-up of 3 years for censored (surviving) patients or until death was available in all cases. Initial biopsies, fixed in 10% formalin and embedded in paraffin, were stained with the AgNOR

Table 3. Cell proliferative activity in pharyngeal carcinoma

Parameter	Reference	No. of cases	Response
Prognostic value			
Ki67/MIB-1 immunostaining	Pich et al. [89][a]	28	OS[b]
	Raybaud et al. [31][a]	56	Recurrence[b]
PCNA immunostaining	Pich et al. [26]	45	OS
TH3/BrdU-LI/T_{pot}	Chauvel et al. [50][a]	87	OS[b]
	Corvò et al. [21]	31	Recurrence
	Benazzo et al. [90]	52	N
DNA-SPF	Myers et al. [91]	91	OS, N, grade
AgNORs	Pich et al. [25][a]	61	OS[b]
Total		451	
No prognostic value			
Ki67/MIB-1 immunostaining	Roland et al. [23][a]	79	Metastasis, OS
	Shnayder et al. [92]	54	Recurrence OS
BrdU-LI/T_{pot}	Bourhis et al. [29][a]	70	Recurrence T, N, DFS, OS
DNA-SPF	Del Valle-Zapico et al. [93][a]	51	T, OS
AgNORs	Pak et al. [37][a]	63	OS, DFS
Total		317	

[a]Studies with multivariate analysis.

[b]Studies in which the proliferative activity has prognostic significance in multivariate analysis.

DFS, disease-free survival; N, N stage; OS, overall survival; T, T stage.

method [11] and PCNA and MIB-1 immunostaining using PC10 and MIB-1 monoclonal antibodies.

In carcinomas of the oro-rhinopharynx, the median AgNOR count was 10.32 AgNORs/cell and the median PCNA score 36.12. No association was found between AgNOR counts or PCNA scores and tumor histological grade or stage. The median OS for the whole series was 48 months, and the 3- and 5-year survival rates were 60% and 49%, respectively. Cell proliferation was the only significant prognostic factor: using the median values as the cut-off, 3-year survival rates

Table 4. Cell proliferative activity in laryngeal carcinoma

Parameter	Reference	No. of cases	Response
Prognostic value			
Ki67/MIB-1 immunostaining	Hake et al. [94]	47	Recurrence, DFS
	Welkoborsky et al. [95][a]	40	Recurrence[b]
	Franchi et al. [96][a]	60	N[b]
	Kropveld et al. [17]	36	Recurrence
	Golusinski et al. [97]	120	T, N, grade
	Valente et al. [98][a]	102	DFS[b]
	Sittel et al. [38]	47	Recurrence, DFS
	Lazaris et al. [99][a]	96	Recurrence, N[b]
	Wozniak et al. [100]	55	T, N
PCNA immunostaining	Munck-Wikland et al. [27]	28	Recurrence
	Welkoborsky et al. [95][a]	40	OS[b]
	Franchi et al. [96][a]	60	N[b]
	Dobros et al. [101]	90	N, OS
	Krecicki and Jelen [102][a]	154	OS[b]
	Sarac et al. [103][a]	92	Grade, N, DFS[b]
	Dong et al. [104][a]	54	OS, DFS
	Sittel et al. [38]	47	Recurrence, DFS
	Dong et al. [105]	102	OS
BrdU-T_{pot}	Alsner et al. [32]	58	OS
DNA-SPF	Tennvall et al. [18]	23	Recurrence
	Tomasino et al. [106][a]	99	OS[b], DFS[b]
	Danic et al. [107][a]	36	OS[b]
	Myers et al. [91]	91	OS, Grade, N
	Dobros et al. [108][a]	90	OS[b]
	Bazan et al. [109][a]	64	OS
AgNORs	Bockmuhl et al. [110][a]	30	OS[b], DFS[b]
	Xie et al. [111][a]	93	DFS[b]
	Xie et al. [112]	69	DFS
	Krecicki et al. [113][a]	154	OS[b]
Total		2077	
No prognostic value			
Ki67/MIB-1 immunostaining	Roland et al. [23][a]	79	N, OS
	Resnick et al. [24]	88	N, OS
	Spafford et al. [114][a]	70	Metastasis, OS
	Hirvikoski et al. [115][a]	103	DFS, OS
	Pulkkinen et al. [116][a]	100	OS
	Krecicki et al. [117][a]	154	T, N
PCNA immunostaining	Resnick et al. [24]	88	N, OS
	Tomasino et al. [106][a]	99	OS, DFS
	Hirvikoski et al. [115][a]	103	DFS, OS

Table 4. (*Continued*)

Parameter	Reference	No. of cases	Response
DNA-SPF	Resnick et al. [24]	88	N, OS
AgNORs	Yamamoto et al. [118]	73	OS
	Barnes et al. [119]	53	T, N, recurrence, OS
	Pulkkinen et al. [120]	66	DFS, OS
	Klatka and Skomra [121]	48	T, N, OS
Total		1212	

[a]Studies with multivariate analysis.

[b]Studies in which the proliferative activity has prognostic significance in multivariate analysis.

