Decreased urokinase receptor expression by overexpression of the plasminogen activator in a colon cancer cell line

William HOLLAS,† Emilia SORAVIA,* Andrew MAZAR,† Jack HENKIN,† Francesco BLASI* and Douglas BOYD‡§

* Institute of Microbiology, University of Copenhagen, Oster Farimagsgade 2A, Copenhagen, Denmark,

† Thrombolytics Venture, Abbott Laboratories, Abbott Park, IL 60064, U.S.A.,

and ‡ Tumor Biology Department, Box 108, M. D. Anderson Cancer Center, Houston, TX 77030, U.S.A.

There is now ample evidence that the proteolytic action of urokinase (UK) is potentiated by a specific cell surface receptor. The present study was undertaken to determine the role of UK as a modulator of its binding site. GEO colonic cells, which secrete low levels of UK (~2.5 ng/ml per 72 h per 10⁶ cells) and display approx. 10⁴ receptors per cell, the majority of which are vacant, were transfected with an exogenous UK gene driven by the RSV long terminal repeat (LTR) promoter (pRSVUK). Several UK-overexpressing pRSVUK clones were identified by an e.l.i.s.a., Northern blotting and Southern blotting, and analysed for receptor numbers after an acid pretreatment which dissociates receptor-bound UK. pRSVUK GEO clones, expressing high levels of UK, consistently bound 50–75% less radioactive di-isopropylfluorophosphate (DFP)-UK than clones harbouring the selectable marker gene *neo* only or control GEO cells. Cross-linking experiments with a radioactive *N*-terminal fragment of UK which binds to the receptor showed a decreased amount of a binding protein of approx. 51 kDa in representative pRSVUK-transfected cells. Saturation and Scatchard analysis indicated that this reduction in radioligand binding reflected a 40–70% decrease in the number of UK receptors, rather than a change in the dissociation constant. The reduction in receptor display could be accounted for by a decrease in the amount of steady-state mRNA encoding the receptor. Radioactive DFP-UK binding to pRSVUK GEO clones, which display two-thirds less receptors than their *neo* counterparts, could be restored to control levels (untreated cells harbouring *neo*) by cultivating them in the presence of an antibody which inhibits the interaction of UK with its receptor. These data suggest that for one colonic cell line at least, UK reduces the expression of its own binding site via an autocrine stimulation of its cell surface receptor.

INTRODUCTION

The plasminogen activator (PA), urokinase (UK), is thought to play a key role in tissue remodelling and cell migration in a variety of physiological and pathological states [1,2]. UK promotes these phenomena via the conversion of the inactive zymogen, plasminogen, into the serine protease, plasmin [3], which cleaves extracellular matrix components including laminin, fibronectin and type IV collagen [4,5].

There is an increasing body of evidence indicating that UK-dependent proteolysis is contingent on the PA being bound to a specific cell surface receptor [6–8]. Thus laminin degradation by colon cancer cells and tissue invasion by monocytes, Hela cells, and HEp3 cells could be ascribed to receptor-bound UK [9–11].

The UK receptor is a heavily glycosylated protein (55–60 kDa) anchored to the plasma membrane via a phosphatidylinositol moiety [12,13]. The UK receptor binds its PA with high affinity (0.4–2.0 nM) [7] via the *N*-terminal fragment of the UK A chain [14,15], allowing activation of plasminogen by the enzyme active site on the B chain. A 1.4 kb cDNA to the receptor has been cloned and its authenticity verified by transfection into receptor-negative cells [16].

Currently, however, the regulation of this binding site is poorly understood. Previous studies have reported on the up-regulation of the UK binding site (with or without a decrease in affinity) by a variety of unrelated agents, including phorbol esters, planar polar solvents, γ -interferon, epidermal growth

factor (EGF) and tumour necrosis factor [17–20]. In contrast, the UK receptor number was decreased by EGF [21] in a colon cancer cell line, GEO. Interestingly, the change in receptor number caused by the growth factor was accompanied by an opposite shift in UK mRNA expression and protein levels, leading us to speculate on the possible existence of a homeostatic link between UK expression and receptor number in the colonic cells. To examine this contention, we have determined the effect of overexpressing the UK gene in GEO cells on receptor expression.

