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HEPARIN-BINDING GROWTH FACTOR/PROSTATROPIN ATTENUATES INHIBITION OF RAT PROSTATE TUMOR EPITHELIAL CELL GROWTH BY TRANSFORMING GROWTH FACTOR TYPE BETA

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SUMMARY

Normal rat prostate epithelial cell growth requires both epidermal growth factor and heparin-binding growth factor/prostatropin. In contrast, epithelial cells derived from the transplantable Dunning R3327H rat tumor require either epidermal growth factor or heparin-binding growth factor/prostatropin. Transforming growth factor type beta inhibited normal epithelial cell growth. Transforming growth factor beta inhibited epidermal growth factor-dependent growth of tumor epithelial cells, independent of epidermal growth factor concentrations. Transforming growth factor beta increased the effective dose of heparin-binding growth factor type 1 required to support tumor epithelial cell growth by 10-fold but saturating levels of heparin-binding growth factor type 1 (290 pM) completely attenuated the inhibitory effect of transforming growth factor beta. These results suggest that prostate tumor epithelial cells may escape the inhibitory effect of transforming growth factor beta as a consequence of alteration of the concurrent requirement for both epidermal growth factor (or homologues) and heparin-binding growth factors.

Key words: epithelial cells; cancer; growth factors; hormones.

INTRODUCTION

Increasing evidence suggests that polypeptide growth factors, not androgen, directly regulate proliferation of both normal and tumor prostate epithelial cells (1). Recently, we demonstrated that in contrast to normal prostate epithelial cells which required both epidermal growth factor (EGF) and heparin-binding growth factors/prostatropins (HBGF), growth of epithelial cells from the androgen-responsive Dunning R3327H rat tumor required *either* EGF *or* HBGF (2). The HBGF family of polypeptide growth factors consists of two characterized gene products, HBGF-1 and HBGF-2. The HBGF polypeptides account for the growth-promoting activity of neural extracts on normal and tumor prostate epithelial cells (1-4). HBGF-1 is also referred to as acidic fibroblast growth factor (aFGF) or endothelial cell growth factor (ECGF) (5). HBGF-2 is also referred to as basic fibroblast growth factor (bFGF) (6). Neural tissue-derived HBGF-1 was initially characterized as "prostatropin" (3). Subsequent experiments have revealed that purified HBGF-1 and HBGF-2 have equal mitogenic activity for prostate epithelial cells and compete for the same 130 kD cell surface binding site (unpublished results). At optimal concentrations, either EGF or HBGF supported a proliferative response of the tumor cells that was 70 to 100 percent of that supported by the other factor. Transforming growth factor type beta (TGF-β), a multi-functional bioresponse modifier that is widely distributed in tissues, is a potent inhibitor of proliferation of normal bronchial epithelial cells (7), keratinocytes (8) and hepatocytes (9). However, malignant counterparts often have acquired the ability to escape the inhibitory action of TGF-β (8,10,11). In this report, we show that normal prostate epithelial cell proliferation is inhibited by TGF-β. In contrast, TGF-β inhibited tumor-derived epithelial cell proliferation dependent on the status of EGF or HBGF-1 in the culture medium. TGF-β inhibited EGF-stimulated proliferation of prostate tumor cells, independent of EGF concentrations. Although TGF-β increased the HBGF-1 requirement for tumor cell proliferation by over ten-fold, inhibition of tumor cell growth by TGF-β was attenuated by saturating amounts of HBGF-1.

MATERIALS AND METHODS

Materials. Cell culture materials, media, and defined growth factors were prepared or obtained as described (1,2). Purified porcine platelet-derived TGF-β was purchased from R&D Systems (Madison, WI). The 17.4 and 15.5 kD forms of HBGF-1 with aminotermini acetylalanine and asparagine, respectively, (3), were isolated as described (4) and used interchangeably or as a mixture throughout the study. EGF (receptor grade) was obtained from Collaborative Research, Inc. (Waltham, MA) or Upstate Biotechnology, Inc. (Lake Placid, NY).

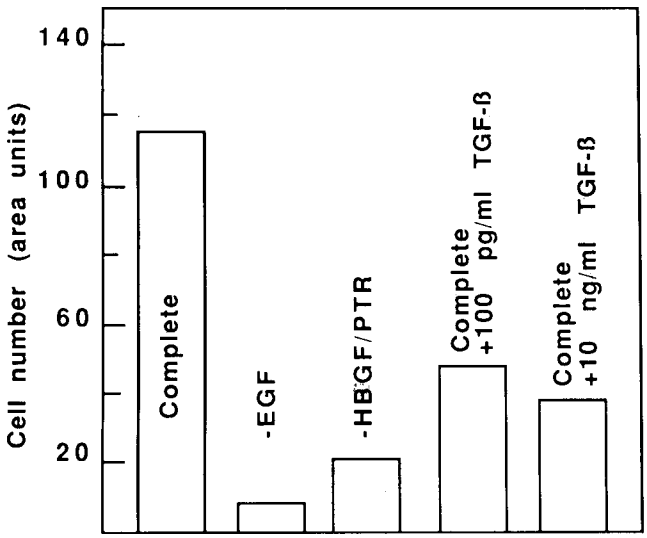

FIG. 1. Effect of TGF-β on normal prostate epithelial cell growth. Cell growth assays were determined in 24-well culture dishes containing 1 ml "complete" medium WAJC 404, cholera toxin (10 ng/ml), insulin (5 μg/ml), dexamethasone (1 μM), EGF (10 ng/ml), HBGF-1 (10 ng/ml) and heparin (25 μg/ml) for 9 days. The indicated factors were deleted or added where indicated. Cell number is expressed in arbitrary densitometric area units (1,12).

