Hormonal Influences on the Expression of Casein Messenger RNA during Mouse Mammary Tumorigenesis

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ABSTRACT

The expression of a differentiated mammary gland function, the production of casein messenger RNA (mRNA), was examined in preneoplastic outgrowth lines of BALB/c hyperplastic alveolar nodules (HAN) and a ductal hyperplasia (DH) as well as tumors that arose from the outgrowth lines. HAN and DH were induced in virgin BALB/c mice by hormonal stimulation and/or treatment with 7,12-dimethylbenz(a)anthracene. Outgrowth lines were established by serial transplantation of the HAN and DH. Casein 15S mRNA was quantitated in total cellular RNA by molecular hybridization with a labeled complementary DNA probe synthesized in vitro from purified mouse casein 15S mRNA. The level of casein 15S mRNA observed in BALB/c outgrowth lines and tumors ranged from 0.01% to less than 0.0005% of total cellular RNA. In comparison, about 0.05% casein mRNA was found in midpregnant BALB/c mammary glands. Virgin BALB/c mice bearing transplanted outgrowth lines or tumors were treated with prolactin (500 µg/day) and/or dexamethasone (100 µg/day) for 5 days to test the effect of these lactogenic hormones on the level of casein 15S mRNA. Significant increases in the level of casein 15S mRNA were observed with the combined hormone treatment in three of five HAN outgrowth lines, whereas the same hormone treatments did not significantly increase casein mRNA levels in the DH outgrowth line or any tumors derived from HAN or DH outgrowth lines. Therefore, the expression of a differentiated mammary gland function is hormonally responsive in BALB/c mRNAs of HAN outgrowth lines but not mammary tumors.

INTRODUCTION

Mammary tumorigenesis in the mouse is influenced by a variety of agents including the milk-transmitted murine mammary tumor virus (MMTV), chemical carcinogens, the genetic complement, and hormones. Mammary lesions classified as HAN have been observed in mouse strains with a high incidence of mammary tumors as well as low-tumor-incidence BALB/c mice treated with hormones and/or DMBA or infected with an exogenous milk-transmitted MMTV (4, 7, 14). HAN outgrowth lines, with stable morphological, biological, and tumorigenic properties, have been established by serial transplantation of primary HAN (14). HAN are characterized in mammary tissue of virgin mice as regions of alveolar hyperplasia that resemble the midpregnant mammary gland (4, 7, 14). HAN outgrowths give rise to mammary tumors earlier and at a higher frequency than does normal mammary tissue, suggesting that HAN are preneoplastic mammary lesions (5, 13, 16–18). In addition, DH outgrowth lines, which also appear to be preneoplastic mammary lesions, have been established from primary DH that occurred in virgin BALB/c mice treated with DMBA (15). Both in vivo (1, 21) and in vitro (6) studies have demonstrated that maintenance and growth of HAN were dependent upon the same hormones required for normal mammary development (20). However, growth and secretory activity of HAN are less dependent upon hormones than are normal lobule growth and secretory activity (2, 6). Tumors derived from HAN appear to be ovarian independent (22).

Previous studies have shown that casein 15S mRNA accumulation is correlated with hormonally regulated normal mammary gland development in BALB/c mice (26). The studies described in this report have examined the expression of a normal differentiated mammary gland function, the production of casein 15S mRNA, during neoplastic mammary development in BALB/c mice. Particular attention was directed toward the influence of the lactogenic hormones prolactin and glucocorticoids on casein 15S mRNA levels. Casein 15S mRNA was present in several HAN outgrowth lines, and the level of casein 15S mRNA was increased significantly by the lactogenic hormone combination. The same hormone treatments had no effect on the low or undetectable levels of casein 15S mRNA in a DH outgrowth line and tumors that arose from 5 different HAN and DH outgrowth lines.

MATERIALS AND METHODS

Animals and Tissues. BALB/cCrjIme mice were bred and maintained in a controlled environment at the mouse colony of the Department of Cell Biology. A BALB/cCrj breeding pair, which was obtained from the Cancer Research Laboratory, Berkeley, Calif., in 1970, has been brother x sister mated and had a mammary tumor incidence of less than 1% (2 of 206) between 1975 and 1977 (24). BALB/c HAN and DH outgrowth lines were maintained by serial transplantation into gland-free mammary fat pads of 3-week-old BALB/cCrjIme mice by the method of DeOme et al. (5). The characteristics of the BALB/c HAN and DH outgrowth lines that were used in this study are summarized in Table 1. Mammary tumors that arose spontaneously from the BALB/c HAN or DH lines were serially transplanted into 8- to 12-week-old virgin BALB/c mice. The transplant generations of each of the HAN lines and tumors that were used are indicated in the text.

