

# Ki-67 index and skin carcinomas with skull base invasion: a case–control study

## Research Article

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**Abbreviations:** Basal cell carcinoma, (BCC); Squamous cell carcinoma, (SCC)

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## Summary

Skin carcinomas may be very aggressive. Cell proliferation, measured by expression of Ki-67 antigen, has been associated with tumor aggressiveness, but controversy still persists. In this study, the Ki-67 index in basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) with skull base invasion was compared with tumors with good outcome. Expression of Ki-67 was graded as mild (if present in <30% of tumor cells), moderate (between 30% and 50%) and intense (more than 50%), in 24 BCCs and 11 SCCs with skull base invasion. Control group included 23 BCCs and 10 SCCs. Intense expression of Ki-67 was noted in 37.50% of BCCs with skull base invasion, compared to 13.04% in the control group ( $p=0.155$ ). Regarding SCCs, intense expression of Ki-67 was found in 72.73% of aggressive tumors, compared to 20.00% in the control group ( $p=0.050$ ). Ki-67 index was higher among skin cancers with skull base invasion, compared to controls with good outcome. However, this difference reached statistical significance only in SCCs.

## I. Introduction

Basal cell carcinoma (BCC) of the skin is the most common human malignant tumor (Lang and Maize, 1991). Squamous cell carcinoma (SCC) is the second most frequent type. Both occur more frequently in caucasians after the sixth decade, who had experienced prolonged exposure to sunlight (Freeman, 1976). Several factors have been associated with increased aggressiveness of these tumors: histopathological subtype, differentiation, depth of invasion and perineural invasion, among many others (Ruhoy et al, 2001). In addition, some authors have showed the relationship between some biological factors and cancer behavior (Cernea et al, 2004).

Sometimes, these skin carcinomas may be extremely aggressive, and despite adequate treatment, they may recur and invade fascia, muscle and bone. Due to the deformity, some authors call them horrifying tumors (Jackson and Adams, 1973; Bianchini and Wolter, 1984; Horlock et al,

1998). As a matter of fact, skin carcinomas with skull base involvement represent the main histological types of tumors in some series of oncological base of skull operations (Medina dos Santos et al, 1994; Cernea et al, 1997; Dias et al, 1997).

Ki-67 is a nuclear protein expressed during active phases of cell cycle (G1, S, G2 and M), being absent in G0 “resting” phase (Kanitakis et al, 1997). The precise mechanism of its action is still unknown, but its presence is essential for cell proliferation (Healy et al, 1995). Immunohistochemical detection of Ki-67 expression allows a quantitative measure of proliferation potential of a particular neoplasia (Gonzalez-moles et al, 1996). Hence, less proliferative tumors exhibit Ki-67 expression in a percentage lower than 30% of its cells. In contrast, more aggressive cancers have Ki-67 expression in more than 50% of cells (Bacchi and Gown, 1993).

Some authors have analyzed the prognostic value of

Ki-67 as a tumor marker, and suggest an important prognostic role in different types of cancer, but results have been somewhat controversial (Lavertu et al, 2001; Fumic-Dunkic et al, 2003; Liu et al, 2003; Koch and Sidransky, 2004).

Regarding BCC, Baum et al, 1993 noted intense Ki-67 expression index in 32.90% of 62 BCCs. Abdelsayed et al, 2000 found a 51% increased expression rate in a group of 20 cancers. Healy et al, 1995 compared three groups of BCC surgical specimens: 17 non-recurrent tumors, 17 initial specimens of cancers that recurred later and their corresponding specimens of the recurrences. They found a statistically significant higher Ki-67 expression among the recurrent tumors. However, Horlock et al, 1998 compared Ki-67 expression of 81 BCCs with 22 horrifying cancers, with no statistical difference. Interestingly, this group reported an increased frequency of expression among aggressive histological subtypes (mainly morphea-like), and this finding was confirmed by other authors (Barrett et al, 1997).