EXPERIMENTAL

Materials

Two-chain UK (TC-UK; 55 kDa) was kindly supplied by Dr. Genesio Murano, Center for Biologics Evaluation, Bethesda, MD, U.S.A. Monoclonal antibodies to the UK A (#3921) and B (#394) chains were kindly provided by Dr. Richard Hart, American Diagnostica, Greenwich, CT, U.S.A. G418 was obtained from GIBCO. Disuccinimidyl suberate (DSS) was provided by Pierce Chemicals, Rockford, IL, U.S.A. Helena Laboratories (Beaumont, TX, U.S.A.) provided the UK-specific substrate pyro-Glu-Gly-Arg-*p*-nitroanilide (pNA) (S2444).

Cell culture

The colon cancer cell line GEO, which is PA inhibitor-1-negative, secretes small amounts of UK (approx. 2.4 ng/ml per

Abbreviations used: PA, plasminogen activator; UK, urokinase; DSS, disuccinimidyl suberate; EGF, epidermal growth factor; TC-UK, two-chain urokinase; SSC, standard saline citrate (0.15 M-NaCl/0.015 M-sodium citrate, pH 7.4); DFP, di-isopropylfluorophosphate; LTR, long terminal repeat; PBS, phosphate-buffered saline (30 mM-sodium phosphate, pH 7.4, 0.15 M-NaCl); pNA, *p*-nitroanilide.

§ To whom correspondence should be addressed.

72 h per 10^6 cells) and has 1.2×10^4 binding sites/cell, 10 % of which are occupied with the PA [9,22]. The cells were maintained in serum-free medium consisting of McCoy's medium 5A supplemented with 4 μ g of transferrin/ml, 5 μ g of insulin/ml and 10 ng of EGF/ml [23].

Transfection of GEO cells with pRSVUK/pSV2neo

The pRSVUK plasmid was obtained by substituting *neo* in pRSVneo [24] with the 7.0 kb human genomic sequence of UK [25,26]. GEO cells were trypsin-treated and suspended in fresh serum-free medium. The cells were mixed with 10 and 1 μ g of linearized pRSVUK and pSV2neo, respectively, and the DNA was delivered into the cells by electroporation using a Bio-Rad Gene Pulser set at 960 μ F and 250 V. Control transfections were done using pSV2neo only. The cells were cultured in serum-free medium containing 325 μ g of G418/ml (active concentration), a concentration previously shown to be lethal to GEO cells not harbouring the *neo* gene. *Neo*-resistant cells were selected by their ability to propagate indefinitely (≥ 10 weeks) in the presence of G418-containing medium, and cloned.

Southern analysis of the transfectants for the integration of the exogenous UK gene

These were carried out as described previously [27] to verify integration of the exogenous gene. Briefly, genomic DNA ($A_{260}/A_{280} \geq 1.9$) was prepared [27] and digested at 37 °C overnight with *Eco*RI. The digested DNA was electrophoresed in a 1 % agarose gel and transferred to Nytran modified nylon (Schleicher & Schuell, Keene, NH, U.S.A.) after acid and base treatment to cleave the larger fragments. Probing of the blot for the UK gene was accomplished using a radioactive cDNA as described previously [27].

UK receptor assays

These were carried out as described previously [28]. The enzyme active site of affinity-purified TC-UK was inactivated with 10 mM-di-isopropylfluorophosphate (DFP), which was verified using the UK-specific chromogenic substrate pyro-glutyl-arg-pNA (Helena Laboratories, Beaumont, TX). The inactivated UK (DFP-TC-UK) was subsequently radiolabelled using Iodogen. The specific radioactivity of the radioactive DFP-TC-UK was 5–20 μ Ci/ μ g.

Cultured colon cancer cells (95 % confluent) were acid-pretreated for 3 min (50 mM-glycine/0.1 M NaCl, pH 3.0) unless otherwise stated, and incubated at 4 °C for 3 h in binding buffer (McCoy's 5A medium containing 50 mM-Hepes, and 0.1 % BSA, pH 7.4) with a single concentration, or a range of concentrations (0.1–12 nM), in the absence or presence of a 50-fold excess of unlabelled DFP-TC-UK to determine non-specific ligand interactions. Where indicated, the 50-fold excess of unlabelled DFP-TC-UK was replaced with various amounts of antibodies to the UK A and B chains. The cells were washed with 0.1 % BSA in phosphate-buffered saline (PBS) and lysed with a 1 % Triton X-100 solution, and radioactivity was counted. The specific binding data, derived using a range of radioligand concentrations, were plotted by the Scatchard [29] method to yield values for the binding capacity and dissociation constant.