The hormone treatments were initiated about 10 and 3 weeks after transplantation of the outgrowth lines and tumors, respectively. Bovine prolactin (NIH-P-B4), which was provided by the National Pituitary Agency through the auspices of the National Institute of Arthritis, Metabolism and Digestive Diseases, was...
dissolved in 0.9% (w/v) NaCl solution, pH 9, at 5 mg/ml. Hydrocortisone sodium succinate (Sigma Chemical Co., St. Louis, Mo.), 10 mg/ml, was dissolved in 0.9% (w/v) NaCl solution. Dexamethasone acetate (Sigma), 1 mg/ml, was dissolved in 5% ethanol-95% sesame oil (v/v). The hormones were injected s.c., 0.1 ml/day, for 5 days. Approximately 6 outgrowth lines and tumors were removed, and the tissues were frozen and stored at −70°.

**RNA Extraction.** Total cellular RNA was isolated by sodium dodecyl sulfate-phenol extraction of pulverized frozen tissue followed by proteinase K (EM Biochemicals, Elmsford, N. Y.) digestion, 40 μg/ml, for 30 min at 37° as described previously (24). Contaminating DNA was extracted with 3 M ethanol-95% sesame oil (v/v). The hormones were injected s.c., 0.1 ml/day, for 5 days. Approximately 6 and 3 animals were present in each treatment group of the outgrowth lines and tumors, respectively. Animals were sacrificed by cervical dislocation 8 hr after the last injection. HAN or tumors were excised, areas of necrosis and hemorrhagia were removed, and the tissues were frozen and stored at −70°.

**Synthesis of Casein cDNA.** Mouse casein 15S mRNA was purified from total cellular RNA, isolated from pooled 1- to 7-day lactating BALB/c mammary glands, by affinity chromatography on deoxyxymethylcellulose and molecular sizing on Sepharose 4B as described previously (28). The purified casein mRNA was resolved as a doublet of 15.2S and 13.9S on agarose-urea gels and is referred to as casein 15S mRNA. The conditions for synthesis of a full-length casein cDNA probe were similar to the previously described method (29). The reaction mixture contained: 50 mM Tris-HCl, pH 8.3; 150 mM KCl; 6 mM MgCl₂; 60 μg actinomycin D per ml (Calbiochem, La Jolla, Calif.); 20 mM diithiothreitol (Calbiochem); 4 mM Na₃P₂O₇ (Chelex-100 treated; Bio-Rad Laboratories, Richmond, Calif.); 400 μM concentrations each of dATP, dGTP, and dTTP (PL Biochemicals, Milwaukee, Wis.); 100 μM [5-³H]dCTP (21 Ci/mmol; Schwarz/Mann, Orangeburg, N. Y.); 50 μg of purified mouse casein 15S mRNA per ml; 2.5 μg oligodeoxythymidylylate per ml (PL Biochemicals); and 124 enzyme units of avian myeloblastosis virus RNA-dependent DNA polymerase per ml (Division of Cancer Cause and Prevention, National Cancer Institute). After incubation at 46° for 15 min, the reaction was stopped by addition of sodium dodecyl sulfate and disodium EDTA to final concentrations of 1% (w/v) and 20 mM, respectively. The cDNA product was purified by Sephadex G-50 column chromatography, the RNA template was digested by treatment with 0.1 n NaOH at 60° for 1 hr, and the cDNA was fractionated by alkaline sucrose density gradient centrifugation (31) as previously described (24). Labeled cDNA in the 9 to 13S region of the gradient was recovered and represents essentially full-length cDNA with a specific activity of 15,000 cpm/ng, assuming a 50% (C + T) content in the cDNA. The yield of casein cDNA, following sucrose density gradient fractionation, was approximately 8%, based on the input RNA in the cDNA synthesis reaction. The mouse casein cDNA is a representative copy of the mouse casein 15S mRNA on the basis of its ability to protect 125I-labeled casein 15S mRNA from nuclease digestion.

