Proliferation indexes – a comparison between cutaneous basal and squamous cell carcinomas

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Abstract

Aims—To compare differences in cell proliferation indexes and apoptotic indexes between cutaneous basal and squamous cell carcinomas, in an attempt to suggest an explanation for the differences in their biological behaviour.

Methods—Forty cases of cutaneous basal cell carcinoma (BCC) and 40 cases of moderately and well differentiated squamous cell carcinoma (SCC) were retrieved from the archives. Sections, 4 μm thick, were cut from formalin fixed, paraffin wax embedded tissue in each case and stained with haematoxylin and eosin. These were then examined for mitotic and apoptotic figures per 1000 cells. Sections from the same cases were also immunostained with the mouse monoclonal antibody Ki67 (MIB1); positive nuclear staining was counted per 1000 cells.

Results—No significant differences were found between the mitotic indexes and apoptotic indexes in these tumours. There was, however, a significant difference in Ki67 (MIB1) staining, with greater staining in the squamous cell carcinomas.

Conclusion—Estimation of the mitotic and apoptotic indexes did not reveal any differences between these two tumour types. The proliferation indexes, assessed by Ki67 immunostaining, did differ. This may be one of the factors underlying the more aggressive behaviour of SCC.

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There is considerable difference in behaviour between cutaneous basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The former will rarely form metastases, whereas SCCs are not only more locally aggressive, but also have a much greater metastatic potential. Cutaneous BCCs and SCCs both arise from the epidermis and both tumours are exposed to a similar host environment in the skin; however, we have little idea why BCCs are so reluctant to form metastases.

We attempted to explain the variable biological behaviour of these tumour by comparing the mitotic, apoptotic and proliferation indexes.

Methods

Formalin fixed, paraffin wax embedded tissue (40 cases for each tumour type) was retrieved from the archives at the Pathology Department of The Royal College of Surgeons in Ireland. By means of the indirect immunoperoxidase technique, representative sections from each case were stained with a monoclonal antibody directed against Ki67 (MIB1). The MIB1 monoclonal antibody (Immunotech, S.A., France) reacts with the Ki67 nuclear antigen associated with cell proliferation and is expressed throughout the cell cycle (G1, S, G2, M phases) and is absent in resting (G0) cells.

Sections, 4 μm thick, were cut on to APES (3-Aminopropyltriethoxysilane) coated slides and dried overnight at 55°C, dewaxed in xylene and rehydrated through industrial methylated spirits. Endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol. Before immunohistochemical staining, the slides were pretreated in a microwave oven (Phillips Whirlpool, VIP27) in 0.01 M citrate buffer (pH 6.0) for 15 minutes at 750 W to enhance antigen retrieval. The slides were left to cool to room temperature for 20 minutes before removal. Sections were incubated for 10 minutes in normal rabbit serum (Dako, Glostrup, Denmark), diluted 1 in 10. Excess serum was removed and sections were incubated with primary layer antibody for 60 minutes at room temperature. Biotinylated rabbit secondary layer antibody (Dako), diluted 1 in 200, was applied for 40 minutes at room temperature. Bound antibody was visualised using the avidin-biotin peroxidase conjugate (ABC, Dako); diamobenzidine (Sigma, St Louis, Missouri, USA) was used as chromogen. Sections were lightly counterstained with Mayer’s haematoxylin. Primary antibody was replaced with normal rabbit serum as a negative control.

Mitotic figures, apoptotic cells and Ki67 (MIB1) positive cells were counted per 1000 cells. Each index was assessed by three independent observers (MHAI-S, ED, CBW). If there was disagreement, this was resolved by discussion around a multi-viewer microscope. The sections were examined at high power (×40) and 10 fields were chosen in the area showing most proliferation (that is, the area of the section that showed the most positive nuclear staining with MIB1); 100 cells were assessed in each field.

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staining. The staining intensity was such that almost no weakly stained nuclei were seen.

Unequivocal mitoses were counted per 1000 cells. Apoptotic cells (showing shrinkage or fragmentation of the nucleus and cytoplasm with no associated inflammatory infiltrate) were also counted per 1000 cells.\(^\text{11}\)

**Results**

The mean, median and range of values for mitotic indexes, apoptotic indexes and Ki67 (MIB1) staining, per 1000 cells, are shown in table 1. The results show no significant differences in the mean values for the mitotic (\(p = 0.621\)) and apoptotic indexes (\(p = 0.405\)) using a two tailed \(t\) test.

