Detection and Partial Characterization of Collagen Synthesis Stimulating Activities in Rat Mammary Adenocarcinomas

(Received for publication, April 9, 1982)

Mozeena Bano, James A. Zwiebel, David S. Salomon, and William R. Kidwell†‡
From the Laboratory of Pathophysiology, National Cancer Institute, Bethesda, Maryland 20205

Rat mammary epithelium and adenocarcinomas derived from it synthesize type IV collagen, a structural protein of basement membranes. In cultures of cells, net production of collagen is stimulated 2-fold more than total cell protein by epidermal growth factor. Mammary adenocarcinoma cells also respond to epidermal growth factor but to a much reduced extent. This difference in growth factor responsiveness appears to be due to the production of collagen synthesis stimulation factors by the mammary tumor cells. Such factors have been partially purified and shown to differentially stimulate the incorporation of proline into collagenase-sensitive protein by 2.5-10-fold in normal rat mammary epithelium, normal rat kidney, and mouse 3T3 cells. The tumor factors do not stimulate net collagen production in cultures of tumor cells from which the factors are derived, suggesting that tumor cells produce sufficient stimulatory factors for optimal synthesis of collagen. Pulse-chase studies indicate that the tumor factors stimulate collagen synthesis rather than block collagen turnover. The activities in the extract have been partially purified by gel filtration, ion exchange column chromatography, and isoelectric focusing. The major species has a molecular weight of about 68,000 and a pI of 5.9. A smaller peak of activity with a molecular weight of 8,000 is also present. Since collagen synthesis appears to be necessary for the growth of mammary adenocarcinomas in vivo, production of these collagen synthesis stimulating factors may be important for tumor growth.

The epithelium of the normal rat mammary gland synthesizes type IV collagen, a constituent of the basement membrane upon which these cells rest in vivo (1). Formation of this protein is also characteristic of differentiated mammary tumors such as those induced by DMBA1 or NMU (2, 3). Synthesis of type IV collagen by these tumors is of considerable interest because their growth can be arrested by several proline analogs which are selective inhibitors of collagen production. One such inhibitor is CHP. It blocks collagen synthesis 10 times more efficiently than total protein synthesis in cultured mammary epithelium (2) and blocks the growth of both DMBA- and NMU-induced mammary tumors in vivo (2, 3). CHP effects on tumor growth are related to the ability of mammary tumors to synthesize type IV collagen since certain transplantable tumors no longer make this protein and their growth in vivo is unaffected by CHP (3, 4).

The growth of the normal mammary rat epithelium also appears to require the production of type IV collagen. CHP blocks the growth of the epithelial cells both in vivo and in vitro, but the inhibitory effect of CHP can be reduced by plating the normal mammary epithelium on type IV collagen-coated culture dishes (5, 6), suggesting that the type IV collagen-cellular interaction is important for cell growth and/or survival.

These observations have prompted us to study the control mechanisms for collagen synthesis and deposition in both normal and neoplastic mammary epithelium. We have shown that production of type IV collagen is controlled both at the level of alterations in biosynthetic and in degradative rates (7). In the present report, we show that normal and tumor cells differ in that collagen production is stimulated by EGF in cultures of normal cells but not tumor cells. In attempting to explain this difference, we found that the tumor cells contained factors that stimulate net collagen production. This suggests that these factors might be important for tumor cell growth. We also report here on the partial purification and characterization of the tumor factors.

EXPERIMENTAL PROCEDURES

Cell Cultures—The epithelium from normal rat mammary glands or from DMBA-induced rat mammary tumors was isolated according to Wicha et al. (8) and cultured in a serum-free growth medium as described by Salomon et al. (7). NRK (clone 49-F) cells were obtained from Dr. Joseph DeLarco, National Cancer Institute, and Balb/c 3T3 fibroblasts were supplied by Dr. Janice Chu, National Institute of Child Health and Human Development. Both cell types were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

Tumor Extraction—Acidified ethanol extracts of tumor tissue were prepared according to Roberts et al. (9). Briefly, the material solubilized in acidified ethanol was adjusted to pH 5.3 and precipitated by the addition of 2 volumes of ethanol and 4 volumes of ether. The precipitate was collected, dialyzed against 0.17 M acetic acid, and lyophilized to dryness. This residue was dissolved in phosphate-buffered saline (pH 7.4) giving a final concentration of 0.5-1 mg/ml after removal of insoluble residue by low speed centrifugation. The soluble tumor extract was sterilized by filtration through 0.2 μm Millipore filters.