DFS, disease-free survival; N, N stage; OS, overall survival; T, T stage;

were 81% for patients with ≤10.32 AgNORs/cell but only 38% for those with higher counts ($P = 0.0002$); 87% for patients with PC10 scores ≤36.12, but only 40% for those with higher scores ($P = 0.0002$). In Cox multivariate survival analysis, only PCNA appeared as an independent variable ($\chi^2 = 13.56$; $P < 0.001$; rr = 8.16). When PCNA was excluded, AgNOR count emerged as an independent variable ($\chi^2 = 13.79$; $P < 0.001$; rr = 5.71).

In carcinomas of the hypopharynx-pyriform sinus, the median AgNOR count was 11.27 AgNORs/cell and the median PCNA score 40. No association was found between AgNOR counts or PCNA scores and tumor histological grade or stage. The median MIB-1 score was 33.87 and was higher in G_3 than in G_2 carcinomas ($P = 0.008$). The median OS for the whole series was 20 months, and the 3- and 5-year survival rates were 26% and 22%, respectively. Of the classical parameters, histological grade ($P = 0.009$) and T stage ($P = 0.04$) were associated with OS. Using the median values as a cut-off, the 3-year survival rates were 48% for patients with ≤11.27 AgNORs/cell, but only 4% for those with higher counts ($P < 0.0001$); 71% for patients with PC10 scores ≤40 but only 14% for those with higher scores ($P = 0.01$); 47% for patients with MIB-1 scores ≤33.87 and 0% for those with higher counts ($P < 0.0001$). In Cox multivariate survival analysis, only MIB-1 scores ($\chi^2 = 18.53$; $P < 0.001$; rr = 6.06) and AgNOR counts ($\chi^2 = 3.89$; $P = 0.04$; rr = 3.34) appeared as independent prognostic variables.

Comments

Even if most of the papers published to date show a correlation between high proliferative activity and poor prognosis in head and neck tumors, a large number of reports failed to show any significant association with prognosis. There are several reasons which may account for this discrepancy.

Anatomic site

In the last edition of the TNM classification of malignant tumors [19], head and neck tumors include tumors of the lip, oral cavity, pharynx, larynx, maxillary sinus, nasal cavity and ethmoid sinus, salivary gland and thyroid gland. Therefore, the series which generically deal with tumors of the 'head and neck' [20–23] are heterogeneous, mixing tumors of different histological type, biological activity and, consequently, clinical behavior. Our experience with pharyngeal carcinoma indicates that it is better not to consider pharyngeal tumors from different anatomic sites as a whole. In fact, the median OS was 23 months when the whole series of 94 cases was considered; however, when cases were subdivided according to the anatomic site, the median OS of the 42 oro-rhinopharyngeal carcinomas was 48 months, significantly longer than that of the 52 hypopharynx-pyriform sinus cases (20 months, $P = 0.002$). The different survival rates were not dependent on differences in clinical stage, since the proportion of low and advanced cases was comparable in the two anatomic sites ($P = 0.3$).

Type of therapy

Literature reports some series of head and neck tumors treated with surgery alone [14, 24], and others with radiotherapy alone [18, 21, 25–32]. Other series were treated with combined surgery and radiotherapy [33, 34], combined surgery and chemotherapy [35], or combined radio- and chemotherapy [36, 37], and there were also a few cases treated with laser therapy [38]. Since different therapies, especially chemo- or radiotherapy, interfere with biological tumor activities, only homogeneously treated series of head and neck carcinomas should be compared if the prognostic value of cell proliferation is to be assessed reliably. For instance, TH3-LI provided independent prognostic information in oral and oropharyngeal carcinomas treated with surgery alone [14], but did not appear to be a predictive factor in patients with carcinoma of the same anatomic site submitted to primary chemotherapy followed by surgery [35].

Standardization

There is a lack of standardization in the various methods for assessing cell proliferation. The number of mitotic figures can be evaluated in several ways: (i) the mitotic index (MI) (total number of mitoses in 10 high power fields); (ii) the mitotic rate (MR) (number of mitoses per 1000 cells); or (iii) the number of mitoses for the area percentage of epithelium, which yields the mitoses per volume (M/V) index [1]. Of course, the different procedures lead to different mitosis

counts. The use of various antibody dilutions, or antigen retrieval, can lead to different results in immunohistochemical staining e.g. most of our PC10 immunonegative pharyngeal carcinomas became positive after microwave irradiation. Moreover, different DNA indices and SPF values are obtained when fresh or fixed tissues are processed. Lastly, the positivity or negativity of a case may depend on the different cut-off values of the proliferation markers. For instance, we chose the median MIB-1 value (33.87) as a cut-off in our series of hypopharyngeal carcinomas and found 14 (50%) negative cases; however, only between one and three cases would have been negative if a cut-off of 15–20% (similar to the cut-off currently used for mammary carcinomas) had been applied.

Site of measurement

The site of measurement is another point, as heterogeneity is one of the hallmarks of malignancy, and heterogeneity for several proliferation markers has been documented for many types of tumors. A large tumor specimen may be classified positive or negative depending on whether the proliferation markers are evaluated only at the most 'active' sites (e.g. the invasive tumor front) [15], in randomly selected areas [25, 26] or in the maximally positive tumor field [17].

