Cell Proliferation in Human Basal Cell Carcinoma

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SUMMARY

Information about cell proliferation kinetics in human basal cell carcinomas has been limited by the inaccessibility of most tumors. Basal cell carcinomas are very common, slowly growing, locally invasive tumors of skin that have been studied by autoradiographic techniques using tritiated thymidine in vivo. The reproductive cycle of the germinative cell population was analyzed. The DNA synthesis period was found to be 20 hr; the G1 period, 188 hr; the G2 period, 7 hr; the mitotic period, 1.5 hr; and the total germinative cycle, approximately 217 hr. These results indicate that there is more cell proliferation activity in a basal cell carcinoma than suggested by the clinically apparent slow growth of the tumor. Therapeutic aspects of basal cell carcinoma are discussed relating to chemotherapeutic drugs.

INTRODUCTION

In recent years the kinetics of cell proliferation in normal and tumor tissues has been actively studied for pathophysiological and chemotherapeutic clues. Almost all these investigations have been performed in experimental animal tumor systems rather than in human tumors, because of the difficulties in obtaining multiple tissue samplings (1). With thymidine-3H as a DNA marker most of the studies in humans have provided only a single determination of the labeling index, the proportion of proliferative cells synthesizing DNA at 1 time (15). An analysis of other cell cycle parameters requires, however, multiple specimens taken at different times after exposure to thymidine-3H in vivo. In humans, the few studies of the complete cell cycle have been performed on the hematopoietic system, gastrointestinal tract, neoplastic effusions, solid tumors, and psoriasis (3, 5, 7, 12, 14, 18, 20).

BCC's are one of the most common cancers known to man. While having a negligible rate of mortality and metastatic activity, they nonetheless are progressively enlarging, locally invasive tumors. When 1 or more cutaneous tumors are present in an individual patient, multiple biopsy samples can be easily obtained for studies. The techniques used in this report have permitted a detailed analysis of cell proliferation kinetics of BCC in vivo with relatively small amounts of thymidine-3H (19).

MATERIALS AND METHODS

Cell proliferation kinetics as studied by autoradiographic analysis, with thymidine-3H as a DNA label, has been reviewed in several publications (1, 9). Adaptation of these methods to study cutaneous diseases has been described in previous reports by us and is briefly reviewed here (18, 20).

The 9 patients studied to determine the duration of DNA synthesis had multiple nodular and/or superficial BCC that permitted an average of 10 biopsy specimens to be obtained from each patient. Additional single specimens were taken from other patients to determine 1-hr labeling indices. All patients were over 40 years old with clinically diagnosed and histologically proven BCC's. In the first group of patients multiple sites of tumor were injected superficially with 0.1 ml (5 μCi) thymidine-3H (specific activity, 1.9 Ci/m mole, Schwarz BioResearch, Inc., Orangeburg, N. Y.) and the sites were carefully marked. At serial intervals between 1 and 33 hr, 4-mm punch biopsies were obtained, fixed in Bouin's solution, and prepared histologically for autoradiography. Injection schedules were arranged to permit all biopsies to be taken between 9 a.m. and 9 p.m. The slides were dipped in Kodak NTB-3 emulsion and exposed for 1 to 4 weeks. Black silver grains from tritium emissions over nuclei indicate that those cells were synthesizing DNA (S phase) during the pulse labeling period of the thymidine-3H injection.

For a short interval following injection, only unlabeled mitoses are seen within the tumor (Fig. 1). Within a few hours, labeled mitoses appear, representing the movement of cells labeled previously in the S phase as they migrate through the G2 period into the mitotic period. From sequential specimens the ratio of labeled mitoses to total mitoses is determined and plotted in a classical labeled mitoses versus time after injection curve. The S phase is measured as the interval (in hr) between the 37% points on the ascending and descending limbs of the labeled mitoses curve. The use of 37% intercepts was suggested as a theoretical analysis by Quastler (10) while other workers more commonly use 50% intercepts for the measurement of the S and G2 periods. In the several studies we have performed on human cutaneous tissues, the...
Cell Proliferation in BCC

Fig. 1. a, BCC 1 hr after the intralesional injection of thymidine-\textsuperscript{3}H. Labeled cells are scattered throughout the tumor. b, higher magnification of similar specimen showing unlabeled mitoses.

Labeled mitosis curve never reaches 100\% presumably for technical reasons as discussed below. The use of the 37\% intercepts therefore has appeared to be a more reasonable level at which to determine the S periods for comparative purposes in human cutaneous tissues. The time from the thymidine-\textsuperscript{3}H injection until the appearance of a significant number of labeled mitoses (defined as the 37\% point on the ascending limb) is the mean G\textsubscript{2} period.