**Nucleic Acid Hybridization.** The level of casein 15S mRNA in total cellular RNA was determined by molecular hybridization performed by the titration method (34). The titration method involves hybridizing RNA to a fixed excess of cDNA for sufficient time to assure completion of the annealing reaction. Hybridization reactions were carried out in a final volume of 20 μl containing various amounts of RNA, 0.6 M NaCl, 0.01 M N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (pH 7.0), 2 mM disodium EDTA, 8 μg of chick liver RNA, and 0.6 ng of [³H]casein cDNA. Hybridizations were carried out at 68° for 48 hr. The equivalent C₀, the product of the cDNA concentration in mol/liter times the time in seconds, normalized to 0.1 M NaCl, was calculated. The amount of hybridized [³H]casein cDNA was quantitated by digestion with the single-strand specific S1 nuclease as previously described (24).

The concentration of casein 15S mRNA in cellular RNA was determined by comparing the kinetics of hybridization between the [³H]casein cDNA and total cellular RNA to the kinetics of hybridization between the [³H]casein cDNA and purified mouse casein 15S mRNA, as described for the titration method (34). Under these annealing conditions, the amount of hybrid formed during the initial portion of the hybridization reaction is proportional to the concentration of RNA in the annealing reaction as previously shown (25, 26). The initial slope of the hybridization reaction was determined, using at least 3 data points, by least-square analysis yielding correlation coefficients ≥0.90. The lower limit of detection of casein 15S mRNA by molecular hybridization is 0.0005% of the total cellular RNA. The statistical significance of the difference in the slope of the hybridization data between a given hormone treatment and the control for each experiment was quantitated and evaluated by the Student t test.

**RESULTS**

Casein mRNA in BALB/c HAN Lines and Tumors. HAN outgrowth lines morphologically resemble the midpregnant mammary gland. The C3, C4, and C5 HAN lines were derived by transplantation of a primary DH that was observed in a virgin 8-month-old BALB/c mouse that had received pituitary isografts alone (D1) or in combination with estradiol (D2) starting at 2 months of age (13, 17). The tumors that arose from HAN lines were alveolar adenocarcinomas. The CD8 line gave rise to ductal adenocarcinomas.

**Table 1**

<table>
<thead>
<tr>
<th>Line</th>
<th>Origin</th>
<th>Type</th>
<th>Tumor-producing capability</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Hormonal</td>
<td>Alveolar (HAN)</td>
<td>12</td>
</tr>
<tr>
<td>D2</td>
<td>Hormonal</td>
<td>Alveolar (HAN)</td>
<td>51</td>
</tr>
<tr>
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<td>Alveolar (HAN)</td>
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</tr>
<tr>
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<td>DMBA</td>
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<td>84</td>
</tr>
<tr>
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<td>DMBA</td>
<td>Alveolar (HAN)</td>
<td>85</td>
</tr>
<tr>
<td>CD8</td>
<td>DMBA</td>
<td>Ductal (DH)</td>
<td>72</td>
</tr>
</tbody>
</table>

* J. Rosen, personal communication.
mammary gland (4, 7, 14) and contain milk-like material (11). To determine if the preneoplastic HAN contain casein milk protein mRNA, the level of casein 15S mRNA in HAN as well as tumors was quantitated by molecular hybridization. Initially, the in vitro-synthesized casein cDNA was hybridized to various amounts of casein 15S mRNA, and the amount of [3H]cDNA annealed to RNA was quantitated. Approximately 55% of the casein cDNA hybridized to the purified template casein 15S mRNA at a RNA:cDNA concentration ratio of 1, and 95% of the cDNA was hybridized at a RNA:cDNA concentration ratio of 3 or greater (Chart 1). In comparison, at a RNA:cDNA concentration ratio of 10,000 with D2 HAN RNA, about 22% hybridization was observed, indicating that approximately 0.004% of total cellular RNA was casein 15S mRNA in the D2 HAN outgrowth line (Chart 1). Similar levels of casein 15S mRNA were observed over several transplant generations of the D2 HAN outgrowth line. A mammary tumor that spontaneously arose from the D2 HAN outgrowth line contained 0.002% casein 15S mRNA (Chart 1). In comparison, 4- to 7-day and 10- to 13-day pregnant BALB/c mammary glands contained approximately 0.007 and 0.05% casein mRNA, respectively (26).