Bio-Gel P100 Column Chromatography—Approximately 20 mg of soluble protein from the extract was applied to a column (1.5 × 90 cm) of Bio-Gel P100 equilibrated with phosphate-buffered saline. The column was eluted with the same buffer at a flow rate of 12 ml/h. Column fractions were collected into tubes to which 0.1 ml of 1% BSA

---

1 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; NRK, normal rat kidney; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; EGF, epidermal growth factor; NMU, N-methylN-nitrosourea; T-DMBA, transplantable 7,12-dimethylbenz(a)anthracene-induced rat mammary tumor; T-NMU, transplantable N-methylN-nitrosourea-induced rat mammary tumor; CHP, cis-hydroxyproline; BSA, bovine serum albumin; MTW9, a highly differentiated rat mammary tumor; MTW9a, a less-well differentiated tumor derived from the MTW9 tumor.

† To whom correspondence and requests for reprints should be addressed.

‡ The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Solubilized fractions were dialyzed against 1 liter of 0.2 M acid ([$^{3}$H]lysine or [$^{14}$C]proline, were determined three days after factor addition during which the cell layers were analyzed for the amount of collagen either by quantitating the amount of collagen-sensitive labeled cell-associated protein, or by measuring the mass of hydroxyproline present following acid hydrolysis of the cell layer. The values depict the amount of collagen produced normalized against total labeled cell protein or normalized against the total cellular lysine, respectively. These normalized values were then compared to normalized values for control cultures with EGF omitted. A value of 0% means that collagen production is not stimulated more than total protein synthesis is stimulated. The tumor cells were prepared from primary NMU-induced tumors.

### Effect of EGF on Collagen Production in Cultures of Normal and Neoplastic Mammary Epithelium

Cultures of cells (2 x $10^5$/dish) were propagated in serum-free medium ± EGF (10 ng/ml) and ([14C]proline). Three days after plating, the cell layers were analyzed for the amount of collagen either by quantitating the amount of collagen-sensitive labeled cell-associated protein, or by measuring the mass of hydroxyproline present following acid hydrolysis of the cell layer. The values depict the amount of collagen produced normalized against total labeled cell protein or normalized against the total cellular lysine, respectively. These normalized values were then compared to normalized values for control cultures with EGF omitted. A value of 0% means that collagen production is not stimulated more than total protein synthesis is stimulated. The tumor cells were prepared from primary NMU-induced tumors.

### Differential Stimulation

<table>
<thead>
<tr>
<th>Cells</th>
<th>Collagenase sensitive protein</th>
<th>4-Hydroxyproline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mammary epithelium</td>
<td>103</td>
<td>106</td>
</tr>
<tr>
<td>Mammary adenocarcinoma</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

### Differential Effects of EGF on Normal and Tumor Cells of the Mammary Gland

The net incorporation of proline into cell-associated collagen in cultured normal mammary epithelium has previously been shown to be differentially stimulated by EGF (12). Mammary tumor cells such as those derived from primary mammary tumors induced by NMU or DMBA show a reduced response to EGF as is demonstrated in Table I. EGF produces about a doubling in the net percentage of incorporation of precursor amino acids into collagen-sensitive protein in the cell layer in cultures of normal cells whereas it produces only an 8% differential labeling of collagen in the tumor cell layer. This difference in the EGF responsiveness of the tumor and normal cells is also apparent when the effects of the growth factor are assessed on the basis of the mass of cell-associated hydroxyproline in cultures propagated with and without EGF (Table I) as shown by amino acid analysis. In our attempts to explain this difference in EGF responsiveness, we found that there were acid-ethanol extractable factors in tumors which differentially promoted the amount of labeled collagen in the cell layer of cultures of normal mammary epithelium. This factor(s) had relatively little effect on the relative amount of labeled collagen found in association with tumor cells, however. For example, cell-associated collagen was labeled 300% more efficiently than total cell protein in cultures of normal cells containing the tumor factors than in cultures without tumor factors (Table II). With cultured tumor cells, however, there is only a 25% differential increase in cell-associated collagen label produced by adding the same amount of the tumor extract (Table II); i.e., the normal cells were 10 times as responsive to the tumor extracts than were the tumor cells. At the concentration of tumor extract utilized (~ 100 µg of protein/ml), there was only a 40% and 70% stimulation of total protein synthesis for normal and tumor cells, respectively. As will be subsequently demonstrated, the differentially enhanced labeling of collagen in the tumor cells can be altered by altering collagen turnover rates or by altering the relative distribution of the newly synthesized collagen between the cell layer and culture medium.

