Ki67 as a Biologic Marker of Basal Cell Carcinoma: A Retrospective Study

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ABSTRACT: **Background:** Basal cell carcinoma (BCC) is the most common malignancy in humans. Several factors have been associated with the biological behavior of these tumors, including histopathologic type, depth of tumor invasion, perineural invasion, and the expression of several biological markers including Ki67, a proliferative marker. Previous studies assessing the relationship between the proliferative fraction, as expressed by Ki67, and the histological variants of BCC as well as its association with the tendency to recur, failed to illustrate significant statistical correlation.

Objectives: To examine the proliferative index, as expressed by Ki67, in various subtypes of basal cell carcinoma, and to assess its relationship to various histological and clinical variables.

Methods: In this retrospective study 51 lesions of BCC were examined. In each case, the following data were gathered: demographic (age and gender), anatomic location, size of the lesion, and clinical follow-up. Each case was stained immunohistochemically with anti-Ki67 antigen (MIB-1), and the proliferative index was determined. Histological analysis was performed for the following data: presence of an ulcer, intensity of inflammatory infiltrate, histologic subtype, mitotic count, and the presence of perineural invasion.

Results: Basal cell carcinoma exhibited a wide variation of proliferative indices, ranging from 1% to 61%. A significant statistical correlation was observed between the proliferative index and the mitotic activity, tumor ulceration and brisk tumor-infiltrating lymphocytes.

Conclusions: The wide variation in the degree of proliferation (from almost no activity to highly proliferative tumors) suggests that basal cell carcinoma exhibits a wide spectrum of biological characteristics. Ulcerated lesions were characterized by high proliferative index. No true correlation was demonstrated between the proliferative index and the aggressive histological subtypes, implying that other factors were more biologically significant. The degree of proliferation also showed significant statistical correlation with the degree of tumor infiltration by lymphocytes. The significance of this proliferation-associated increased immunogenicity needs to be further studied.

IMAJ 2014; 16: 229–232

KEY WORDS: basal cell carcinoma (BCC), Ki67 antigen, skin tumor, immunohistochemistry, mitotic count

Basal cell carcinoma is the most common skin cancer in Caucasians worldwide [1-3]. It is associated with prolonged sun exposure, and in 85% of the cases occurs in the head and neck region [4,5]. This slow-growing tumor is locally destructive and has low metastatic potential [4-6]. Several parameters have been associated with increased aggressiveness of this lesion: tumor diameter, occurrence in the head, the aggressive-growth histological variants (infiltrative and morpheaform), depth of invasion, perineural invasion, and the expression of several biological markers including cyclin D1, p53, and Ki67 [6-11]. The Ki67 antigen, a high molecular weight non-histone nuclear protein, is generally accepted as the most reliable cell proliferative marker. Being expressed in all active cell cycle phases, the Ki67 antigen is superior by far to the mitotic count for the assessment of tumoral proliferative activity [9,12]. Ki67 labeling has been used as a diagnostic and prognostic tool in other tumors such as lymphomas, soft tissue sarcomas, and recently melanoma [12].

Healy et al. [13] examined the relationship between Ki67 proliferative index and the histopathological variants of BCC, as well as the recurrence rate. The study showed that in cases of local recurrence, the primary tumors demonstrated significantly higher proliferative indices by Ki67, while there was no correlation seen between the BCC histological type and pattern of KI67 antigen expression [13]. The aim of the present study was to examine the relationship between the proliferative activity of BCC, as expressed by the Ki67 index, and its microscopic and clinical variables such as histological subtype, mitotic count, the presence of ulceration documented histologically, inflammation, tumor diameter measured macroscopically, and anatomic location.

