X-Ray Microanalysis of Electrolyte Content of Normal, Preneoplastic, and Neoplastic Mouse Mammary Tissue

Nancy K. R. Smith,1 Sidra B. Stabler, Ivan L. Cameron, and Daniel Medina

Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio 78284 [N. K. R. S., S. B. S., I. L. C.], and Department of Cell Biology, Baylor College of Medicine, Houston, 77030 [D. M.], Texas

ABSTRACT

Intracellular sodium, chlorine, and potassium concentrations (mmol/kg dry weight) were determined by electron probe X-ray microanalysis of individual epithelial cells in freeze-dried 2-μm sections of mouse mammary tissue which were cut at −30°. A model system was utilized in order to compare elemental content of cells from normal pregnant mammary tissue and preneoplastic and neoplastic mammary tissues from female BALB/cCrI Med mice. Animals were killed by cervical dislocation, and tissue was rapidly frozen in liquid propane. Normal mammary glands were obtained from primiparous mice at 16 to 17 days of gestation. Tissue from the hyperplastic alveolar nodule line D1 was removed from donor mice 12 to 16 weeks after transplantation into the cleared mammary fat pad. All mammary adenocarcinomas, D1T, were primary tumors which developed in mice with transplants of nodule line D1.

Data were collected from five animals (10 cells/animal) in each of the three groups. It was found that the electrolyte content of cells of preneoplastic tissue was the same as that of the normal mammary tissue but was significantly elevated in neoplastic tissue (162, 130, and 48% increases for sodium, chlorine, and potassium, respectively). Thus, an increase in electrolyte content seems to be associated with the transformation to a neoplastic state and not associated with conversion to the preneoplastic state.

INTRODUCTION

Our laboratory is interested in the role of intracellular ions, particularly sodium, and changes in their concentration with regard to mitogenesis, growth control, and oncogenesis. It appears that changes in the intracellular concentration of inorganic substances and small ions might cause a cascade of physiological, biochemical, and morphological events leading to a coordinated and appropriate response on the part of the cell. Our findings have been presented in recent reviews (5, 8, 9, 28) which discuss the importance of the intracellular environment in cellular regulation. We have used electron probe X-ray microanalysis to test the hypothesis of Cone (10, 11) and Hazelwood (18) that the intracellular concentration of sodium should be greater in transformed cells than in their normal counterparts. In 1978, we reported that sodium and chlorine were more than twice as high in transplantable H6 hepatoma cells as compared to hepatocytes from the host mice or from control mice (30). No significant differences were found between nuclear and cytoplasmic concentrations for either element. Several other normal and tumor populations were examined and similar results were found (3, 5–9). Other elements (potassium, magnesium, phosphorus, sulfur) did not show a consistent pattern of elemental concentration differences between tumor and nontumor populations.

The concept of multistage development of neoplasia is generally accepted. The qualitative and quantitative changes undergone by a normal cell population exposed to a carcinogen are called preneoplastic progression. The neoplasm formed continues to evolve, undergoing "tumor progression" (15, 16). Having established that transformed cells have higher sodium and chlorine concentrations than their normal counterparts, it was of great interest to investigate the electrolyte content of cells which might be at an intermediate stage of transformation. Cameron et al. (6) performed an X-ray microanalytical study of hepatocytes from animals to which a carcinogen, hydrazine sulfate, had been administered and found that the sodium and chlorine levels were intermediate between those for normal and transformed hepatocytes. These data support our basic hypothesis but are subject to criticism in that the carcinogen undoubtedly had toxic effects and those effects could not be distinguished from the carcinogenic effects. In order to study populations of cells which are between normal cells and fully transformed cells and for which the acute toxic effects of a carcinogen can be ruled out as a source of confusion, it is important to utilize a model system in which the intermediate populations can be visualized, defined, and manipulated. The model system which was used in the present study is the HAN,2 which is the principal preneoplastic lesion in mouse mammary glands. The HAN is a focus of hyperplastic lobuloalveolar development in an area of nonstimulated mammary gland. HAN’s can be induced in mice by mammary tumor viruses, chemical carcinogens, irradiation, or prolonged hormone stimulation (21). All of these agents can also induce the formation of neoplastic tissue in the mammary gland. DeOme et al. (14) demonstrated the high-risk preneoplastic nature of HAN’s, showing that HAN’s transplanted into the mammary gland-free fat pads of isogenic mice gave rise to hyperplastic alveolar outgrowths which produced mammary tumors sooner and in greater frequency than did normal tissue transplanted into the contralateral mammary fat pads. Samples of the alveolar outgrowth tissues can be serially transplanted from one fat pad to another indefinitely, always giving rise to lobuloalveolar tissue which completely fills the fat pad. By this technique, nodule outgrowth lines can be established. These lines can be considered the in vivo equivalent of in vitro cell lines (21).