Cross-linking of radioactive UK N-terminal fragment to the GEO cell surface

This was carried out as described previously [18], but with modifications. Near-confluent (95 %) cells were acid-pretreated and incubated with 2.0 nM radioactive UK N-terminal fragment under identical conditions to those used for the receptor assay. In some instances, a 50-fold excess of unlabelled N-terminal fragment was included in the incubation mixture. At the end of the

incubation period, the cells were washed with PBS. The cross-linking agent DSS was added to achieve a final concentration of 1 mM and, after 15 min at ambient temperature, the reaction was terminated with 10 mM-ammonium acetate. The cells were extracted at 4 °C for 1 h with a solution containing 0.5 % CHAPS, 0.1 M-Tris (pH 8.1), 10 mM-EDTA, 1 mM-phenylmethanesulphonyl fluoride and 10 μ g of aprotinin/ml. The extract was clarified by centrifugation (10000 g, 20 min), heated at 95 °C in the presence of 2.3 % SDS and 5 % β -mercaptoethanol, and equal amounts (10–50 μ g) of protein were electrophoresed in a 12.5 % polyacrylamide gel. The gel was subsequently stained with 0.25 % Coomassie Blue, dried and subjected to autoradiography.

Northern blotting

RNA was extracted from the cells with 5.0 M-guanidinium isothiocyanate and purified by centrifugation (150000 g, 20 h) on a 5.7 M-caesium chloride cushion [21]. Purified RNA (20 μ g) was electrophoresed in a 1.5 % agarose gel and transferred to Nytran-modified nylon filters by capillary action using $10 \times$ SSC. Filters were incubated at 42 °C for 16–18 h with either a radiolabelled 1.5 kb cDNA specific for the UK mRNA or a radioactive 0.6 kb cDNA which hybridizes with the receptor transcript [16]. Stringency washes (UK, $0.5 \times$ SSC/0.5 % SDS at 65 °C; UK receptor, $1 \times$ SSC/0.5 % SDS at 50 °C) were undertaken to eliminate non-specific probe interactions. Where indicated, blots were checked for loading equalities by re-probing with a cDNA corresponding to a housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase).

Purification of the N-terminal fragment of UK

Preparation of the N-terminal fragment (amino acids 6–135 of the UK A chain) by autodigestion of TC-UK (55 kDa) was as described previously [30]. The N-terminal fragment was purified by reverse-phase h.p.l.c.

RESULTS AND DISCUSSION

Screening of the conditioned medium of G418-resistant clones for production of UK by an e.l.i.s.a. [31] led to the identification of several over-producers (Table 1). pRSVUK GEO clones 5, 7/9, 9 and 14 secreted between 2 and 8 times more UK than GEO cells (2.4 ng of UK/ml per 72 h per 10^6 cells) or pSV2neo GEO clones 3 and 6 (2.5 and 2.8 ng of UK/ml per 72 h per 10^6 cells respectively). In contrast, the secretion rate of pRSVUK GEO clone 10 (2.7 ng of UK/ml per 72 h per 10^6 cells) was identical to that of non-transfected GEO cells or pSV2neo GEO clones 3 and 6.

Northern blotting (Fig. 1) indicated that the increase in UK secretion by representative pRSVUK GEO clones (9 and 14) was a consequence of the translation of a more abundant UK-specific transcript. The relationship between mRNA levels and secreted UK protein was not, however, linear. Laser densitometer scanning of the Northern blot indicated that while pRSVUK GEO clone 14 contained between two and three times more mRNA than pRSVUK GEO 9 cells, the former secreted only 30 % more UK.

To demonstrate that the increased expression of the UK receptor transcript was a consequence of the integration of the exogenous gene, DNA was digested with *Eco*RI and subjected to Southern blotting. Restriction mapping [25,26] had indicated the presence of *Eco*RI sites both to the RSV long terminal repeat (LTR) heterologous promoter and in the coding region of the UK gene (Fig. 2), which should yield a 3.5 kb fragment upon digestion with the enzyme. In contrast, no *Eco*RI site exists in the homologous promoter of the UK gene [32]. The integration of the exogenous gene is indicated by the presence of a 3.5 kb band

Table 1. UK secreted into the conditioned medium by GEO cells and transfectants

The indicated cells were grown to approx. 90% confluency and replenished with fresh serum-free medium. The conditioned medium was harvested 72 h later and assayed for UK by an e.l.i.s.a. The data are expressed as means±s.d. The experiment was repeated three times.