The hormone-induced BALB/c D1 HAN outgrowth line contained similar levels of casein 15S mRNA to the D2 HAN outgrowth line, approximately 0.004% (Table 2). Tumors that spontaneously arose from the D1 HAN line hybridized to the casein cDNA with similar initial kinetics as D1 HAN RNA, indicating that approximately 0.005% of the total cellular RNA was casein 15S mRNA (Table 2). The carcinogen-induced BALB/c HAN lines C3, C4, and C5 contained low levels of casein mRNA, ranging from 0.001 to 0.003% of total cellular RNA. Tumors arising from C4 or C5 HAN outgrowth lines lacked or had barely detectable levels of casein 15S mRNA (Table 2). Casein 15S mRNA levels in the DH and a tumor that arose from the DH were at or below the limit of detection by molecular hybridization (Table 2).

**Hormone Effects on Casein Expression in Hormone-induced HAN Lines and Tumors.** The effects of the lactogenic hormones prolactin and glucocorticoids on casein mRNA levels were examined in the hormone-induced HAN outgrowth lines and tumors. The hormone-induced BALB/c HAN outgrowth lines D1 and D2 (16, 17) were serially transplanted into the gland-free mammary fat pads of virgin BALB/c mice. Tumors

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue</th>
<th>Control</th>
<th>Prolactin</th>
<th>Dexamethasone</th>
<th>Prolactin + Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1 HAN</td>
<td>0.0038</td>
<td>0.0236</td>
<td>0.0122</td>
<td>0.0420</td>
<td></td>
</tr>
<tr>
<td>D1 Tumor</td>
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<td>0.0253</td>
<td></td>
</tr>
<tr>
<td>D2 Tumor</td>
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<td>0.0011</td>
<td>0.0012</td>
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<td></td>
</tr>
<tr>
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<td>0.0009</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>C5 HAN</td>
<td>0.0026</td>
<td>0.0110</td>
<td>0.0062</td>
<td>0.1082</td>
<td></td>
</tr>
<tr>
<td>C5 Tumor</td>
<td>0.0006</td>
<td>&lt;0.0005</td>
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<td></td>
</tr>
<tr>
<td>CD8 DH</td>
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</tr>
</tbody>
</table>

- The transplant generation (TG) of each tissue was: D1 HAN, TG-48; D1 tumor, TG-2; D2 HAN, TG-45; D2 tumor, TG-36; C3 HAN, TG-27; C4 HAN, TG-25; C4 tumor, TG-2; C5 HAN, TG-23; C5 tumor, TG-2; CD8 DH, TG-12; CD8 tumor, TG-2.
- The level of casein 15S mRNA was determined by molecular hybridization between RNA extracted from tissue of each treatment group and the casein cDNA using the titration method. The level of hybridizable RNA is expressed as a percentage of the total cellular mRNA.
- Virgin BALB/c mice bearing transplanted tissues were treated with 0.9% NaCl solution, 500 µg prolactin, 100 µg dexamethasone, or both prolactin and dexamethasone daily for 5 days and sacrificed. D2 HAN and tumors received 1000 µg hydrocortisone instead of dexamethasone.
- *p < 0.05 between control and hormone-treated groups.
- ND, not determined.

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Chart 1. Hybridization of casein cDNA to RNA. Hybridizations with various RNA's were carried out as described in "Materials and Methods." The amount of S, nuclease-resistant labeled casein cDNA, expressed as a percentage of input labeled casein cDNA, is plotted as a function of the ratio of the input concentration of RNA and cDNA. The RNA's that were used were purified mouse casein 15S mRNA (B), total cellular RNA isolated from a 12-day pregnant mouse mammary gland (B), total cellular RNA isolated from the D2 HAN outgrowth line (C), and total cellular RNA extracted from a D2 mammary tumor (C). The level of casein 15S mRNA, expressed as a percentage of total cellular RNA, was 0.048% in the 12-day pregnant mammary gland, 0.004% in the D2 HAN outgrowth line, and 0.002% in the D2 mammary tumor.