### Effect of the Tumor Extract on Collagen Turnover

In order to characterize the activities in the tumor extract, an abundant supply of extract-responsive cells was desirable. Consequently, several cell types were screened as potential candidates. As shown either by the increased production of labeled collagen-sensitive protein formed or by the mass of hydroxyproline formed, normal rat kidney cells were quite responsive. That is, the relative amount of cell-associated collagen was increased by the extract (Table III). 3T3 cells were also responsive but an EGF receptorless cell line (NR6) (13), derived from 3T3, was not. Because the NRK cells were from the same species as the tumors (rat), we opted to use these cells for determining the biological properties of the tumor extract and for routine screening during purification of the active factors in the extract.

Previously, type IV collagen accumulation was shown to be favored in mammary cell cultures supported with glucocorticoid hormones. The hormones acted by blocking the turnover of newly synthesized collagen (7). This was deduced by pulse labeling the collagen in the presence of the hormone followed...
The tumor extracts, prepared as described under "Experimental Procedures," were added to cultures at a final concentration of 100 µg of protein/ml. Three days later the amount of radioactively labeled proline in collagen and total cell protein in the cell layer was estimated using the collagenase digestion technique. Control cultures received the same buffer in which the tumor extract was solubilized.

### Table II

**Collagen Synthesis Stimulating Activities**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Additions</th>
<th>Total Protein</th>
<th>Collagen</th>
<th>cpm in Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mammary epithelium</td>
<td>Control buffer</td>
<td>164,970</td>
<td>5,299</td>
<td>3.1</td>
</tr>
<tr>
<td>Normal mammary epithelium</td>
<td>Tumor extract</td>
<td>232,020</td>
<td>25,231</td>
<td>9.0</td>
</tr>
<tr>
<td>Tumor epithelium</td>
<td>Control buffer</td>
<td>53,100</td>
<td>580</td>
<td>1.1</td>
</tr>
<tr>
<td>Tumor epithelium</td>
<td>Tumor extract</td>
<td>89,535</td>
<td>1,360</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Effect of primary NMU tumor extract on collagen production in different cell types**

Assays were performed as described in the legend of Table II. In the case of the NRK, 3T3, and NR6 cells, growth medium contained 10% fetal calf serum, whereas the mammary epithelium was cultured in serum-free medium. The effects of the tumor extract on hydroxyproline production in the NRK cells was also determined. After three days of incubation, the control cultures had only 1/4 as much 4-hydroxyproline/unit of cell protein as did the cultures grown in the presence of the tumor extract.

### Table III

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Differential stimulation of collagen production (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRK</td>
<td>150-940</td>
</tr>
<tr>
<td>3T3</td>
<td>190</td>
</tr>
<tr>
<td>Normal mammary epithelium</td>
<td>210</td>
</tr>
<tr>
<td>Mammary tumor epithelium</td>
<td>25</td>
</tr>
<tr>
<td>NR6</td>
<td>0</td>
</tr>
</tbody>
</table>

The tumor extracts, prepared as described under "Experimental Procedures," were added to cultures at a final concentration of 100 µg of protein/ml. Three days later the amount of radioactively labeled proline in collagen and total cell protein in the cell layer was estimated using the collagenase digestion technique. Control cultures received the same buffer in which the tumor extract was solubilized.