Numerous nodule outgrowth lines have been developed us-

1 Supported by Grant RR07187-01, Biomedical Research Grant Program, Division of Research Resources, NIH, and in part by Grant PCM-804084, National Science Foundation. To whom requests for reprints should be addressed.

Received May 15, 1981; accepted July 7, 1981.

2 The abbreviations used are: ANOVA, analysis of variance statistical test; HAN, hyperplastic alveolar nodule; NMR, nuclear magnetic resonance.
These lines have been characterized with regard to numerous parameters (Refs. 21, 22, and 24; see ‘‘Discussion’’) in an effort to identify a marker(s) for mammary preneoplasia and to determine whether such a marker(s) would provide information on the essential differences among normal, preneoplastic, and neoplastic cells. For these reasons, it was of great interest to us to utilize this model system to compare the electrolyte content of the 3 types of mammary epithelial cells.

RESULTS AND DISCUSSION

Examination of tissue prepared for light microscopy showed the morphology of mammary tissue from the 3 groups of mice to be as described by Medina (21). In the scanning electron microscope, morphology was readily identified by means of the secondary electron image. The 16- to 17-day pregnant mouse was chosen as the control animal in our model system since, morphologically, HAN’s are indistinguishable from the lobuloalveolar development seen in normal prelactating mammary cells (21). Histologically, alveoli in the preneoplastic outgrowth are composed of a single row of epithelial cells surrounding a small lumen. Fat and protein droplets are commonly seen in the apical cytoplasm. Since the cytoplasm of the cells was scant, making it difficult to locate an area of cytoplasm of sufficient size to raster with certainty and not be picking up a signal from excitation of extracellular contents, only data from nuclei were analyzed. Previous studies from our laboratory (4, 7–9) have consistently not found a significant gradient between nucleus and cytoplasm for any of the elements analyzed in any of the tissue types analyzed (control, tumor, rapidly proliferating normal, slowly proliferating normal) in rat or mouse. A consistent criterion used for choice of cells for analysis was that they be located near the blood supply in order to ensure that the cells were viable and not necrotic. Only cells in the outermost layer of a group of cells were analyzed. Areas of obvious damage were avoided. Myoepithelial cells, which were present in the normal and preneoplastic tissue but not in the neoplastic tissue, were avoided. In nuclei which were analyzed, excitation of the nucleolus was also avoided.

Results of a one-way ANOVA for each of the 3 elements (sodium, chlorine, potassium), as well as the average concentration values (mmol/kg dry weight) and the standard errors of the means, are given in Table 1. For each of the 3 elements, concentration values for the control and preneoplastic cells are not different from each other but are significantly different from values for the tumor cells. On a dry weight basis, sodium is

### Table 1

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Preneoplastic</th>
<th>Neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>116 ± 7</td>
<td>122 ± 7</td>
<td>304 ± 6</td>
</tr>
<tr>
<td>Chlorine</td>
<td>144 ± 6</td>
<td>156 ± 5</td>
<td>332 ± 7</td>
</tr>
<tr>
<td>Potassium</td>
<td>287 ± 13</td>
<td>253 ± 9</td>
<td>426 ± 10</td>
</tr>
</tbody>
</table>

$^*_{ Each concentration value is the mean of 50 nuclei (5 animals, 10 cells/animal) ± S.E.}$

---

approximately 3 times higher in the tumor cells than in the preneoplastic and normal mammary cells and chlorine is twice as high. Potassium is elevated in the tumor cells but not to the same extent as sodium and chlorine.