PATIENTS AND METHODS

The study group comprised 51 patients histopathologically diagnosed with BCC who were operated consecutively (28 by Mohs and the remainder by conventional excisions) during the year 2010. The corresponding paraffin-embedded blocks were retrieved from the archive of the Institute of Pathology at the Wolfson Medical Center, Holon, Israel. The selected samples

BCC = basal cell carcinoma

included the major tumoral bulk. Only primary lesions were included. The original hematoxylin and eosin-stained sections were reviewed and histological subtypes were determined according to the conventional classification (keratotic, infiltrative, superficial, morpheaform, nodular, pigmented, mixed). The accompanying inflammatory reaction was graded as None, Non-brisk (lymphocyte infiltrate only focally or not along the entire base of the tumor), and Brisk (dense tumoral infiltration or along the entire base of the tumor). This system was the same as used in melanoma reports [15]. Mitotic activity of the tumor cells was examined with x400 objective, using an Olympus microscope (Japan). The mitotic count was expressed as a number/mm^2 starting with the most mitotically active area ("hot spot") and proceeding to adjacent areas consecutively. Only tumoral mitotic figures were counted. The macroscopic maximal diameter of the tumor (in centimeters) was determined. Perineural invasion and ulceration were also assessed histologically. Data regarding the patient's age, gender, additional skin tumors, postoperative follow-up (for recurrence), and tumoral anatomic location were collected from the medical files of the patients.

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Formalin-fixed, paraffin-embedded tissue blocks were cut into 4 μm thick sections. The sections were incubated with anti-Ki67 antibodies (SP6 clone, 1/150, SPING Bioscience, USA). Immunoreactivity was illustrated with Ultra View Universal 3, 3'-diaminobenzidin (DAB) detective kit, using the VENTANA Automatic Benchmark Ultra System (Ventana Medical Systems, Inc. Tucson, AZ, USA). Nuclear staining was counted per 200 tumor cells with x400 objective, using an Olympus microscope. Proliferation activity of the tumor cells was defined as the percentage of positively stained cells.

STATISTICAL ANALYSIS

Data were stored on an Excel spreadsheet and analyzed on SPSS version 19.0 (IBM Inc., USA). Distribution of continuous data was assessed for normality using the Kolmogorov-Smirnov test (cutoff at $P < 0.01$). Continuous data were described as mean ± SD. Associations between proliferation and other characteristics were described by calculating Pearson's correlation coefficient. The *t*-test for independent samples or one-way analysis of variance (ANOVA) was used to compare proliferation by independent variables such as gender, location, growth type, etc., in a series of analyses. All tests were two sided and considered significant at $P < 0.05$.

RESULTS

The study group comprised 51 patients: 37 males and 14 females (72.5% and 27.5% respectively). The mean age of the patients was 74.6 years (range 37–90 years). The postoperative follow-up period ranged from 1 to 12 months (median 8.4 months), during which no recurrence was observed. The most common site in this study was the head and face (70%). The anatomic distribution of the tumors is shown in Table 1. Nodular growth pattern was noticed in the majority of cases (n=36, 70.6%). The distribution of the histological growth pattern (microscopic subtypes) is also shown in Table 1. Half the cases (n=27, 52.9%) were not associated with any inflammatory reaction, but brisk inflammatory response was observed in 7 (13.7%) [Table 2]. None of the specimens showed perineural invasion, while ulceration was demonstrated in 19 cases (37.3%) [Table 2]. No significant statistical correlation was found between the histologically aggressive subtypes and the intensity of the inflammatory infiltrate ($P = 0.566$), patient's gender ($P = 0.432$), or occurrence of ulceration ($P = 0.597$). The maximum diameter of lesions ranged from 0.3 to 7.2 cm, with a mean diameter of 1.4 cm [Table 3]. The mean mitotic count was 10.39/mm^2. The mean Ki67 labeling index was 12.3%, ranging from 1% to 61% [Table 3] [Figure 1]. The proliferative index showed no statistical correlation with the histological growth pattern ($P = 0.617$), tumor diameter ($P = 0.214$), anatomic location ($P = 0.253$), gender ($P = 0.63$), and age of the patients ($P = 0.45$). Brisk inflammatory response and

Table 1. Clinicopathological data

Variables	Cases	%
Age (yr)		
Mean	74.6	
Range	37–90	
Gender		
Male	37	72.5
Female	14	27.5
Site		
Nose	9	17.6
Scalp	2	3.9
Ear	3	5.9
Face	17	33.3
Neck	5	9.8
Back	8	15.7
Extremities	7	13.7
Histological growth patterns		
Nodular	36	70.6
Mixed	8	15.7
Superficial	2	3.9
Infiltrative	3	5.9
Keratotic	1	2.0
Morphea	1	2.0