**Effect of primary NMU tumor extract on collagen production in cultures of epithelial cells from the normal mammary gland or mammary adenocarcinomas**

by a chase with the hormone present or absent. The same strategy was employed to determine if the tumor extract affected collagen degradation. As shown in Fig. 1, the amount of ["C"]proline incorporated into cell-associated collagen sensitive protein by the NRK cells in a 60-min pulse was not degraded during a subsequent 24-h chase. This was true regardless of whether or not the tumor extract was present in the cultures. Since the processing time for newly made collagen (fibroblasts) can be very short (14), a 15-min pulse with ["C"]proline followed by a 6-h chase without label was also performed to assess effects of tumor extract on collagen turnover. As shown in Table IV about 3% of the cell-associated collagen labeled in 15 min was still present in the cell layer at the end of the chase period whether the cultures were grown in the presence of tumor extract, EGFB, or control buffer. Thus, we can conclude that for NRK cell cultures the effects of the tumor extract or EGFB on collagen degradation in the accumulation of newly synthesized collagen in the cell layer is not via inhibiting the turnover of cell layer collagen. Likewise, neither factor affected the relative amount of collagen secreted into the growth medium. Analyses of acid precipitable, collagen sensitive labeled protein in the cell layer and growth medium after the 6-h chase showed that 90, 83, and 87% of the acid precipitable labeled collagen was cell-associated in the control, in tumor extract, or EGFB supplemented cell cultures, respectively. An analysis was also made of the total ["C"]hydroxyproline (acid soluble and acid insoluble) in the cell layer and cell medium; after 3 days of labeling, there was 2,061 cpm in medium hydroxyproline and 2617 cpm in cell-associated hydroxyproline in the control cultures. For the cultures with tumor extract added, the values were 20,100 and 28,930 cpm, respectively. Thus, 53% of the total labeled hydroxyproline was in the cell layer for the controls and 58% for the tumor extract-treated cultures. The above results strongly indicate that the activities in the primary mammary tumor extract differentially promote collagen synthesis.

**Sources of the Collagen Synthesis Stimulating Activity**

Before attempting to purify and characterize the collagen synthesis stimulating activity, a number of tissues were screened as potential sources of starting material. Using identical fractionation procedures, extracts were prepared from proliferating rat mammary tissue, adult rat liver, primary rat mammary tumors induced by NMU or DMBA, and several transplantable tumors including MTW9, MTW9A, T-NMU, and T-DMBA. None of the extracts of normal tissues were active in differentially stimulating collagen synthesis in cultures of NRK cells or in normal rat mammary epithelium cultures. Activity was highest in extracts of primary mammary tumors induced by NMU or DMBA. Activity was moderate in MTW9 and MTW9A tumors, low but detectable in T-NMU and T-DMBA tumors. In general, the activity in the tumors correlated with the degree of differentiation of the tumor tissue as estimated from histological examination. Because the primary NMU tumor extracts possessed the most activity, extracts of this tissue were utilized for further characterization and purification of the collagen synthesis stimulating activity.

**Characterization of the Active Fractions in the Tumor Extract**

**Gel Filtration**—When the soluble fraction prepared from the tumor extract was chromatographed on Bio-Gel P100 columns and the fractions screened for effects on collagen production by NRK cells, two major peaks of activity were seen. These had elution positions approximately equal to bovine serum albumin and porcine insulin markers and, therefore, had apparent molecular weights of 68,000 (the major species) and 6,000 (the minor species), respectively, under neutral conditions. The fractions were tested in cultures grown in medium containing 5 or 10% fetal calf serum. Total protein synthesis stimulating activities were detected in the column fractions, but the peaks of activity were not coincident with the collagen synthesis stimulating activity peaks. In the most active fraction, collagen synthesis was stimulated 7.5-fold.
while total protein synthesis was increased by 3.5-fold (Fig. 2).

The nonidentity of the collagen synthesis stimulating activities and total protein synthesis stimulating activities deduced from analysis of the high molecular weight fractions from the Bio-Gel P100 column was also evident since the two activities were differentially heat sensitive. Thus, a 2-min heating of the tumor extract at 90 °C reduced the collagen synthesis stimulating activity by 95%, while only reducing the total cell protein synthesis stimulating activity by 53% (Table V).