Kerschmann et al, 1994 noted Ki-67 expression in 46% of 20 patients with SCC. Mansoor et al, 1996 studied Ki-67 expression in a series of 175 SCCs, finding a positive statistical correlation with differentiation, thickness and depth of invasion; however, they could not demonstrate any statistical relationship with recurrence. Similarly, Kanitakis et al, 1997 compared Ki-67 expression between 14 aggressive and 28 non-aggressive SCCs, with no difference.

The objective of this study was to analyze the proliferation index, using immunohistochemical evaluation of Ki-67 with antibody the monoclonal antibody MIB-1, in a consecutive series of very aggressive skin carcinomas with skull base invasion submitted to combined craniofacial oncological operations. In addition, these findings were compared with skin carcinomas with good outcome, treated in the same Institution within the same time frame, in a case-control study.

## II. Materials and Methods

### A. Patients

A retrospective review the cases with very advanced BCC or SCC with skull base involvement treated at the Department of Head and Neck Surgery of the University of São Paulo Medical School, Brazil, was undertaken. Only cases with enough tumor tissue in the paraffin-embedded blocks to harvest slides for immunohistochemistry were included. Thirty-five patients constituted the two study groups: Group 1: 24 BCCs (**Figure 1**) and Group 2: 11 SCCs. Seventeen patients (71%) in patients of Group 1 and five patients (50%) in Group 2 had recurrent tumors, after surgery and/or radiotherapy. One patient (4.17%) in Group 1 and four patients (36.36%) in Group 2 had lymph node metastasis. Two control groups included patients with BCCs and SCCs located on the head and neck area, treated at the Dermatology Department of the same Institution, with no recurrence for a minimum follow-up of 24 months (median follow-up period: 32.2 months): Group 3: 23 BCCs and Group 4: 10 SCCs.

### B. Immunohistochemical analysis

The procedure described by Hsu et al, in 1981 was employed. Representative slides obtained from the tumors were washed with a buffered saline solution at pH 7.4. They were then

incubated in a buffered citrate solution at pH 6.4 for 15 minutes (Gown et al, 1993). Then, slides were incubated with specific primary antibody against Ki-67 (clone MIB-1), diluted 1:50, for 12 hours, at temperature of 4°C. After washing with buffered saline solution, slides were incubated for 60 minutes with biotinylated antibodies anti-IgG (Vector Corp., USA). Then, they were incubated for 45 minutes with ABC Elite complex (Vector Corp., USA). Finally, slides were treated with 3.3' diaminodibenzidine (Sigma Chemical Company, USA) and with peroxide 0.1% (Sigma Chemical Company, USA). Counter staining was performed with methylated green for 5 minutes. The immunohistochemical positive control was a lymph node with lymphoid hyperplasia.

### C. Criteria for interpretation of immunohistochemistry staining for Ki-67

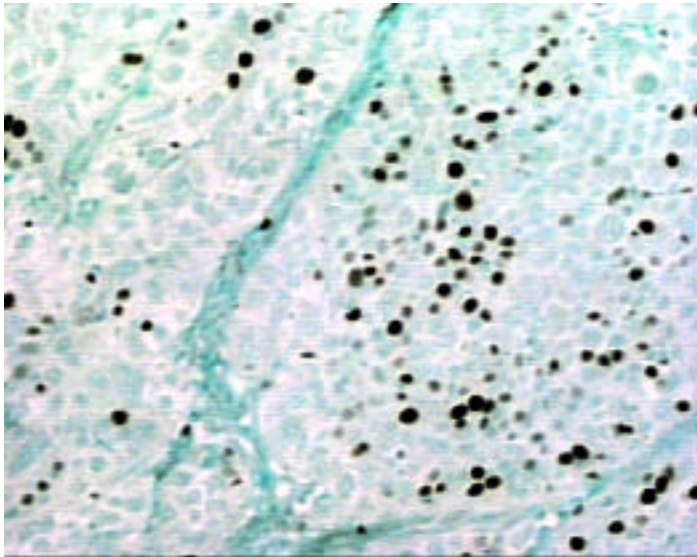
Grading was semi-quantitative. Immunoreactivity was considered positive when nuclear staining in cells of tumor was found. The intensity was graded as mild, when positive in less than 30% of tumor cells (**Figure 2**), moderate, when positive between 30% and 50% of tumor cells (**Figure 3**), and intense, when positive in more than 50% of tumor cells (**Figure 4**). All slides were blindly graded by two co-authors (Angela F. Logullo, Carlos E. Bacchi), with good correlation scores between them.