Identification of neoplastic cells

There is a difficulty in identifying the neoplastic cells. It is often hard to distinguish by MIB-1 or PCNA immunostaining small neoplastic cells from reactive proliferating lymphocytes in poorly differentiated rhinopharyngeal carcinomas. AgNOR staining is particularly suitable in such cases, since it allows for an easy distinction between neoplastic and non-neoplastic cells, avoiding the need for double (Citokeratin/MIB-1) staining.

Proliferative activity

Finally, what was indeed measured in most studies reported is not the actual proliferative activity of a tumor, in as much as the mechanisms responsible for proliferative activity are the proportion of cells committed to cycle (growth fraction, or G) and the speed of the cycle, which is inversely proportional to the generation time (*T*) [1]. The mathematical relationship between proliferative activity (*PA*), growth fraction (*G*) and generation time (*T*) is $PA = G/T$ [1]. It is clear from this equation that neither the growth fraction nor the generation time can by itself define the actual proliferative activity of a tumor [39]. The tumor growth fraction can be easily assessed on routinely processed tissues by the Ki67 or MIB-1 labeling index, since Ki67 or MIB-1 antibodies recognize an antigen expressed in all cycling cells [3, 4]. On the contrary, evaluation of the speed of cell cycle by the potential doubling time (T_{pot}) is a rather lengthy procedure and is not suitable for retrospective studies. However, since the AgNOR quantity reflects the rapidity of cell proliferation [12, 13], and can be detected in routinely fixed and embedded tissues [11], AgNOR analysis can be regarded as an easy and reliable technique to evaluate the tumor cell doubling time on histological preparations. Therefore, the 'actual' proliferative activity of a tumor can be simply expressed by the equation (*PA* = Ki67 or MIB-1 scores × AgNOR quantity). To the best of our knowledge, this equation, which represents the 'true' proliferative activity of a tumor, has never been applied to head and neck tumors. We used this formula to assess cell proliferation in 28 carcinomas of the hypopharynx-pyriform sinus. The median OS for the whole series was 18 months, and the 3- and 5-year survival rates were 24% and 19%, respectively. Using the median (MIB-1 × AgNOR) value as a cut-off, the median survival for cases with (MIB-1 × AgNOR) ≤407.61 was 23 months, but only 11 months for those with higher values (P <0.0001). Noteworthy, after 20 months of follow-up, 79% of patients with a low (MIB-1 × AgNOR) index were alive, whereas patients with higher values were not (Figure 1). We are aware that the number of our cases is rather small and this approach should be validated in a large series of patients.

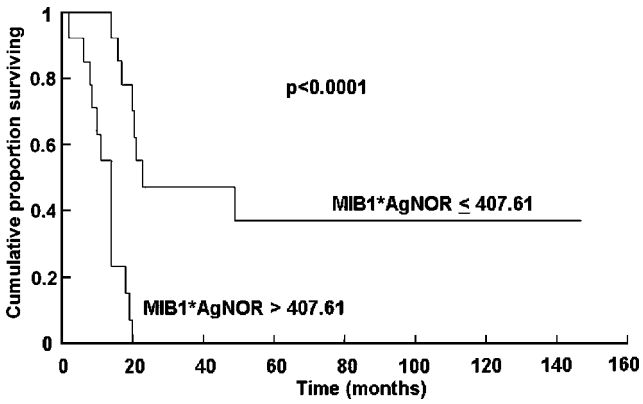

Figure 1. Kaplan–Meier survival curves (OS) for 28 hypopharynx-pyriform sinus carcinomas categorized according to the median (MIB-1 × AgNOR) value.

Summary

Despite the many negative reports in the literature, cell proliferative activity is a reliable prognostic factor in head and neck tumors, especially when studies are performed with well standardized methods that take into account the type of treatment used. Indeed, a high cell proliferation, as expressed by the MIB-1 labeling index, was a significant indicator for treatment failure in a large matched-pair study design of recurrent and non-recurrent oral and oropharyngeal carcinomas initially treated with primary surgery combined with curative post-operative radiation [34]. Also, in another large matched-pair study on recurrent and non-recurrent laryngeal carcinomas, homogeneous for site (glottis), stage (T1 and T2) and treatment (transoral laser surgery alone), investigated using well standardized MIB-1 and PCNA staining and scoring, high proliferative activity appeared to be a significant prognostic factor. Lastly, the standardized AgNOR analysis showed the strong independent prognostic value of cell proliferation in a large series of oral squamous cell carcinoma [15].

An increasing number of sophisticated techniques, such as molecular biology, are currently being applied to tumor pathology to investigate the oncogene alterations responsible for cell proliferation changes. However, since the proliferation changes during the cell cycle reflect the net effect of genetic damage and, therefore, include the accumulated changes in genes, they may be regarded as more useful prognostic indicators than individual oncogene alterations [40].

In conclusion, provided that large series of cases, homogeneous for anatomic site and type of treatment, are evaluated by well standardized methods, cell proliferative activity still seems to be an inexpensive and reliable prognostic factor in head and neck tumors.

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