**CM-cellulose Column Chromatography—**Ion exchange chromatography also resulted in the separation of the collagen synthesis stimulating activity into two major peaks, the larger eluting from the column at 0.28 M ammonium formate and the less abundant species at 0.56 M salt. The major collagen synthesis stimulating activity was partially resolved from the total protein synthesis stimulating activity but the two activities fractionated coincidently in the case of the lesser abundant, higher salt eluting species (Fig. 3). As was the case with the Bio-Gel P100 column fractionation, both the collagen synthesis and total protein synthesis stimulating activities were only partially separated from the bulk protein of the tumor extract, especially the higher molecular weight species. At the peak of the collagen synthesis stimulating activities there was a 3.1- and 2.0-fold differential stimulation of collagen labeling for the major and minor activity peaks, respectively.

**Isoelectric Focusing—**Upon isoelectric focusing of the crude tumor extract from the primary NMU tumor, one major peak of collagen synthesis stimulating activity was detected (Fig. 4, fraction 15). The pI of this fraction was 5.9. Total protein synthesis stimulating activity was also present as a broad peak focusing between a pI of 5.6 and 6.3. Several minor collagen synthesis stimulating peaks were also present but most of the activity was found in fraction 15. Gel electrophoresis was performed on fractions 13–17 for further characterization of the proteins present in the active fractions. The profiles are depicted in Fig. 5. The major stainable protein bands were broad and diffuse, with apparent molecular weights ranging from 25,000 to 45,000. Fraction 15, having the most prominent collagen synthesis stimulating activity, contained several minor bands not present in the other fractions. One band had a molecular weight of 88,000, which is the molecular weight of the major collagen synthesis stimulating activity as detected by Bio-Gel P100 column chromatography. Other protein bands were also present only in this fraction. These are estimated to have molecular weights of 16,000, 11,000, and smaller. Whether more than one band possesses collagen synthesis stimulating activity is not certain, but the

### Table IV

<table>
<thead>
<tr>
<th>Addition</th>
<th>Pulse-labeled collagen at the end of chase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control buffer</td>
<td>69%</td>
</tr>
<tr>
<td>Tumor extract</td>
<td>61%</td>
</tr>
<tr>
<td>EGF</td>
<td>72%</td>
</tr>
</tbody>
</table>

**Effect of primary NMU tumor extract and EGF on the turnover of newly synthesized collagen**

NRK cells were plated for 1 day in medium with the buffer, tumor extract (100 ug of protein/ml), or EGF (10 ng/ml) in medium containing 10% serum. [3H]lysine (50 μCi/ml) was added for 15 min, then quickly aspirated, and the cell layers washed 3 times with medium without [3H]lysine. Dishes were returned to the incubator for 6 h or immediately frozen on dry ice. The cpm collagenase sensitive protein in the cell layer was determined for the pulse or pulse-chase samples. In this experiment, labeled collagen production was differentially stimulated 4-fold by the tumor extract and 2-fold by EGF when normalized against total cell protein counts.

**Table V**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total protein (×10⁻⁹)</th>
<th>Collagen (×10⁻¹⁰)</th>
<th>cpm in collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>5.7</td>
<td>6.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tumor extract</td>
<td>14.4</td>
<td>38.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Tumor extract (heated)</td>
<td>9.5</td>
<td>10.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**Effect of heating on collagen synthesis stimulating activity**

Primary NMU tumor extracts were solubilized in phosphate buffered saline (1 mg/ml) and half of the sample was heated for 2 min at 90 °C, then rapidly cooled. The solutions were sterilized by filtration and 100 μl of the extract was added to the culture of NRK cells grown in 10% fetal calf serum-supplemented medium. [14C]Proline was added and cultures incubated for 3 days. The collagenase assay was utilized to quantitate effects on labeled collagen present in the cell layer.

**Fig. 2. Fractionation of primary NMU tumor extract on Bio-Gel P100 columns.** 20 μg of protein was applied to a Bio-Gel column (1.5 × 90 cm) and eluted with phosphate-buffered saline at a flow rate of 12 ml/h. Fractions were collected into tubes containing 0.1 ml of 1% bovine serum albumin (see "Experimental Procedures"). The fractions were tested for their ability to enhance the incorporation of [14C]proline into collagenase-sensitive protein in NRK cell layers. O — O, A₃₄₀; Δ—Δ, cpm total protein; O — O, cpm collagen.