### D. Statistical analysis

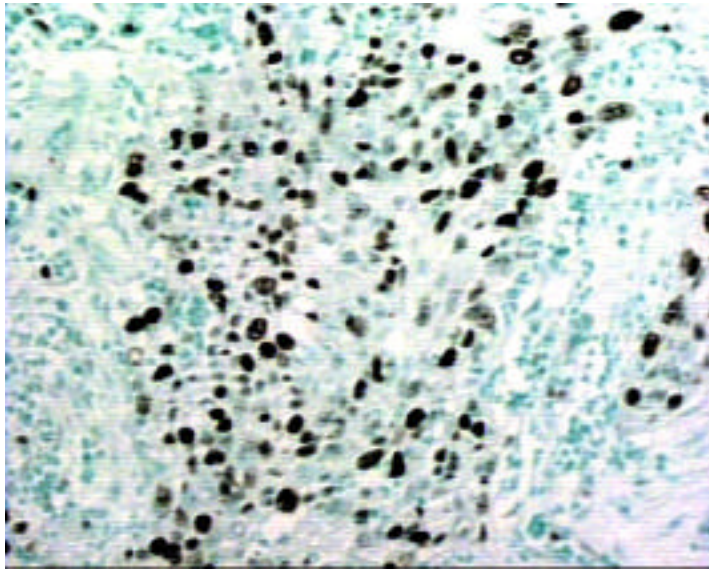
Data were collected in a databank, using a software Excell (Microsoft Corp., USA), in a personal computer Pentium II 400 MHz (LG Electronics, South Korea). For the statistical analysis, either the chi-square test or the Fisher exact test were employed, to a significance level of 0.050.



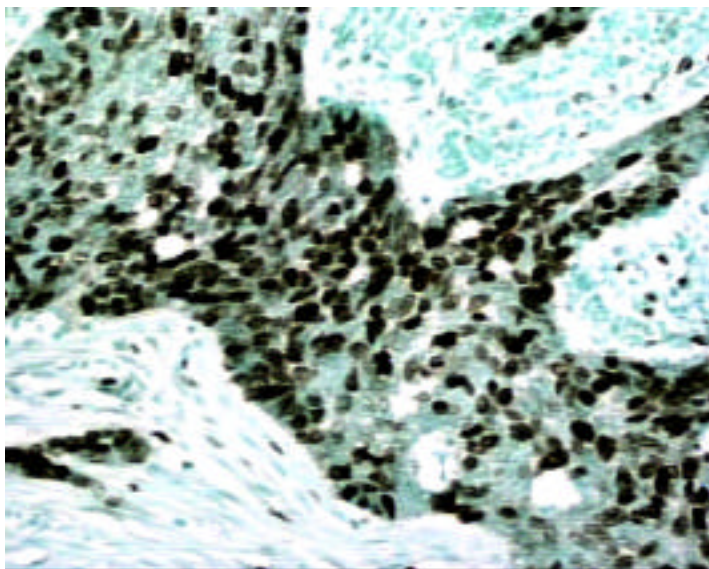
**Figure 1.** Extensive SCC, involving left orbit, frontal region and anterior skull base, with parotid and cervical metastases.



**Figure 2.** Immunostaining showing Ki-67 nuclear expression in less than 30% of cancer cells (ABC technique; counter-staining with methylated green; original magnification: 400X).