Cell cultures and cell proliferation assays. Normal and tumor rat prostate epithelial cells were isolated and maintained as described (1,2). Cell proliferation assays were carried out and quantified by computerized videometry as described (12). All assays were in triplicate. Specific assay conditions are described in the text.

RESULTS

In the presence of the complete hormone mixture (cholera toxin, insulin, glucocorticoid, EGF and HBGF-1), TGF-β inhibited normal cell proliferation (Fig. 1). Cell numbers in the absence of either EGF or HBGF-1 were not significantly inhibited by TGF-β (not shown). In contrast to normal epithelial cells from rat ventral or dorsal prostate, epithelial cells derived from the androgen-responsive rat Dunning R3327H tumor exhibited a density-dependent proliferative response to HBGF-1 and EGF (2). Although tumor cell growth was responsive to either HBGF-1 or EGF at low density, the response to either or both factors became attenuated with increasing cell density (2). Figure 2 shows that TGF-β also exhibited a dose-dependent inhibition on the endogenous increase in tumor epithelial cell number at moderate cell density. Inhibition was half of maximum at about 30 pg/ml TGF-β. Since tumor-derived epithelial cell proliferation was supported at low cell density by either HBGF-1 or EGF (2), we examined the effect of TGF-β on stimulation of tumor epithelial cell number by either factor at low cell density (Fig. 3). HBGF-1 supported a half-maximum increase in tumor cell number (ED_{50}) at 8.8 pM. TGF-β (0.3 ng/ml) increased the apparent ED_{50} for HBGF-1 to 100 pM without effect on the maximal cell number achieved at saturating concentrations of HBGF-1. In contrast, TGF-β had a less significant effect on ED_{50} for EGF, but markedly reduced the maximum cell number supported by saturating concentrations of EGF (Fig. 3).

When plotted as inhibition curves, the data indicated that HBGF-1, but not EGF, attenuated the inhibitory effect of TGF-β on tumor epithelial cell proliferation (Fig. 4). Half-maximal attenuation of inhibition occurred at about 60 pM HBGF-1 and attenuation was complete at about 290 pM HBGF-1 (Fig. 4).

DISCUSSION

The proliferation of isolated epithelial cells from androgen-dependent normal rat prostate and androgen-responsive rat prostate tumors is androgen-independent (1,2). Normal prostate epithelial cell maintenance and regeneration was directly supported by multiple polypeptide hormone-like growth factors acting in synergy (1). The normal cell requirement for multiple factors appeared stringent and independent of cell density (1). Although the origin and control of the direct-acting growth factors that support normal prostate epithelial cells has not been established, the density-independent requirement for multiple factors suggested an origin of the growth factors that is external to the epithelial cells. In contrast, isolated epithelial cells derived from androgen-responsive Dunning R3327H tumors exhibited dramatic and specific alterations in response to factors required for normal epithelial cell growth (2). Tumor-derived epithelial cells exhibited a density-dependent alteration in responsiveness to EGF and HBGF-1 (2). In contrast to normal prostate epithelial cells, the tumor epithelial cells responded to *either* HBGF-1 *or* EGF at low cell density (2). Either factor attenuated the response to

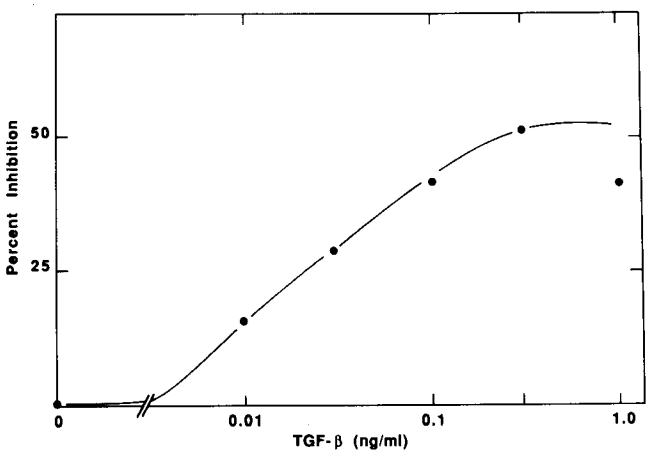

FIG. 2. Effect of TGF-β on endogenous prostate tumor epithelial cell growth. The indicated amounts of TGF-β were added to cell growth assays containing 1 ml of medium WAJC 404, insulin (5 μg/ml), dexamethasone (1 μM) and bovine serum albumin (100 μg/ml)/oleic acid (4 μg/ml). Initial cell density was 6400 cells/cm^2 and cell number was measured videometrically after 2 days.