**Fig. 3. CM-cellulose column chromatography of primary NMU tumor extract.** 55 μg of tumor extract in 10 ml of 0.01 M ammonium formate (pH 5.2) was applied on a column (1.5 × 25 cm) of CM-cellulose-52 resin. Samples were eluted with a linear gradient of ammonium formate (0.01–1.0 M, pH 5.2). 5-ml fractions were collected in tubes containing 1% BSA, lyophilized, and tested for activity with cultures of NRK cells as described in Fig. 2. O — O, cpm collagen; O — O, A₃₄₀; Δ—Δ, cpm total protein; O — O, salt concentration.
68,000 molecular weight species is active. The gel slices corresponding to the 68,000 molecular weight species were extracted and the extracts tested for activity with NRK cells (Table VI). Compared to control gels with no extract applied, the 68,000 molecular weight species stimulated collagen synthesis 2.6-fold, while stimulating total protein synthesis by only 1.1-fold.

In addition to the direct demonstration that the 68,000 molecular weight protein recovered from the SDS-gels was biologically active (Table VI), further evidence that this protein was the entity responsible for stimulating collagen synthesis was obtained as follows. Equal amounts of extracts (100 mg of protein) of tumors possessing collagen synthesis stimulating activity or tumors not having this activity were isoelectrically focused and the PI-5.9 fraction recovered and subsequently electrophoresed on SDS-gels as described in Fig. 5. As shown in Fig. 6, a stainable protein band coincident with the bovine serum albumin marker was present in the extracts of primary NMU tumors (lane 2), primary DMBA tumors (lane 3), and MTW9 tumors (lane 4). No stainable bands were seen in this region of the gel in the case of the transplantable NMU tumor (lane 5) or the transplantable DMBA tumor (lane 6). Thus, the relative abundance of the 68,000 molecular weight protein with PI 5.9 correlates roughly with the amount of collagen synthesis stimulating activity detected in the extracts of the various tumor types.

A rough estimate is that the 68,000 molecular weight collagen synthesis stimulating species represents about 0.0001% of the protein in the tumor. From the relative staining intensity of the 68,000 molecular weight band in fraction 15 from the isoelectric focusing column compared to the albumin standard staining intensity, we can estimate that there was approximately 100 ng of this material added in the assay for effects of fraction 15 on collagen synthesis in the NRK cell culture. This produced a 300% differential stimulation of collagen synthesis over and above the stimulation of total protein synthesis (Fig. 4). A maximal stimulation of collagen synthesis of about 100% is observed with 10 ng of EGF, which was the optimal amount of this factor for the NRK cells in so far as stimulation of collagen synthesis is concerned (not shown). Thus, the 68,000 molecular weight tumor collagen synthesis stimulating factor is a more effective stimulator of collagen synthesis than an equal molar concentration of EGF. It is clear that the tumor derived activity is not rat EGF.
Collagen Synthesis Stimulating Activities

because the latter species has a pI of 4.6 (15), because EGF does not bind to CM-cellulose at pH 5.2 (15), and because the molecular weight of EGF is ½ that of the tumor factor.

In addition to the collagen synthesis stimulating activities, the tumors also contain activities that stimulate the growth of NRK cells in soft agar. In preliminary gel filtration studies, these activities, the soft agar growth promoting and collagen synthesis stimulating activities, co-fractionated but they were not coincident in the isoelectrically focused fractions, the collagen synthesis stimulating activity being localized in fraction 15 while the soft agar growth promoting activities were present in fractions 13, 14, and 17 but absent from fraction 15. The characteristics of the soft agar growth promoting activity will be described more fully in a separate publication.

DISCUSSION

The cellular controls for modulating the amount and types of collagen synthesis are poorly understood. With normal mammary epithelium, collagen production is alterable depending on the substratum upon which the cells are plated. For example, these cells produce about twice as much type IV collagen when plated on tissue culture plastic surfaces or on type I collagen-coated dishes as the cells produce on type IV collagen-coated dishes (16). Collagen production by the normal rat mammary cells is also influenced by modulating turnover and, apparently, by enhancing the biosynthetic rate. Glucocorticoids have been shown to inhibit the production of type IV collagenase while EGF promotes collagen production in these cells by another mechanism (7). Substratum effects on collagen production by other cells has also been observed. Thus, Enders et al. (17) have found that limiting the degree of flattening of cells by varying the degree of coating of culture dishes with polyhemin selectively affects collagen production. Another example of a surface effect is in the change from the production of interstitial type to a basement membrane type of collagen by kidney mesenchyme as it interacts with embryonic notochord (18) Thus, cells can respond to physical, hormonal, or growth factors and selectively modify the amount and/or types of collagen they produce. Also, a change in collagen species or amount may or may not accompany transformation (19-21), depending on the cell type.

