Nuclear Binding of Tritiated Actinomycin in Basal Cell Carcinoma and in Normal Human Epidermis

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SUMMARY

The ability of fixed histological samples from normal epidermis and from basal cell carcinoma to bind actinomycin D-3H was measured by autoradiography. Significantly higher nuclear fixation of the labeled antibiotic was measured in all cases in basal cell carcinoma, fixed with formalin or with alcohol:acetic acid. This suggests that chromatin in the carcinoma is less repressed than in normal basal cells, a conclusion which is in agreement with our present knowledge of this particular type of epidermal tumor, known to be able to evolve towards more or less well-characterized differentiated epidermal adnexae.

INTRODUCTION

The most widely accepted theory on the histogenesis of basal cell carcinoma is that it derives from immature pluripotential cells of the epidermis (11). These cells, in contrast to cells of the basal layer of normal epidermis, are able to evolve toward any of the epithelial structures. This is expressed by the appearance of differentiated forms of basal cell carcinoma which seems to represent abortive attempts to form apocrine, eccrine, or sebaceous glands or hair follicles.

As reported in a previous work (10) DNA replication time (S phase) is longer in basal cell carcinoma than in normal epidermal cells. This also suggested a change in chromatin properties in carcinomatous cells. Changes in the ratio of hetero- to euchromatin with an increase in the proportion of chromatin being under this latter state may be expected in instances where genomic repression is decreased (6).

Since the rate of DNA synthesis in euchromatin is slower than it is in heterochromatin, (13) this could be related to the observed increase in S-phase duration in basal cell carcinoma. It appeared interesting, therefore, to investigate by a more direct approach whether a decrease in the repression of chromatin is likely to exist in this carcinoma.

Autoradiographic measurement of the nuclear binding of actinomycin-3H affords a means to test this hypothesis, since various works have indicated that binding reflects the state of chromatin repression (2, 5, 8, 12). Thus, Ringertz et al. (12) observed a good correspondence in vitro between RNA synthesis and actinomycin fixation in lymphocytes stimulated by phytohemagglutinin. Berlowitz et al. (2), investigating the uptake of the tritiated antibiotic in cells of the male mealy bugs, demonstrated a higher binding in euchromatin than in the repressed heterochromatin. Brachet and Hulin (4), after staining of fixed tissue sections, found that the capacity of actinomycin binding decreases with differentiation during embryonic development of urodeles. The present investigation was therefore undertaken in order to test whether differences in the capacity of basal cell carcinoma to bind the antibiotic could be revealed and, if so, whether they were consistent with the hypothesis that these carcinomatous cells are under a less repressed state than are normal basal cells.

MATERIALS AND METHODS

Small nodular basal cell carcinoma excised with normal adjacent epidermis were taken from 7 patients. Tissue samples were fixed for 1 hr at 4°C in 10% neutral buffered formalin or in ethanol:acetic acid (3:1). After paraffin embedding, the tissue was sectioned at 3 μm thickness. Dehydrated sections were immersed for 1 hr at room temperature in a solution of 125 μCi tritiated actinomycin D per ml (Schwarz/Mann, Orangeburg, N. Y.) (specific activity, 4.8 Ci/mmmole) and then rinsed in tap water for 2 hr. Autoradiographs were prepared by the dipping technique using Ilford K2 emulsion and developed in amidol. Autoradiographs from specimens fixed in ethanol:acetic acid were exposed for 24 hr while specimens fixed in neutral formalin were exposed for 48 hr. Finally, the sections were stained by the methyl green-pyronin method.

Grain count was assessed in the same tissue sample in cells from nodules of basal cell carcinoma and in basal cells of normal adjacent epidermis; a minimum of 100 cells was scored in each region.

RESULTS

Labeling intensity in ethanol:acetic acid-fixed tissue was approximately 2 times that observed in formalin-fixed material. Therefore, in order to increase the accuracy of grain count, exposure of autoradiographs from ethanol:acetic acid-fixed tissue was reduced to 24 hr.