The germinative cell cycle duration (from mitosis to mitosis) is calculated as follows:

\[
\frac{N_s}{T_s} = \frac{N_{gc}}{T_{gc}} \tag{A}
\]

\(N_s\) = no. of cells in S phase  
\(T_s\) = duration of S phase  
\(N_{gc}\) = total no. of germinative cells  
\(T_{gc}\) = duration of entire germinative cell cycle  
\(N_s/N_{gc}\) = labeling index (\%)  

Chart 1. Labeled mitoses curve to determine the durations of the DNA synthesis and G\textsubscript{2} premitotic periods. The curve is drawn through points (\textbullet), each of which represents the mean of the percentage labeled mitoses in 9 patients. An average of 114 mitoses was counted at each interval for each patient. Vertical lines, 80\% confidence limits of each mean. \(\bigcirc\)---\(\bigcirc\), corrected data considering the thickness of sections as described in text.

\(T_s\) is obtained from the labeled mitoses curve (Chart 1) which represents the cumulative data from 9 patients. A uniform distribution of labeled cells in tumor areas was a prerequisite for determining the ratios quantitatively, labeled cells to total cells and labeled mitoses to total mitoses. In some specimens all tumor areas did not show labeled cells with uniform distribution. This has been assumed to be a technical artifact from inadequate distribution of the labeled compound at the time of injection or the biopsy being obtained slightly off center from the injection site. An alternate explanation is that there are isolated areas of tumor which contain no active cell proliferation, but this is doubtful since mitotic figures are present in all areas. Since the data reported here are taken from areas selected for uniform labeling the results may represent the maximum DNA-synthesizing activity within these tumors, rather than the average activity.

The number of germinative cells (\(N_{gc}\)) in the BCC was obtained by counting the individual viable cells in enlarged photomicrographs of selected well-labeled islands of tumor. In each of these areas, the number of labeled cells (\(N_s\)) was counted and related to the \(N_{gc}\) to obtain the labeling index (\(N_s/N_{gc}\)). The labeling index was measured in specimens taken from 18 patients 30 to 60 min after thymidine-\textsuperscript{3}H injection (Table 1).

In the analysis of cell proliferation in the BCC, it has been assumed that the viable cells of the tumor are a homogeneous proliferating cell population in a steady state with uniform phases in the cell cycle. Evidence for these assumptions and possible variations are discussed below.

Since the histological sections were cut 6 \(\mu\) thick (human skin cannot reliably be cut much thinner) and tritium
Table 1

Labeling index in BCC

<table>
<thead>
<tr>
<th>Patient</th>
<th>( N ) (_{v}) germinative cells</th>
<th>( N_{v}/N_{v}^{*} ) labeling index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1869</td>
<td>5.0</td>
</tr>
<tr>
<td>B</td>
<td>1930</td>
<td>5.1</td>
</tr>
<tr>
<td>C</td>
<td>1217</td>
<td>6.9</td>
</tr>
<tr>
<td>D</td>
<td>1495</td>
<td>13.2</td>
</tr>
<tr>
<td>E</td>
<td>1834</td>
<td>7.2</td>
</tr>
<tr>
<td>F</td>
<td>3390</td>
<td>6.9</td>
</tr>
<tr>
<td>G</td>
<td>1365</td>
<td>12.8</td>
</tr>
<tr>
<td>H</td>
<td>1221</td>
<td>9.6</td>
</tr>
<tr>
<td>I</td>
<td>1359</td>
<td>8.6</td>
</tr>
<tr>
<td>J</td>
<td>5419</td>
<td>8.7</td>
</tr>
<tr>
<td>K</td>
<td>670</td>
<td>11.7</td>
</tr>
<tr>
<td>L*</td>
<td>4190</td>
<td>5.6</td>
</tr>
<tr>
<td>M</td>
<td>2749</td>
<td>11.0</td>
</tr>
<tr>
<td>N</td>
<td>1513</td>
<td>10.3</td>
</tr>
<tr>
<td>O</td>
<td>2365</td>
<td>5.9</td>
</tr>
<tr>
<td>P</td>
<td>1685</td>
<td>8.2</td>
</tr>
<tr>
<td>Q</td>
<td>1845</td>
<td>16.8</td>
</tr>
<tr>
<td>R</td>
<td>1090</td>
<td>11.5</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>9.2% ± 3.2</td>
</tr>
</tbody>
</table>

\( a \) Labeling index corrected for thickness of sections by factor of 1.4 (see text).