The results of Ki67 (MIB1) immunostaining, using the same statistical analysis, show a significant difference between BCCs and SCCs, with greater staining in the latter (\(p = 0.029\)). The staining was most noticeable at the invasive fronts. The values are plotted on a logarithmic scale with confidence intervals for the mitotic and apoptotic indexes and Ki67 (MIB1) staining (fig 1).

Figures 2 and 3 show examples of Ki67 immunostaining in BCCs and SCCs.

**Discussion**

Cutaneous BCCs and SCCs differ with respect to behaviour and metastatic potential.\(^\text{1,2}\) Why BCCs have a more indolent course remains an enigma. Researchers have investigated a number of avenues in an attempt to find an explanation for this. Studies have investigated cell proliferation by assessing S-phase fractions using flow cytometry; p53 immunostaining has also been studied.\(^\text{11,12}\) Another aspect examined was tumour–stromal interaction by investigating expression of metalloproteinases and cathepsin B in these tumours, as proteases play an important role in the proliferative, invasive and metastasising effects exerted on the extracellular matrix.\(^\text{16-24}\) A high apoptotic index in BCCs has also been suggested as a possible cause for this difference in behaviour.\(^\text{25}\)

None of these investigations of cell proliferation, apoptotic indexes or oncoprotein expression have produced a satisfactory explanation for the difference observed between BCCs and SCCs. BCCs and SCCs also did not differ in their production of the proteases studied.

As it may be difficult to identify mitotic and apoptotic figures in haematoxylin and eosin stained sections,\(^\text{26}\) the MIB1 monoclonal anti-

![Figure 1](image1.png)

**Figure 1** Box plots on logarithmic scale of BCC and SCC values per 1000 cells. \(*, ** =\) outliers; shaded areas = 95% confidence intervals. LB = Log BCC; LS = Log SCC; \(m\) = mitotic index; \(A\) = apoptotic index; \(M\) = MIB1 staining.

![Figure 2](image2.png)

**Figure 2** Example of Ki67 (MIB1) staining in a BCC (original magnification, \(\times 400\)).

![Figure 3](image3.png)

**Figure 3** Example of Ki67 (MIB1) staining in a SCC (original magnification, \(\times 400\)).

<table>
<thead>
<tr>
<th></th>
<th>BCCs</th>
<th>SCCs</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotic index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
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<td>0–23</td>
<td>0.621</td>
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<tr>
<td>Mean</td>
<td>5.375</td>
<td>4.925</td>
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</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Apoptotic index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2–33</td>
<td>1–29</td>
<td>0.405</td>
</tr>
<tr>
<td>Mean</td>
<td>12.675</td>
<td>11.25</td>
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</tr>
<tr>
<td>Median</td>
<td>10.5</td>
<td>10</td>
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</tr>
<tr>
<td>MIB1 (Ki67)</td>
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<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>0.742</td>
<td>41–774</td>
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<tr>
<td>Mean</td>
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<tr>
<td>Median</td>
<td>231</td>
<td>339.5</td>
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</table>

Table 1. BCC and SCC mitotic, apoptotic and proliferative indexes, scored per 1000 cells.

A quantitative estimate of the nuclear labelling index for MIB1 immunoreactivity was made by scoring positive nuclei per 1000 nuclei per section.\(^\text{8-10}\) Two categories of staining were used: positive and negative nuclear
body was used as an additional marker of mitosis. (A recent study comparing various mono- and polyclonal antibodies used as proliferation markers obtained the most reliable results with MIB1.23) The results of our study showed no differences in mitotic or apoptotic indexes for the two tumour types. However, there was a significantly higher proliferation index in SCCs compared with BCCs.

This study proves that the metastatic potential in SCC is not the result of either a raised mitotic index or a depressed apoptotic index. However, our study did show a significantly different proliferation index in these tumours.

The difference in results for mitotic and proliferation indexes may be due to the fact that Ki67 (MIB1) identifies cells throughout the cell cycle (except for the G0 resting phase) and thus may be a more reliable indicator of proliferation than the assessment of mitoses which evaluates cells only in the M phase of the cell cycle.24 This difference may therefore be one of the factors underlying the more aggressive behaviour of SCCs.

In conclusion, whilst a higher proliferation index in SCCs may not be the only factor responsible for the different biological behaviour of these tumours, it is reasonable to suggest it may be a factor as there is correlation between increased cell proliferation and aggressive behaviour in many tumour types. Of course, one must recognise that SCCs and BCCs are tumours with different phenotypes and this may also play a role in their behaviour.

7 Weidner N, Moore DH, Vartanian R. Correlation of Ki-67 antigen expression with mitotic figure index and tumour grade in breast carcinomas using the novel "Paraffin-reactive MIB1 Antibody. Hum Pathol 1994;25:337-42.