There is evidence suggesting that collagen production can be regulated at the level of translation of the collagen mRNA as well as by altering the amount of mRNA produced. In vitro translation systems, for example, the NH₂-terminal peptides from procollagen selectively inhibit collagen mRNA translation (22-25). Quantitation of collagen mRNA levels during development has revealed that the level of collagen production may be proportional to the level of the collagen messenger RNA but in some cases it is not (26). Regulation of collagen production may be effected by modulation of the degradative rate of collagen within the cell. Berg et al. (27) have proposed that such a mechanism of control could be provided by changes in the ability of newly formed collagen to form a mature triple helix depending on the degree of hydroxylation of proline. These authors demonstrated that about ½ of the collagen labeled in a short pulse was degraded, a value identical to our results with the NRK cells. In our present studies, the amount of turnover of collagen was the same for control cultures or cultures with the tumor factors (or EGF) present. Thus, we can conclude that the effects of the tumor collagen synthesis stimulating activity (or EGF) in enhancing net collagen production is not via inhibiting intracellular turnover. Based on the lack of turnover of labeled collagen formed during long labeling times during a subsequent chase, it is also unlikely that extracellular turnover is affected by the tumor factors or EGF. The probability is, then, that these two agents act by similar mechanisms, i.e. by enhancing collagen mRNA production or translation, possibilities that are currently being investigated.

Because of the complexities in the regulation of collagen production and the possibility that such changes might not be of physiological significance, some caution in interpreting the observed effects of factors on collagen production in culture is necessary. In a search for such factors that might be physiologically important, some criteria for their identification can be formulated. One would anticipate that a cell that produced such a factor would not be responsive to the exogenous addition of that factor. Additionally, a producer cell might be expected to be unresponsive to other growth factors that stimulated collagen production by a similar mechanism. One would also anticipate that such a factor would be active at low concentrations and it would probably be produced in small amounts.

Based on our present knowledge of the collagen synthesis stimulating activities we have detected in some mammary tumors, it would appear that most of these criteria are met and that such factors are present in these tumors. Thus, collagen synthesis in cultures of primary NMU tumor cells is only marginally affected by exogenously added collagen synthesis stimulating activity derived from the primary NMU tumor. The same cells are also unresponsive to EGF, a growth factor that, like the collagen synthesis stimulating activity derived from primary NMU tumors, modulates collagen pro-
ducation independently of affecting intracellular or extracellular turnover of collagen. That is, the primary tumor-derived factor is EGF-like since neither the tumor-derived factor nor EGF stimulates collagen synthesis in cultures of primary NMu tumor cells. As demonstrated, the 68,000 molecular weight factor is also present in very low concentrations. Based on the intensity of the staining of the 68,000 molecular weight species with a pl of 5.9, we can roughly estimate that this species represents about 0.0001% of the protein in the tumor. This is a rough estimate because recoveries may be poor. The 6000 molecular weight species was not recovered at all following isoelectric focusing, for example. Using the same rough estimate of the abundance of the 68,000 molecular weight protein, we can calculate that it is more active than EGF on NRK and normal mammary cells.

What would be the significance of the production by the mammary adenocarcinomas of a collagen synthesis stimulating activity? In these tumors, as in the normal mammary epithelium, collagen synthesis appears to be restricted to a minority population, the basal or myoepithelial cell (4). One might speculate that such an activity is produced by one cell type in the gland (possibly the epithelial cell) and it acts on the basal cells to enhance collagen production. This condition might favor tissue growth since it has been demonstrated that a variety of proline analogs such as cis-hydroxyproline, thio-

REFERENCES

Detection and partial characterization of collagen synthesis stimulating activities in rat mammary adenocarcinomas.
M Bano, J A Zwiebel, D S Salomon and W R Kidwell


Access the most updated version of this article at http://www.jbc.org/content/258/4/2729

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/4/2729.full.html#ref-list-1