Selective Reduction in Receptors for Epidermal Growth Factor-Urogastrone in Chemically Transformed Tumorigenic Syrian Hamster Embryo Fibroblasts

Morley D. Hollenberg, J. C. Barrett, P. O. P. Ts'o, and Paulos Berhanu

Division of Clinical Pharmacology, Department of Medicine and Department of Pharmacology and Experimental Therapeutics, School of Medicine [M. D. H., P. B. J.], and Division of Biophysics, School of Hygiene and Public Health [J. C. B., P. O. P. T.], The Johns Hopkins University, Baltimore, Maryland 21205

ABSTRACT

Receptors for insulin and epidermal growth factor-urogastrone (EGF-URO) have been measured in Syrian hamster embryo fibroblasts and in a benzo(a)pyrene-transformed tumorigenic cell line (BP6T). Compared with the parent Syrian hamster embryo cells, the BP6T cells exhibit a marked reduction in the binding of EGF-URO, whereas the binding of insulin is the same in both cell types. The selective reduction in EGF-URO receptors in BP6T cells cannot be attributed to changes in receptor-binding kinetics, to the presence in BP6T cultures of either receptor blocking- or receptor-destroying activities, to differences in EGF-URO degradation, to effects of cell density on receptor numbers, or to a glycosylation defect that can be corrected by supplementing cells with N-acetylglucosamine. It is suggested that changes either in receptor turnover rates or in receptor characteristics apart from ligand recognition may account for reduced receptor availability in the transformed cells. Like BP6T cells, other chemically transformed tumorigenic cell lines derived from Syrian hamster embryo cells also exhibit reduced EGF-URO binding compared with the parent Syrian hamster embryo cell strain. However, the amount of binding of EGF-URO by transformed cells does not correlate either with the cellular growth characteristics (generation time and cloning efficiency in agar) or with the ability of cells to cause tumors in vivo. Thus, while the selective reduction in EGF-URO receptors may be a feature of cell transformation, a direct relationship between this receptor reduction and cellular tumorigenesis appears to be nonexistent.

INTRODUCTION

EGF-URO is a single-chain polypeptide with a molecular weight of about 6000, isolated from the mouse (4-6) and the human (8-10, 14). EGF-URO is both a potent stimulant of cell proliferation and an inhibitor of gastric acid secretion. Specific membrane receptors for EGF-URO can be detected in many tissues, including various normal and tumor-derived cell lines (3, 15, 16, 18, 22) originating from humans and other mammals. Recent interest in EGF-URO and its receptor is not only due to the potent mitogenic and acid-inhibitory actions of the polypeptide, but is also due to the association of an apparently selective reduction in cell receptors for EGF-URO with viral (26) and spontaneous (24) transformation of cells. In view of the previous observation, it was of interest to us to determine whether chemically induced cell transformation might also lead to changes in EGF-URO receptors analogous to those observed consequent to transformation of cells with RNA tumor viruses. Furthermore, it was of importance to determine whether or not the changes in EGF-URO receptors might be correlated either with the cellular growth properties or with cellular tumorigenicity. Therefore, we have examined the receptors for both EGF-URO and for insulin (13, 16, 17) in normal Syrian hamster embryonic fibroblasts in comparison to a highly tumorigenic hamster embryonic fibroblast cell line BP6T derived from Syrian hamster embryo cells by benzo(a)pyrene-mediated transformation (1). Additionally, we have compared the binding of EGF-URO among a number of similarly derived benzo(a)pyrene-transformed cell lines of differing tumorigenicity and growth properties (2). We report here that BP6T cells, compared with the parent Syrian hamster embryo cells, exhibit a marked reduction in the binding of EGF-URO, whereas the binding of insulin remains unchanged. Nonetheless, while all of the transformed cell lines possess fewer EGF-URO receptors than does the parent cell strain, no simple correlation can be found between the number of EGF-URO receptors present on the various cell lines and the tumorigenicity of these cells in vivo.

