Stimulation of Mammary Carcinoma Cell Proliferation by Epithelial Growth Factor in Vitro

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Mammary carcinogenesis in the C3H mouse involves a complex interaction of viral, genetic, and hormonal factors, and this experimental model for breast cancer has been the subject of extensive investigation. Hormonal factors may be important both in the neoplastic transformation (6) and in the regulation of the rate of tumor growth (9). Previous studies demonstrated that cell proliferation in C3H mammary carcinoma explants is stimulated by the addition of insulin to the synthetic medium, and the rate at which cells enter the DNA-synthetic (S) phase of the cell cycle may be further modified by estrogenic hormones (9).

A protein has been isolated from the submaxillary salivary glands of mice which stimulates growth of epidermal cells (2). Under appropriate conditions the purified epidermal growth factor (EGF) causes increased numbers of epidermal cells to synthesize DNA (3). It may stimulate several types of rat epithelial cells to proliferate and has consequently been termed the “epithelial growth factor” (4). Although the biologic function of EGF has not been clearly established, the studies reported here have examined its potential role in the regulation of growth of mammary carcinoma cells. The results demonstrate that EGF can act as a potent regulator of mammary carcinoma cell proliferation and suggest that it may be an additional hormonal factor governing the rate of mammary cancer growth.

Explants of small, nonnecrotic, spontaneous mammary adenocarcinomas of C3H/HeJ mice were prepared and cultured in Medium 199 as previously described (9). Highly purified EGF was prepared as described previously (2) and was added to the medium at a final concentration of 0.5 μg/ml, unless otherwise stated. The rate of DNA synthesis was measured by allowing explants to incorporate thymidine-3H (Schwarz, methyl-labeled, sp. act. 8.0 c/mmole; 1.0 μc/ml medium) into trichloroacetic acid-precipitable material during 4-hour labeling periods as previously described (7, 9). Explants taken for histologic examination were fixed in Bouin's solution, sectioned at 5 μ, and stained with hematoxylin and eosin.

Slides for autoradiography were dipped in Kodak NTB-3 emulsion.

Chart 1 shows the time course of DNA synthesis in mammary carcinoma explants incubated on medium containing EGF. After an initial lag period of 8—12 hours, the rate of DNA synthesis is stimulated, and after 48 hours it is approximately 4-fold greater than in control explants. As shown in Table 1, this increased rate of DNA synthesis reflects primarily an increased number of cells engaged in DNA synthesis. A parallel increase in the mitotic index indicates that the thymidine labeling index reflects increased cell proliferation rather than merely a change in the uptake of the radioactive precursor.

Chart 2 shows the rate of DNA synthesis as a function of the molar concentration of EGF in the medium (based on a...
Table 1

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>Mitotic figures per 1000 cells</th>
<th>% cells labeled by thymidine-(^{3}H), 44–48 hr</th>
<th>Average grain count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>6</td>
<td>50 ± 18</td>
</tr>
<tr>
<td>EGF</td>
<td>31</td>
<td>33</td>
<td>52 ± 20</td>
</tr>
</tbody>
</table>

Mitotic and labeling indices of C3H mouse mammary carcinoma cells in explants cultured for 48 hours in Medium 199 with or without epidermal growth factor (EGF). The results are representative of experiments on 3 tumors.

These results demonstrate that EGF can act as a potent stimulus for cell proliferation in C3H mouse mammary carcinomas. EGF stimulates the rate at which these cells initiate DNA synthesis but does not detectably alter the rate at which DNA is replicated per cell. Its stimulatory effect \textit{in vitro} is similar to that of insulin in its magnitude and time course (9). EGF is effective in a concentration range in which many polypeptide hormones are found in plasma (1), and it may act as a hormonal factor which is either synthesized or accumulated in the salivary glands. EGF has been positively identified in relatively large amounts in the salivary glands of male mice only. Its concentration in salivary gland is markedly increased by testosterone (2), and it is likely that, as in the case of nerve growth factor (5), it will also be found in significant amounts in female animals. Recent studies indicate that EGF can stimulate DNA synthesis in normal mouse mammary epithelial cells \textit{in vitro} and that EGF may play a role in the growth and development of mammary gland (8). Its stimulatory effect upon C3H mammary epithelial cells does not depend upon the presence of the mammary tumor virus since a stimulation of DNA synthesis of equal magnitude was observed in tissue derived from C3H mice known to be free of the virus. Elucidation of the potential role of EGF in the regulation of normal and neoplastic cell proliferation in the human may prove relevant to the control of human cancers.

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\textbf{REFERENCES}

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