Table 2. Distribution according to inflammation and ulceration

	No. of cases	%
Inflammation		
None	27	52.9
Non-brisk	17	33.3
Brisk	7	13.7
Ulceration		
Present	19	37.3
Not present	32	62.7

Table 3. Distribution of the lesions according to age, diameter, mitotic activity, and proliferation index

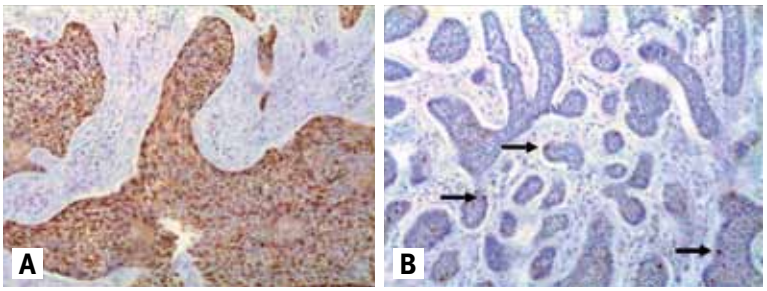
	Mean	Median	SD	Minimum	Maximum
Age (yr)	74.86	77.0	11.20	37.00	90.00
Diameter (cm)	1.41	1.00	1.09	0.30	7.20
Proliferative index (%)	12.37	9.00	12.35	1.00	61.00
Mitotic count (per mm2)	10.39	7.00	8.64	1.00	36.00

high proliferative index were correlated statistically ($P = 0.002$). In addition, a statistically significant correlation between the mitotic count and the presence of ulceration with the proliferative index was detected ($P = 0.00$, $P = 0.034$ respectively).

DISCUSSION

The function of Ki67 antigen is unknown. Ki67 antigen expression is seen in G1, S, G2, and M phases of the cell cycle but is absent in the G0 phase [11]. In the current study the mean Ki67 index of proliferation was 12.3%, ranging from 1% to 61%, among the various histological subtypes. A low proliferative index can be found in benign tumors or "biologically silent" tumors, such as carcinoid [16]. A high proliferative index is more indicative of malignant tumors [17]. Having such a wide range of proliferative activity demonstrates the fact that BCC exhibits a wide spectrum of biologic activity. The mean mitotic count was 10.3/mm2. Not surprisingly, being a marker of proliferative activity, this parameter also showed a wide range, 1–36 mitoses/mm2, further emphasizing the aforementioned conclusion. In this study, a high proliferative index correlates with the presence of tumor ulceration ($P = 0.034$). Nineteen cases (37.3%) demonstrated ulceration. This can be explained by the fact that highly proliferative tumors are characterized by a rapid growth rate, eventually exceeding their blood supply and culminating in tumor necrosis and ulceration. Surprisingly, no correlation between the diameter of the lesion and its proliferative index was found ($P = 0.214$), indicating, most likely, that large BCCs are also slow growing in most cases. Nodular BCC was found in 70% of the cases, infiltrative in 5.9%, and morpheaform in 2%. In this study, no statistically significant correlation was found between the proliferative index and the histological variant ($P = 0.617$), including the aggressive subtypes. Resit et al. [14] detected higher mean, yet not statistically significant, Ki67 indices in the infiltrative variant, one of the aggressive variants. This study emphasizes, once again, that the aggressiveness ascribed to the so-called aggressive-growth BCCs (morpheaform, infiltrative and metatypical) cannot be explained by their proliferation capabilities but rather by other qualities such as their infiltrative nature of growth (hampering complete excision due to poor gross localization) as well as their higher tendency for perineural invasion.