MATERIALS AND METHODS

Measurements of Ligand Binding and Stimulation of Thymidine Incorporation. Syrian hamster embryo fibroblasts were routinely grown in 75-sq cm T-flasks, were subcultured for study without the use of trypsin (cells just prior to confluency were dissociated in 0.2% (w/v) EDTA-0.9% NaCl solution, pH 7.4) into 24-well, 1.5-cm diameter multidish trays, and were allowed to grow to confluency in antibiotic-free Eagle's minimal essential medium supplemented with 10% (v/v) fetal calf serum. The incorporation of $[^3H]$thymidine (1 $\mu$Ci/ml; 6 Ci/mmoll into trichloroacetic acid-insoluble material, measured at 37$^o$ during a 4-hr period began 16 hr after the addition of increasing amounts EGF-URO was determined as previously described (16). The binding of $^{125}$I-labeled mouse EGF-URO (specific activity, approximately 140 cpm/pg) and $^{125}$I-labeled insulin (specific activity, approximately 270 cpm/pg) at increasing concentrations of ligand was determined at 24$^o$ in Earle's...
buffer, pH 7.4, supplemented with 25 mM Tris-HCl, and 0.1% (w/v) bovine albumin on replicate cell monolayers, as described elsewhere (16), and identical monolayers were used to determine the protein and cell content (hemacytometer counting of trypsin-dispersed cells) of each sample (approximately 28 x 10^4 cells/100 μg protein). Nonspecific binding, determined in the presence of an excess (1 μg/ml) of unlabeled peptide was subtracted from the total amount bound to give the specific binding of each ligand. Further details as to the growth of the transformed cell lines used for binding studies are recorded in the chart and table legends.

RESULTS

Thymidine Incorporation and Ligand Binding in Syrian Hamster Embryo and BP6T Cells. Like other cell strains that have been previously examined simultaneously for the binding and action of EGF-URO (12, 15, 16), Syrian hamster embryo cells possess functional receptors for EGF-URO that can be related to the stimulation of [3H]thymidine incorporation (Chart 1). Although the binding assays, done at 24°, cannot be compared precisely with the bioreponse measured at 37°, it is evident that, as observed in other studies (12, 16), “spare” receptors are present, in that a maximum biological response as measured by stimulation of DNA synthesis is obtained when only about 20 to 30% of the available binding sites are occupied. In the transformed cell lines such as BP6T, unchecked cell division is so rapid under the comparative growth conditions chosen for our study that an augmentation by EGF-URO of thymidine incorporation cannot be observed.

BP6T cells bind considerably less EGF-URO than do the parental Syrian hamster embryo cells (Chart 2, bottom; Table 1). In contrast to the binding of EGF-URO, both cell types bind equivalent amounts of insulin (Chart 2, top; Table 1). The reduction of receptors for EGF-URO may not, therefore, reflect a general reduction in the number of receptors for growth factors. Despite the reduction of EGF-URO binding in the transformed cells, the profile of the EGF-URO-binding isotherm (Chart 2, expanded scale) is the same in BP6T cells as in

---

Chart 1. EGF-URO binding and stimulation of thymidine incorporation in Syrian hamster embryo fibroblasts. The incorporation of [3H]thymidine (C) and binding of [125I]labeled EGF-URO (O, •) at increasing ligand concentrations were measured in confluent monolayers as described elsewhere (16), and outlined in "Materials and Methods." Binding was measured either before (C) or after (•) treatment of the monolayers (24 hr: 37°) with depleted growth medium from confluent monolayers of BP6T cells. EGF, mouse epidermal growth factor.

Chart 2. Binding of insulin and EGF-URO to Syrian hamster embryo fibroblasts (SHE). Monolayers (1.5 cm diameter) of either Syrian hamster embryo fibroblasts (C: seeded at 2 to 5 x 10^5 cells/well) or BP6T (•, •; seeded at approximately 2 x 10^5 cells/well) cells were allowed to attain confluency (approximately 3 days for BP6T; 6 to 8 days for Syrian hamster embryo fibroblasts) in multidish trays. Cells were rinsed free from growth medium (0.5 hr at 37° in 1 ml of Earle’s buffer, pH 7.4, supplemented with 25 mM Tris-HCl and 0.1% (w/v) bovine albumin), and the binding of either [125I]labeled insulin or EGF-URO was determined at 24°. Protein (20) was measured in the solubilized monolayers for which radioactivity (85% counting efficiency) had been determined; cell counts were done on replicate monolayers using a hemacytometer. The binding of EGF-URO to BP6T cells is also displayed with an expanded scale (right ordinate, □).