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**Table 2**

Hormonal responsiveness of casein 15S mRNA accumulation in HAN, DH, and tumors

<table>
<thead>
<tr>
<th>Cell line</th>
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<td>0.0067</td>
<td>0.0253</td>
<td></td>
</tr>
<tr>
<td>D2 Tumor</td>
<td>0.0015</td>
<td>0.0011</td>
<td>0.0012</td>
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</tr>
<tr>
<td>C3 HAN</td>
<td>0.0013</td>
<td>0.0019</td>
<td>0.0009</td>
<td>0.0014</td>
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<tr>
<td>C4 HAN</td>
<td>0.0013</td>
<td>0.0019</td>
<td>0.0009</td>
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<td>C4 Tumor</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
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<tr>
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- The transplant generation (TG) of each tissue was: D1 HAN, TG-48; D1 tumor, TG-2; D2 HAN, TG-45; D2 tumor, TG-36; C3 HAN, TG-27; C4 HAN, TG-25; C4 tumor, TG-2; C5 HAN, TG-23; C5 tumor, TG-2; CD8 DH, TG-12; CD8 tumor, TG-2.
- The level of casein 15S mRNA was determined by molecular hybridization between RNA extracted from tissue of each treatment group and the casein cDNA using the titration method. The level of hybridizable RNA is expressed as a percentage of the total cellular mRNA.
- Virgin BALB/c mice bearing transplanted tissues were treated with 0.9% NaCl solution, 500 µg prolactin, 100 µg dexamethasone, or both prolactin and dexamethasone daily for 5 days and sacrificed. D2 HAN and tumors received 1000 µg hydrocortisone instead of dexamethasone.
- *p < 0.05 between control and hormone-treated groups.
- ND, not determined.
Casein mRNA during Mammary Tumorigenesis

**Chart 2. Effect of hormone treatments on casein mRNA in the D1 HAN outgrowth line.** D1 HAN outgrowths, TG-48, were transplanted into the gland-free mammary fat pads of BALB/c mice. Following treatment with 0.9% NaCl solution (●), prolactin (□), dexamethasone (△), or prolactin and dexamethasone (○), as specified in "Materials and Methods," HAN were removed, and total cellular RNA was extracted. The concentration of casein mRNA in total cellular RNA from each treatment group was determined by molecular hybridization. The level of casein 15S mRNA was 0.0038 (●), 0.0236 (□), 0.0122 (△), and 0.0420 (○) % of the total cellular RNA (Table 2). Statistical analysis of the data illustrated here indicated that the hormone treatments caused a significant increase in the level of casein 15S mRNA (Table 2).

were serially transplanted into virgin BALB/c mice. The mice were treated with prolactin and/or glucocorticoids, and casein mRNA was quantitated in total cellular RNA prepared from each treatment group of HAN or tumors. Prolactin and glucocorticoids exert a lactogenic effect on the mammary gland (12, 20, 32). Importantly, on a morphological basis, a lactogenic effect of prolactin and glucocorticoids was observed on HAN outgrowths in vivo (2) and in vitro (6).

In the hormone-induced D1 HAN outgrowth line, prolactin and glucocorticoids caused about a 6- and 3-fold increase, respectively, in the level of casein 15S mRNA (Chart 2; Table 2). Treatment with prolactin and glucocorticoids in combination resulted in an 11-fold increase in casein mRNA in the D1 HAN outgrowth line (Chart 2; Table 2). A similar effect of prolactin and glucocorticoids on casein mRNA levels was observed in the hormone-induced D2 HAN outgrowth line (Table 2). The number of molecules of casein 15S mRNA per 2c amount of DNA was calculated on the basis of the yield of RNA and the amount of DNA per g tissue and the molecular weight of purified mouse casein 15S RNA using a previously described formula (24, 29). The number of molecules of casein 15S mRNA per cell was 84, 446, 161, and 876 in the control, prolactin-treated, glucocorticoid-treated, and prolactin-plus-glucocorticoid-treated D2 HAN outgrowth lines, respectively. Therefore, the increase in casein 15S mRNA with the hormone treatments was not due to changes in total cellular RNA levels but represents the induction of casein mRNA in the HAN. In D1 and D2 tumors, treatment with prolactin and/or glucocorticoids did not significantly increase casein 15S mRNA (Table 2). These observations demonstrate a hormonal influence on casein gene expression in preneoplastic HAN but not tumors, in agreement with previous observations concerning the effect of prolactin and glucocorticoids on the synthesis of milk-like material (11).

**Hormone Effects on Casein mRNA in Carcinogen-Induced HAN or DH and Tumors.** The carcinogen-induced BALB/c HAN outgrowth lines (13) were transplanted into mice, were similarly treated with prolactin and/or glucocorticoids, and were then assayed for casein 15S mRNA (Table 2). Treatment of the C3 or C4 HAN outgrowth lines with prolactin and/or glucocorticoids caused no increase in the level of casein mRNA relative to the control. Treatment of the C5 HAN outgrowth line with prolactin and/or glucocorticoids increased the level of casein mRNA as much as 40-fold. The same hormone treatments had no effect on casein mRNA levels in tumors that arose from the carcinogen-induced C4 and C5 outgrowth lines (Table 2).