\( b \) Superficial multicentric BCC's.

emissions have a range of only 1.5 to 2.0 \( \mu \), a correction factor of 1.4 is used to compensate for visible nuclei containing tritium that are too far below the photographic emulsion for their emissions to produce grains (6). The same factor presumably contributes to the failure of the labeled mitoses curve to reach 100%. Because some mitoses containing thymidine-\(^{3}H\) are more than 2 \( \mu \) below the emulsion they are spuriously recorded as "unlabeled" mitoses. A corrected curve is also presented in Chart 1. If 50% intercepts are used for analysis on the corrected curve, the S and G\(_2\) period values remain the same.

The duration of mitosis (\( T_m \)) in BCC is obtained by relating the number of mitoses to the number of labeled cells in 1-hr specimens. \( T_m \) can then be calculated by the following equation:

\[
\frac{N_m}{T_m} = \frac{N_s}{T_s}
\]

\( N_m = \) No. of mitoses
\( T_m = \) duration of mitotic period

RESULTS

The duration of the DNA synthesis period (\( T_s \)) in BCC was obtained from a curve of the mean percentage of labeled mitoses at each hour tested in 9 patients. An average of 114 mitoses were counted on each biopsy specimen at each time interval. The S phase, measured by the distance between 37% points on the labeled mitoses curve, is 20 hr. A similar value is found on the corrected curve when using the 50% points, as is often used in animal studies where thinner sections can be obtained. The median G\(_2\) period obtained from the curve is 7 hr. The labeling index determined from tumors in 18 patients is 9.2 ± 3.2% corrected (Table 1). When the BCC's are separated into solitary nodular and superficial, multicentric types, their labeling indices are 8.4 ± 2.9% and 10.6 ± 3.7% respectively. The slightly higher labeling index in the superficial BCC may be accounted for by a greater ease in penetration of thymidine-\(^{3}H\) throughout the smaller islands of these tumors or may reflect a small difference in the proliferative activity of these 2 types of BCC.

The duration of the germinative cell cycle in the BCC calculated from Equation A is 217 hr. Multiple microscopic fields of labeled tumors were examined in 5 patients to obtain \( N_m \) and \( N_s \) (Table 2). The mitotic duration is calculated to be 1.5 hr. The G\(_1\) period is determined from the following equation:

\[
T_{G1} = T_{m} - (T_s + T_{G2} + T_n)
\]

The G\(_1\) period is approximately 188 hr for the average BCC cell. The entire cell cycle is summarized in Chart 2.

DISCUSSION

This report provides, to our knowledge, the most detailed information available regarding the kinetics of cell...
proliferation of a human tumor. BCC's are very slowly growing, locally invasive tumors that rarely metastasize. Multiple tumors of the nodular or superficial types frequently occur, demonstrating the multicentric origins of this type of cancer. They appear most frequently on the face and upper trunk with some relation to sun-exposed areas. BCC's also appear in the basal cell nevus syndrome, as a delayed response to arsenic intake, and in xeroderma pigmentosum, a disease in which an abnormality of the DNA repair mechanism occurs (4).

The utilization of small amounts of isotope (5 μCi) for intralesional (i.d.) injections has been a useful tool for the investigation of cutaneous diseases (18). A comparable study of cutaneous tumors with thymidine-3H administered i.v. would require 200 times more isotope than required in the present study (5). Thymidine-3H is considered to be a physiological marker of DNA synthesis and we have not found evidence that the local injection technique interferes with normal proliferative activity of the cell populations being studied. The similarity of labeled mitoses curves in 9 patients with BCC and the reproducibility of curves in specimens from psoriatic patients (in which there is a much shorter S period) supports the validity of the experimental technique (18). To obtain approximately 10 biopsies for the S phase determination in each patient, several different tumors were usually required. All tumors in a subject were assumed to be similar with respect to cell proliferation kinetics.

In a study in which 20 mCi thymidine-3H were injected i.v. into 2 patients with BCC, thereby eliminating possible effects of trauma from the intralesional injection, an S period duration of 19 hr was found, confirming the present results.