Table 1 Binding of EGF-URO and insulin to Syrian hamster cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Bmax (10^15 x sites/cell)</th>
<th>C1/2max (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syrian hamster embryo fibroblasts</td>
<td>9.1 ± 3.5</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>BP6T</td>
<td>1.0 ± 0.7</td>
<td>0.9 ± 0.7</td>
</tr>
</tbody>
</table>

* Mean ± S.D. for measurements on either 2 (insulin) or 3 (EGF-URO) independently grown monolayer trays.

Syrian hamster embryo cells, indicating that the ligand affinity of the receptor is not changed consequent to neoplastic transformation. Furthermore, the rates of binding of both EGF-URO and insulin (reaching equilibrium at 30 to 50 min at 24°; data not shown) did not differ between the 2 cell types.

Degradation of EGF-URO. An experiment was done to determine if there were differences in EGF-URO degradation caused by Syrian hamster embryo and BP6T cells. EGF-URO was incubated in the presence of both cell types under the conditions of the binding assay and was then tested for its...
ability to bind to liver membranes in a sensitive radioreceptor assay (21). The receptor-binding ability of the EGF-URO samples treated by these 2 cell types in culture did not differ appreciably either from each other or from identical samples that were incubated without cells. These results indicated that differences in the ability of Syrian hamster embryo or BP6T cells to remove EGF-URO from the incubation medium (either by cellular uptake or by ligand degradation) could not account for the reduction of EGF-URO binding observed for the BP6T cells.

**Effects of Growth Medium and Cell Density on EGF-URO Binding.** Two other major possibilities were explored that might account for the reduced EGF-URO binding by BP6T cells. First, the growth medium from BP6T cells was tested to determine if EGF-URO-like substances were present that might block the binding of EGF-URO to its receptor [a possibility raised by Todaro et al. (26)] or, alternatively, to determine if the growth medium for BP6T cells could destroy the receptor, since it is known that BP6T cells produce fibrinolysin (1) and that the EGF-URO receptor is very sensitive to proteolysis (22). When Syrian hamster embryo cell monolayers were incubated with $^{125}$I-labeled EGF-URO either in the presence or absence of the medium in which BP6T cells were grown, there was no diminution in the amount of EGF-URO bound by the cells (Table 2); this result was corroborated with an analogous sensitive radioreceptor assay using placenta membranes (21) as a source of EGF-URO receptor (data not shown). Furthermore, the data in Chart 1 demonstrate that even prolonged exposure (24 hr; $37^\circ$) to the growth medium from BP6T cells did not alter the EGF-URO-binding ability of the parental Syrian hamster embryo cells. Thus, no evidence was obtained to suggest the presence in the BP6T growth medium either of receptor-deactivating activity or of appreciable concentrations of a substance that could compete for the binding of EGF-URO.

Secondly, the effect of cell density on the numbers of EGF-URO receptors was examined (Chart 3). There is a cell density-dependent reduction of EGF-URO receptors in Syrian hamster embryo cells in contrast to the BP6T cells which do not exhibit a cell density-dependent change in EGF-URO receptors (Chart 3). Thus, at low cell density, the differences in EGF-URO binding between Syrian hamster embryo and BP6T cells would be even more striking than would the data we have obtained using confluent cell monolayers. An African green monkey kidney cell line (BSC-1) also exhibits a cell density-dependent reduction of EGF-URO receptors (19). In contrast, BALB/c3T3 cells demonstrate a marked density-dependent increase in EGF-URO binding, whereas the nontransformed mutant derived from BALB/c3T3 (AD6) exhibits only a small increase in EGF-URO binding with increased cell density (23, 24). Unlike the AD6 cells (23, 24), BP6T cells do not demonstrate an increase in EGF-URO binding when cultured in the presence of $N$-acetylglucosamine (data not shown).