Figure 1. Tumoral Ki67 labeling (Ki67 immunostain x100)
[A] Prominent nuclear immunostaining with anti-Ki67 antibodies, exceeding 80% of cells
[B] A different case, illustrating much less Ki67 expression (arrows), not more than 2–3% of the cells



None of the patients in the current study experienced recurrence of the primary tumor, most likely due to a short postoperative follow-up (median 8.4 months). Healy et al. [13] detected a significant increase in Ki67 antigen expression in primary tumors of BCCs, which later recurred as compared to those that did not. The presence of inflammatory cells infiltrating the tumor as a response of the immune system against the tumor was examined in this study. The inflammatory response was graded as follows: None, Non-brisk, and Brisk, following the system used in malignant melanoma [15]. Tumors showing high proliferative indices correlated statistically with a brisk inflammatory response ($P = 0.002$); in other words, tumors with high proliferative activity were characterized by considerable lymphocytic infiltration. This finding, i.e., the increased immunogenicity of highly proliferative BCCs, requires longer periods of follow-up in order to clarify its prognostic significance.

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Capsule

Endothelial *Notch* activity promotes angiogenesis and osteogenesis in bone

Blood vessel growth in the skeletal system and osteogenesis seem to be coupled, suggesting the existence of molecular crosstalk between endothelial and osteoblastic cells. Understanding the nature of the mechanisms linking angiogenesis and bone formation should be of great relevance for improved fracture healing or prevention of bone mass loss. Ramasamy et al. show that vascular growth in bone involves a specialized, tissue-specific form of angiogenesis. *Notch* signaling promotes endothelial cell proliferation and vessel growth in postnatal long bone, which is the opposite of the well-established function of *Notch* and its ligand Dll4 in the endothelium of other organs and tumors. Endothelial cell-specific and inducible genetic disruption of *Notch* signaling in mice not only impaired bone vessel morphology and growth, but also led to reduced osteogenesis, shortening of long bones, chondrocyte defects, loss of trabeculae and decreased bone mass. On the basis of a series of genetic experiments, the authors conclude that skeletal defects in these mutants involved defective angiocrine release of Noggin from endothelial cells, which is positively regulated by *Notch*. Administration of recombinant Noggin, a secreted antagonist of bone morphogenetic proteins, restored bone growth and mineralization, chondrocyte maturation, the formation of trabeculae and osteoprogenitor numbers in endothelial cell-specific *Notch* pathway mutants. These findings establish a molecular framework coupling angiogenesis, angiocrine signals and osteogenesis, which may prove significant for the development of future therapeutic applications.

Nature 2014; 507: 376

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Capsule

Active or passive exposure to tobacco smoking and allergic rhinitis, allergic dermatitis, and food allergy in adults and children

Allergic rhinitis, allergic dermatitis, and food allergy are extremely common diseases, especially among children, and are frequently associated with each other and with asthma. Smoking is a potential risk factor for these conditions, but so far results from individual studies have been conflicting. Saulyte et al. examined the evidence for an association between active smoking (AS) or passive exposure to second-hand smoke and allergic conditions. The authors retrieved 97 studies on allergic rhinitis, 91 on allergic dermatitis, and 8 on food allergy published in 139 different articles. When all studies were analyzed together (showing random effects model results and pooled odds ratios expressed as relative risk), allergic rhinitis was not associated with active smoking (pooled RR 1.02, 95% confidence interval (CI) 0.92–1.15), but was associated with passive smoking (pooled RR 1.10, 95% CI 1.06–1.15). Allergic dermatitis was associated with both active (pooled RR 1.21, 95% CI 1.14–1.29) and passive smoking (pooled RR 1.07, 95% CI 1.03–1.12). In children and adolescents, allergic rhinitis was associated with active (pooled RR 1.40, 95% CI 1.24–1.59) and passive smoking (pooled RR 1.09, 95% CI 1.04–1.14). Allergic dermatitis was associated with active (pooled RR 1.36, 95% CI 1.17–1.46) and passive smoking (pooled RR 1.06, 95% CI 1.01–1.11). Food allergy was associated with second-hand smoke (1.43, 1.12–1.83) when cohort studies only were examined, but not when all studies were combined. The findings are limited by the potential for confounding and bias, given that most of the individual studies used a cross-sectional design. Furthermore, the studies showed a high degree of heterogeneity and the exposure and outcome measures were assessed by self-report, which may increase the potential for misclassification.

PloS Med 2014; DOI: 10.1371/journal.pmed.1001611

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