Table 1 compares the grain counts of basal cell carci-
Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Fixative</th>
<th>Normal basal cells</th>
<th>Basal cell carcinoma</th>
<th>% over normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol:acetic acid</td>
<td>21.8 ± 1*</td>
<td>29.0 ± 1.2</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol:acetic acid</td>
<td>14.0 ± 0.6</td>
<td>23.1 ± 0.7</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol:acetic acid</td>
<td>20.9 ± 1.7</td>
<td>29.8 ± 0.8</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol:acetic acid</td>
<td>10.8 ± 0.5</td>
<td>19.8 ± 0.7</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>Formalin</td>
<td>8.7 ± 0.4</td>
<td>15.0 ± 0.5</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol:acetic acid</td>
<td>10.2 ± 0.5</td>
<td>15.8 ± 0.5</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>Formalin</td>
<td>12.2 ± 0.5</td>
<td>19.7 ± 0.5</td>
<td>82</td>
</tr>
<tr>
<td>8</td>
<td>Formalin</td>
<td>19.2 ± 0.6</td>
<td>26.9 ± 0.8</td>
<td>40</td>
</tr>
<tr>
<td>9</td>
<td>Formalin</td>
<td>15.8 ± 0.6</td>
<td>32.5 ± 0.8</td>
<td>100</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

DISCUSSION

Autoradiographic studies with tritiated actinomycin have shown that the degree of binding is influenced by the state of repression of chromatin. Intact lymphocytes incubated with actinomycin-3H showed an increased fixation after phytohemagglutinin stimulation (12). Conversely, suppression of RNA synthesis by prednisolone in the same cell was accompanied by a decreased binding (8). Brachet and Hulin (3), studying the ability of fixed tissues to bind actinomycin-3H in Pleurodeles larvae, demonstrated that the intensity of binding correlated with the state of differentiation. The same observation was made during spermiogenesis (4) and lymphocyte maturation (5). In the present investigation we demonstrated that basal cell carcinoma binds actinomycin-3H to a greater extent than does the germinative layer of epidermis. Hereabove cited works suggest that this could be a reflection of cell immaturity in basal cell carcinoma. The same observation was made in acute leukemia, a neoplastic state also characterized by a defect of cellular differentiation (5).

In 2 recent works, differences in the rate of DNA replication of heterochromatin and euchromatin have been observed with remarkably longer replication time in euchromatin (7, 13). The long S-phase duration that we measured in basal cell carcinoma (10) could perhaps be explained by a modification of hetero- to euchromatin. Such modification could be expected if one admits that differentiation parallels the conversion of euchromatin to heterochromatin (1). The same hypothesis would account for the increased nuclear fixation of actinomycin-3H in the tumor. In fact, hypercondensed interphasic heterochromat...
tin appears to contain DNA in which genetic information is repressed. Such DNA supports RNA synthesis to a lesser extent than euchromatin does (6). It has also been shown that it binds less actinomycin D in vivo and in vitro than euchromatin. Thus, in male mealy bugs, all nuclei bind 3 times more actinomycin to euchromatin than to heterochromatin (2). It was also observed that the amount of condensed chromatin varies in a given cell with the stage of development, e.g., there is a marked increase in the quantity of condensed chromatin during the formation of polyhormonuclear leukocytes (1).

For a given exposure time of autoradiographic material, tissue fragments fixed in ethanol:acetic acid exhibited 2 times higher grain number than did formalin-fixed material, thus indicating increased actinomycin-3H binding. Dick and Johns (9) have reported that ethanol:acetic acid extracts 10% of the total histones, predominantly from the arginine-rich fraction. Experiments on the effect of removal of lysine-rich and arginine-rich histones on the template activity of DNA indicated that the removal of arginine-rich material considerably modifies RNA polymerase activity (14). That the ratio of labeling in normal epidermis relative to carcinomas remained essentially the same with both fixatives, with unmodified grain count distribution (Chart 1), suggests that arginine-rich histones are not at the basis of the differences between normal epidermis and neoplastic tissue that are evaluated by the actinomycin-3H binding test. Since there are no data in the literature allowing one to quantitate differences between actinomycin D binding in terms of degree of repression, our data must be taken only qualitatively.

In conclusion, our study of cell cycle parameters showing a longer S-phase duration in basal cell carcinoma than in normal basal cells was indicative of changes in the chromatin properties of the cells of this particular tumor. The present data on actinomycin binding further suggest that these changes correlate with a decreased degree of genetic repression in this tumor as compared to that in normal basal cells. Both types of data could be integrated in the admitted view (11) that basal cell carcinoma derives from immature pluripotential cells of the epidermis.

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REFERENCES

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