**Binding of EGF-URO to Tumorigenic Cell Lines.** In view of the reduced binding of EGF-URO by BP6T cells, it was of interest to examine other similarly derived tumorigenic cell lines (Table 3). Although BP6T is the most tumorigenic of the cell lines examined (i.e., the fewest cells of this line cause a tumor in test animals), BP6T cells do not possess the lowest number of receptors for EGF-URO. From our limited data, it is apparent that the cellular content of EGF-URO receptors does not correlate with cell generation time, with cellular tumorigenicity, or with the ability of cells to grow in soft agar.

**DISCUSSION.**

Our observation of the selective reduction of EGF-URO receptors, with retention of receptors for insulin in the chemically transformed BP6T cell line, is strikingly similar to results reported earlier either for cells transformed by murine or feline
sarccoma viruses (26, 27) or for nontransformed cell mutants with defective glycosylation (23, 24). It may be more than fortuitous that, of the 15 chemically transformed cell lines mentioned briefly by Todaro et al. (26), a benzo(a)pyrene-transformed clone (A21) of BALB/c3T3 cells was the only line thought to exhibit reduced EGF-URO binding. Similarly, Yeh and Holley (28) have observed reduced EGF-URO binding in benzo(a)pyrene-transformed 3T3 cells (BP3T3), but in this instance, in contrast to the BP6T cells we have studied, there was an apparent increase in the BP3T3 receptor affinity for EGF-URO.

The mechanism leading to the reduced EGF-URO binding by BP6T cells is as yet a matter for speculation. Our data do not indicate an intrinsic difference between Syrian hamster embryo and BP6T cells in the receptor characteristics (either in ligand affinity or in rates of ligand binding at equivalent receptor concentrations), and we find no evidence for the presence in BP6T cultures either of a receptor-destroying activity or of a substance that could occupy the BP6T receptors for EGF-URO, leading to a masking of cell receptors, as suggested by Todaro et al. (26). Qualitatively, the data with the Syrian hamster embryo and BP6T cells most closely approximate the study of Pratt and Pastan (24) with the AD6 cell line (23). However, unlike the glycosylation-deficient AD6 line, BP6T cells do not demonstrate an augmentation of EGF-URO binding in the presence of N-acetylglucosamine. In future work, it will therefore be of interest to examine [e.g., with the use of recently developed receptor-labeling methods (7, 11, 25)] both the rates of turnover of EGF-URO receptors in BP6T and Syrian hamster embryo cells and the detailed physicochemical characteristics of the EGF-URO receptors in these cells. Possibly, changes in receptor turnover rates or in receptor characteristics, apart from ligand recognition, may lead to reduced availability in transformed cells.

Since the cell membrane represents an important control point for cell growth, it is not unreasonable to expect that alterations in receptors for a growth factor such as EGF-URO may lead to changes in either the cellular growth characteristics or tumorigenicity. Implicit in earlier findings (26, 27) is the possibility that the reduction in EGF-URO receptors may reflect the presence of a transformation-associated growth factor responsible for uncontrolled cell division. However, the results of the present study indicate that a transformation-associated reduction in receptor number per se need not relate to either cellular growth rate or tumorigenicity. The presence in the parental cell strain of receptors far in excess of those required for a maximum biological response may minimize the biological consequences of a reduction in receptor number caused by viral or chemical transformation. Thus, although the reduction in receptors for EGF-URO may prove a useful phenotypic marker for transformation, the casual relationships if any, between cell transformation, tumorigenicity, reduced serum requirements for growth, and the associated changes in receptors for growth factors may be very difficult to determine. Hopefully, detailed comparative studies in transformed tumorigenic cells and their parental cell strains will shed some light on this important area of study.

ACKNOWLEDGMENTS

We thank Brenda Lipp, Lolly Mixter, and W. H. Shackelford for technical assistance.

REFERENCES

Selective Reduction in Receptors for Epidermal Growth Factor-Urogastrone in Chemically Transformed Tumorigenic Syrian Hamster Embryo Fibroblasts


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/39/10/4166

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.