Elevation of sodium and chlorine in the mouse mammary tumor cells as compared to normal counterparts agrees with our previous results for H6 hepatoma in the mouse (30), Morris hepatoma 7777 in the rat (6), and mammary adenocarcinomas C3H in the mouse and 13762 NF in the rat (8). Potassium was also found to have increased for the H6 and 7777 hepatomas but was not different from normal counterpart cells for the 2 mammary adenocarcinomas.

It is of great interest to consider whether the increased electrolyte content, on a dry weight basis, of the mammary tumor cells could be due to their taking on increased water and hence not be a reflection of a true concentration change on a wet weight basis. If such were the case, one might expect each of the major electrolytes to change to the same extent, which they do not do. It has been known for many years that the water content of tumors can be higher than for the host tissues of origin (12). In 1971, Damadian (13) reported that the NMR relaxation times of water protons in Walker sarcoma and in Novikoff hepatoma were significantly higher than in their normal tissues of origin in rats. The relaxation times were correlated with water content and with state of transformation. The findings of Damadian (13) were confirmed by Hazlewood et al. (19, 20) and extended to mammary tissues. Hazlewood et al. (19, 20) concluded that the relaxation times of water protons in tumors were almost doubled relative to normal and preneoplastic mammary gland tissues and that there appeared to be a progressive change in the relaxation times for the normal, preneoplastic, and neoplastic mammary tissues so that the 3 morphological stages could be distinguished by the average values of the relaxation times. Examination of the data showed a definite difference between normal and neoplastic tissue, but the preneoplastic tissue showed high variability among different outgrowth lines. The water content for the preneoplastic lines also showed a high variability among outgrowth lines (31 to 58% water). Interpretation of the data was complicated by the fact that the analyses were carried out on whole tissue. In order to circumvent this problem and to rule out the effects of extracellular contents, connective tissue, fat, etc., primary cell cultures of the 3 tissue types were prepared and analyzed by Beall et al. (1, 2). In the primary culture system, it was possible to distinguish among normal, preneoplastic, and neoplastic mammary epithelial cells with a high degree of accuracy (a < 0.001) by NMR, with the relaxation times for preneoplastic cells being intermediate between those for normal and neoplastic cells. No significant differences were found in hydration for the 3 cell types; each showed a water content of 90 to 91%. Assuming a similar equality of hydration for the 3 cell types in vivo, it can be concluded that our observed differences in electrolyte content between normal, preneoplastic, and neoplastic mammary epithelial cells were not due to differences in intracellular water content. Comparison of our data and those of Hazlewood et al. (19, 20) and Beall et al. (1, 2) indicates that there is not a direct correlation between elemental content and freedom of the water since relaxation times are different for all 3 cell types, being intermediate in value for preneoplastic cells, whereas the electrolyte content is the same for normal and preneoplastic cells, which are both different from neoplastic cells.

Medina (21–23, 25) has compiled data on the various properties of preneoplastic HAN’s as compared to normal and neoplastic mammary tissue. Most of the properties of HAN’s are the same as for normal tissue. HAN’s are different from normal cells and similar to neoplastic cells only with regard to indefinite division potential and loss of steroid regulation of DNA synthesis (24).

One interest in the model system reported herein derives from a quest for markers which will distinguish preneoplastic and/or malignant cells from their normal counterparts (2, 24). The search has proven to be lengthy and frustrating. Measurement of NMR water proton relaxation times does have some potential as a diagnostic tool. Most properties, however, are measured as averages for cell populations in tissue rather than for individual cells. Electron probe X-ray microanalysis has the advantage of measuring intracellular elemental concentration values for individual cells, but with the model used, such analysis has not revealed differences which would enable one to distinguish between normal and neoplastic cells, although one can rather confidently identify a neoplastic cell by its electrolyte content.

It would appear from the electrolyte content data reported herein that an increase in electrolyte content occurs in association with some critical step undergone by a cell when it transforms to the neoplastic state. Whatever this critical step is, a change in electrolyte content does not appear to be a progressive phenomenon with development of preneoplastic HAN tissue.

REFERENCES

10. Cone, C. D., Jr. Unified theory on the basic mechanism of normal mitotic

OCTOBER 1981

3879


X-Ray Microanalysis of Electrolyte Content of Normal, Preneoplastic, and Neoplastic Mouse Mammary Tissue

Nancy K. R. Smith, Sidra B. Stabler, Ivan L. Cameron, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/10/3877

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.