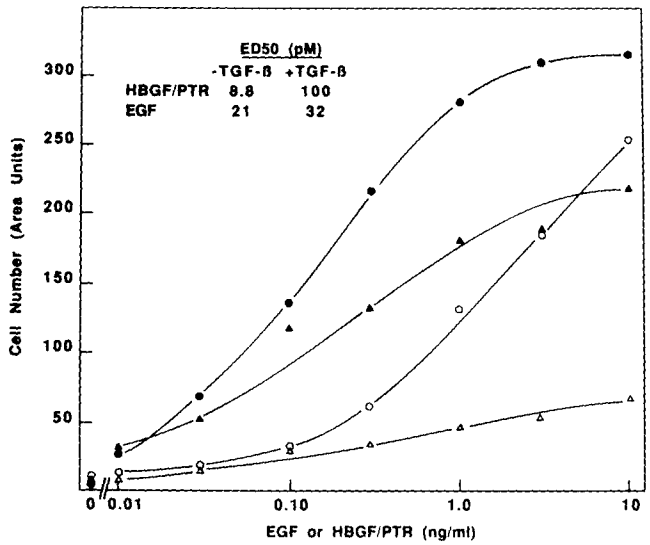

FIG. 3. Effect of TGF-β on prostate tumor epithelial cell number as a function of EGF or HBGF-1 concentration. Cell growth assays were performed at initial cell density of 250 cells/cm² for 7 days in medium WAJC 404, insulin (5 μg/ml), dexamethasone (1 μM) and serum albumin (100 μg/ml)/oleic acid (0.4 μg/ml). EGF or HBGF-1 was added at concentrations indicated. TGF-β was added at 0.3 ng/ml. ●, HBGF-1; ○, HBGF-1 + TGF-β; ▲, EGF; △, EGF + TGF-β. Half-maximal effective dose (ED_{50}) of EGF and HBGF-1 is indicated.

the other. Increasing cell density reduced the responsiveness to either or both factors (2). This indicated that either EGF-like, HBGF-like or both type activities may be endogenously produced by the tumor epithelial cells (2). In this study, we show that the normal epithelial cell growth inhibitor, TGF-β, inhibited proliferation of normal prostate epithelial cells. TGF-β also inhibited proliferation of tumor epithelial cell growth, but inhibition was dependent on the hormonal environment. EGF-stimulated tumor epithelial cell growth was severely inhibited by TGF-β. However, inhibition of tumor epithelial cells was largely attenuated when tumor cell growth was supported by HBGF-1. These results suggest that prostate tumor cell growth may escape the inhibitory effects of TGF-β if HBGF is at adequate levels in the local tumor environment.

The defined differences in the response of normal and tumor prostate epithelial cells to the EGF, HBGF and TGF-β families of polypeptides should allow dissection of the mechanism of action of the three types of factors. TGF-β decreases the number of high-affinity receptors for EGF in rat heart endothelial cells and expression of EGF-responsive genes (13). The same report also noted that acidic FGF (HBGF-1) prevented the inhibition of bovine aortic, but not human umbilical vein endothelial cell growth by TGF-β.

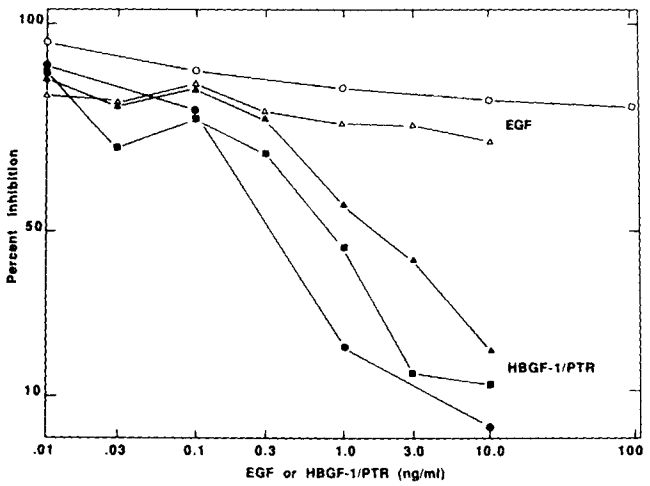

FIG. 4. Effect of EGF or HBGF-1 on TGF-β inibition of tumor epithelial cell number. Cell growth assays were performed as in Fig. 3 and contained TGF-β and the indicated concentrations of EGF or HBGF-1. ○, ●, ■; TGF-β was added at 1 ng/ml. ▲, △; TGF-β was at 0.30 ng/ml. Effect of EGF and HBGF-1 were compared in two independent experiments indicated by circles and triangles. Data showing effect of HBGF-1 (■) from a third experiment containing TGF-β at 1 ng/ml is also indicated.

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EDITOR'S STATEMENT

The observation that heparin-binding growth factor/prostatropin can counteract the inhibitory effect of transforming growth factor beta in prostate epithelial cells may help explain how some cancers avoid the action of growth inhibitors and provides a model for studying how inhibitory peptides overcome the stimulatory signals generated by growth factors.