The analysis of tumor growth must take into consideration the following factors: (a) the duration of the cell cycle and its component parts in the proliferative cell population, (b) the proportion of proliferating and nonproliferating cells (growth fraction) within the tumor, and (c) the rate of cell death (13). In determination of the cell cycle time, the duration of the S period and the labeling index are the primary factors examined. While the labeling index is a function of the size of the proliferative cell pool and is, therefore, subject to less than perfect measurement, the S phase is more easily and reliably obtained. The duration of the S phase in BCC is significantly longer than in normal epidermis (about 16 hr, unpublished observations) and psoriatic epidermis (8.5 hr), (18). In other human cancers, S values have been reported as 17 to 60 hr for neoplastic effusions and 20 hr for leukemic cell populations (3, 12). The few other values available for S phase in normal human tissues are 13 to 14 hr for erythrocyte and granulocyte precursors and 10 to 15 hr for gastrointestinal tract epithelium (7, 14). The data for the BCC provide further evidence that in certain human cancers S phase may be longer than that of the tissue from which the cancer originates. This is contrary to observations in some animal tumor systems in which all parts of the cell cycle are shortened in the neoplastic cell population compared to its normal counterpart (2, 11).

Unlike normal epidermis, psoriatic epidermis, or ichthyotic epidermis, or in squamous cell carcinoma, there is little evidence histologically of cell differentiation and maturation within the BCC and, therefore, the viable cells have usually been considered to be a pure population of germinative cells. Furthermore, thymidine-labeled cells and mitotic figures (16) are found distributed in a random manner throughout the tumor mass rather than compartmentalized in any region of the tumor such as the periphery, where palisading cells are seen. We have, therefore, considered the BCC a relatively homogeneous population of proliferative cells for the purpose of determining its cell proliferation kinetics.

The results of this study show that the average cell of the BCC reproduces every 9 days. If, however, a BCC were not a homogeneous population of cells but contained 2 or more cell populations, i.e., proliferative cells, differentiated cells, and dead cells, the true labeling index would be greater and the G1 portion of the germinative cell cycle would be proportionately shorter, because of a decrease in the size of the germinative cell population. At present, it is not yet possible to ascertain how homogeneous this cell population is, but further studies to determine a growth fraction, as described by Mendelsohn (8), are in progress. Obvious dead pyknotic cells on histological examination were not included in the labeling indices and the questionable presence of differentiated cells cannot be eliminated with certainty. The data obtained are believed to be a reasonable estimate of the cell cycle in the BCC with available techniques.

Clinical experience has shown that the BCC is an extremely slowly growing tumor which may take months or even years to double in size. This does not conform with the experimentally determined cell doubling time of 9 days. The concept of cell loss must then be considered as a significant factor in the net growth of this tumor. Three hypotheses concerning cell loss should be con-

Fig. 2. Histological appearance of a BCC showing pyknotic nuclei suggestive of cell death.
sidered to account for the differences between the doubling times of individual cells and the tumor itself (13). (a) The BCC is a superficial tumor and a significant number of cells could be lost by desquamation at the surface. In actuality, very little desquamation is seen and many tumors are completely surrounded by connective tissue, thereby preventing cell loss at the skin surface. (b) A metastasizing tumor would experience cell loss by the dissemination of cells in the body through the vascular and lymphatic channels, but in the BCC such an occurrence is rare. (c) Within a tumor there is cell death which must occur at a rate almost equal to that of cell division, permitting only a very slight increase in cell number and tumor size. Cell death is difficult to prove; in most histological samples there are some cells that appear to be small in size with staining characteristics of pyknotic nuclei of dying cells (Fig. 2). This observation cannot be proven or measured with reliability at this time, but it remains the most likely explanation to account for the difference between the clinical doubling time of the BCC and the doubling time of the individual cells.

The treatment of BCC's has been restricted almost entirely to the modalities of surgery and X-ray. In most instances, these forms of therapy provide excellent results. However, in the few situations of deep extensive tumor invasion or numerous superficial multicentric tumors systemic or topical chemotherapy would be advantageous. One published report reveals the failure of large systemic doses of methotrexate given at weekly intervals to produce a satisfactory clinical response (17). Based on the germinative cell cycle data in the present report, only 9% of cells in the tumor population in S phase would be affected by the DNA poison, methotrexate, during the several hours of a significant blood level attained from each weekly injection. While the mechanism by which methotrexate achieves a biological result (the death or decrease in the number of proliferative cells in a tumor) is not fully understood, the above drug schedule would not be expected to be effective because such a small percentage of the cell population is attacked by methotrexate. Even if the drug was completely effective in producing a lethal effect in this 9% group of cells, they would still be replaced several-fold within 1 week. More frequent exposure to chemotherapeutic agents that block DNA synthesis like methotrexate and 5-fluorouracil, on a daily basis by topical or systemic administration, would theoretically be more efficacious.

REFERENCES

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