Treatment of virgin BALB/c mice with DMBA gives rise to a variety of mammary lesions including ductal hyperplasias (19). DH lesions were characterized as atypical ductal hyperplasias, made up of several ducts containing intraductal epithelial hyperplasias (19), that were morphologically distinct from HAN. A transplanted DH outgrowth line was established that gives rise to ductal adenocarcinomas at a high frequency (Table 1) when transplanted into cleared mammary fat pads (15). BALB/c mice bearing the transplanted DH outgrowth line or tumor that arose spontaneously from the DH line were treated with prolactin and/or glucocorticoids, and casein mRNA was quantitated. Treatment with prolactin and/or glucocorticoids had no effect on casein 15S mRNA in either the DH or tumor (Table 2).

**DISCUSSION**

Numerous studies have focused upon the elucidation of biochemical differences between normal, preneoplastic, and neoplastic mammary tissues (10, 11, 14, 24). Emphasis has been placed on the milk proteins, the caseins and β-lactalbumin (27, 33), and their mRNA’s (9, 23, 30), because these gene products are markers for hormonally regulated differentiated mammary gland function (12, 32) and have been postulated to be useful prognostic factors for the hormonal responsiveness of mammary tissues. Previous studies in the mouse demonstrated that DMBA-induced hormone-independent mammary tumors lack casein mRNA (30). This study has examined both hormone- and carcinogen-induced preneoplastic and neoplastic mammary lesions for casein mRNA. The preneoplastic HAN outgrowth lines were of particular interest because of the secretory morphology of HAN (4, 7, 14) and the presence of milk-like material in HAN (11).

Tumors that arose from hormone- and/or carcinogen-in-
duced preneoplastic mammary lesions frequently lacked or had lower levels of casein 15S mRNA than did the outgrowth line from which the tumor arose. Importantly, the level of casein 15S mRNA was increased by treatment with the lactogenic hormone combination of prolactin and glucocorticoids in 3 of 5 HAN outgrowth lines, but not in the tumor lines derived from the HAN outgrowth lines. No correlation between the incidence at which tumors arose from a HAN or DH outgrowth line (Table 1) and the presence or hormonal responsiveness of casein mRNA levels in HAN or DH outgrowth lines (Table 2) was apparent from these studies. The observation that casein gene expression was not enhanced by hormones in all HAN outgrowth lines may be due to the heterogeneity of cell populations in the HAN (14) or to a difference in the sensitivity of the HAN lines to the hormone treatment. Further studies will be necessary to identify those cell populations synthesizing casein mRNA and to examine the hormonal responsiveness of casein gene expression by in vitro analyses, in which the hormone environment can be carefully controlled.

The expression (24) and hormonal responsiveness (25) of endogenous MMTV and type C retrovirus sequences in the HAN and DH lines and tumors used in this study were also examined. Glucocorticoid treatment increased MMTV RNA levels in the carcinogen-induced HAN lines and in tumors that arose from either the hormone- or carcinogen-induced HAN outgrowth lines (25). Importantly, in the C5 carcinogen-induced HAN outgrowth line, both MMTV and casein gene expression were enhanced by hormone treatments. On the other hand, in the D1, D2, C3, and C4 HAN outgrowth lines, either MMTV or casein gene expression was enhanced by hormone treatment (24). Therefore, even though casein gene expression was not enhanced by glucocorticoids and/or prolactin in the tumors, MMTV RNA levels were increased by treatment with glucocorticoids demonstrating the hormonal responsiveness of these tissues.

Mammary tumorigenesis in the mouse may in some cases be a multistage process that is influenced by many factors (4, 14, 22). The studies described in this report indicate that cells present in the most common type of preneoplastic mammary hyperplasia, the HAN, express a normal differentiated mammary function and that the expression of this function is hormonally responsive. Similar conclusions have been reached on the basis of morphological criteria (2, 6) and biochemical studies (11). Further studies should be undertaken to examine the hormonal responsiveness of casein gene expression in an organ culture system in which the hormone environment can be carefully controlled. This same organ culture technology could also be used to examine the transcriptional and posttranscriptional effects of prolactin and glucocorticoids on casein mRNA accumulation (8) and to determine if these hormones regulate casein mRNA accumulation by the same mechanism in normal and preneoplastic mammary tissues. In addition, the cell populations synthesizing casein mRNA must be identified and characterized in the hope of understanding their relationship to the complex multistage process of mammary tumorigenesis.

ACKNOWLEDGMENTS

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