**Figure 3.** Immunostaining showing Ki-67 nuclear expression in between 30% and 50% of cancer cells (ABC technique; counter-staining with methylated green; original magnification: 400X).



**Figure 4.** Immunostaining showing Ki-67 nuclear expression in more than 50% of cancer cells (ABC technique; counter-staining with methylated green; original magnification: 400X).

### III. Results

#### A. Ki-67 expression in BCCs

Intense Ki-67 expression was observed in 37.50% of BCCs of Group 1 (Table 1). Despite the fact that this prevalence was more than three times higher than in Group 3 (13.04%), the difference was not statistically significant (p=0.155).

#### B. Ki-67 expression in SCCs

Most cancers in Group 2 (72.73%) showed intense Ki-67 expression, compared to only 20.00% of tumors in Group 4 (Table 2), and this difference was statistically significant (p=0.050).

### IV. Discussion

Sometimes, skin carcinomas are extremely aggressive, and are called by some authors horrifying tumors (Jackson and Adams, 1973; Bianchini and Wolter, 1984; Horlock et al, 1998). Deep anatomical planes may be reached, with skull base involvement. Indeed, these cancers represent the main histological types of tumors in some series of oncological craniofacial operations (Medina dos Santos et al, 1994; Cernea et al, 1997; Dias et al, 1997). In these series, many BCCs and SCCs actually invaded duramater and brain.

Immunohistochemical expression of Ki-67 reflects the cell proliferation status of a neoplasia. When it is present in more than 50% of tumor cells, it is usually associated with increased aggressiveness. However, findings are somewhat controversial.

To our knowledge, this is the first study on proliferation status of a consecutive series of skin carcinomas with skull base invasion. The objective of this study was to analyze this proliferation status, using immunohistochemical evaluation of Ki-67, in a consecutive series of very aggressive skin carcinomas with skull base invasion submitted to combined craniofacial oncological operations. In addition, these findings were

compared with head and neck skin carcinomas with good outcome, with no evidence of disease at a median 36.2-month follow-up, treated in the same Institution within the same time frame, in a case-control study.

In Group 1, 37.50% of BCCs had intense Ki-67 expression, similar to 32.90% reported by Baum et al 1993, but inferior to 51% observed by Abdelsayed et al, 2000. The frequency of intense Ki-67 expression was only 13.04% in Group 3; despite the absence of statistical significance, a clear trend towards an increased proliferation status was suggested.

In Group 2, intense Ki-67 expression was noted in 72.73% of the tumors, statistically higher than 20% encountered in Group 4; and also superior to 46% reported by Kerschmann et al, 1994. Our data did not confirm the findings of Kanitakis et al, 1997 who observes no difference in Ki-67 expression comparing 14 aggressive SCCs with 28 non-aggressive tumors. It is noteworthy the low incidence of this expression in both groups in their series (15.0% and 17.5%, respectively). Our findings confirm the experience of other authors (Kerschmann et al, 1994; Healy et al, 1995), indicating a status of enhanced cell proliferation among these extremely aggressive skin tumors with skull base invasion.

In conclusion, an increased prevalence of intense proliferation among BCCs and SCCs with skull base invasion was noted. This observation could stimulate the analysis of the role of anti-proliferation strategies in the therapy of these tumors. Clearly, the preliminary findings of this study need to be confirmed with larger series.

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Table 1. Expression of Ki-67 in BCC

Group	Positive in less than 30% of cancer cells	Positive in between 30% and 50% of cancer cells	Positive in more than 50% of cancer cells
1	8 (33.33%)	7 (29.17%)	9 (37.50%)
3	10 (43.48%)	10 (43.48%)	3 (13.04%)

p=0.155

Table 2. Expression of Ki-67 in SCC

Group	Positive in less than 30% of cancer cells	Positive in between 30% and 50% of cancer cells	Positive in more than 50% of cancer cells
2	2 (18.18%)	1 (9.09%)	8 (72.73%)
4	3 (30.00%)	5 (50.00%)	2 (20.00%)

p=0.050

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