Clone	UK secreted into culture (ng/ml per 72 h per 10 ⁶ cells)
GEO	2.4±0.3
pSV2neo GEO 3	2.5±0.3
pSV2neo GEO 6	2.8±0.2
pRSVUK GEO 5	5.6±0.6
pRSVUK GEO 7/9	15.4±1.1
pRSVUK GEO 9	14.3±1.3
pRSVUK GEO 10	2.7±0.3
pRSVUK GEO 14	18.5±1.8

Fig. 1. Northern blotting of RNA extracted from GEO cells and pRSVUK GEO clones for UK mRNA

RNA (20 µg) from the indicated cells was electrophoresed in a 1.5% agarose gel and transferred to Nytran modified nylon using 10 × SSC. The blot was probed with a multiprime radiolabelled 1.5 kb cDNA specific for the UK transcript at 42 °C and subsequently washed at 65 °C in 0.25 × SSC/0.5% SDS. The filter was air-dried and exposed to X-ray film. Lane 1, GEO; lane 2, pRSVUK GEO 9; lane 3, pRSVUK GEO 14. Loading equalities were checked by reprobing the blot with a cDNA which hybridizes with the glyceraldehyde phosphate dehydrogenase (GAPDH) transcript. The data are representative of four separate experiments.

in *Eco*RI-digested DNA derived from pRSVUK clones 7/9, 9 and 14, but not control GEO cells or transfectants harbouring *neo* only (clone 6) (Fig. 2). The 1.3 kb band detected in DNA, obtained from both transfectants and non-transfected controls, represents cleavage by *Eco*RI of the coding region present in both the endogenous and exogenous genes [25,26]. The intensity of the 1.3 kb band using DNA from pRSVUK GEO clones 7/9, 9 and 14 appears to be greater than expected from the contribution of the exogenous UK gene. This could, potentially, be explained by a partial digestion at the *Eco*RI site in the promoter region or by an incomplete transfer of the larger (3.5 kb) fragment from the gel to the Nytran filter.

pRSVUK GEO clones 9 and 14, which overexpress UK, and GEO cells harbouring *neo* (clone 6) were subjected to cross-linking experiments using radioactive UK *N*-terminal fragment.

Fig. 2. Southern blotting of DNA extracted from transfected GEO cells

DNA (20 µg) from GEO cells, pSV2neo GEO 6 or the indicated pRSVUK GEO transfectants was digested with 100 units of *Eco*RI at 37 °C overnight. The digested DNA was electrophoresed in a 1% agarose gel and transferred to Nytran modified nylon using 10 × SSC after sequential acid and base treatments. The filter was probed using a radioactive UK cDNA as described in the legend to Fig. 1, and the blot was exposed to X-ray film. Lane 1, pRSVUK GEO 14; lane 2, pRSVUK GEO 9; lane 3, pRSVUK GEO 7/9; lane 4, pSV2neo GEO; lane 5, GEO. The results are typical of two separate experiments. The position of *Eco*RI restriction sites are shown on a linear map of the exogenous gene.

A 66 kDa complex in extracts of GEO cells and pRSVUK GEO 9 and 14 clones could be abolished by inclusion of a 50-fold excess of like-competitor (Fig. 3). Correcting for the molecular mass of the *N*-terminal fragment (15 kDa) would indicate the mass of the binding site to be approx. 51 kDa. This is similar to that published for the glycosylated form of the UK receptor displayed by U937 monocytes and endothelial cells [12,33]. Laser densitometry scanning revealed the signal intensity of the 66 kDa band generated with extracts of pRSVUK GEO clones 9 and 14 to be between 30 and 40% of that achieved with GEO cells.

In receptor assays, the UK overexpressors consistently bound less radioactive PA than did GEO cells transfected with *neo* (Table 2). This only partly reflected the occupation of the binding sites with secreted UK, since a 3 min acid pretreatment could not fully restore ligand binding to control GEO levels. After an acid pretreatment, the specific binding of radioactive UK to pRSVUK GEO clones 7/9, 9 and 14 was only 30–40% of that achieved with pSV2neo GEO 6 cells (Table 2). Longer exposures to the acid buffer (up to 10 min) did not further augment radioligand binding. Interestingly, suppressed binding of the radioligand was not observed for the pRSVUK GEO clone 10, which secretes similar amounts of UK compared with non-transfected GEO cells.

Saturation and Scatchard analyses (Fig. 4) revealed that the decreased radioligand binding to the UK-overexpressing clones pRSVUK GEO 7/9, 9 and 14 was a consequence of a 60–70% reduction in the number of binding sites. Non-transfected GEO cells or GEO cells harbouring *neo* (pSV2neo GEO 3) displayed 12600 and 12900 receptors/cell, whereas pRSVUK GEO clones 7/9, 9 and 14 had only 4800, 4500 and 4200 binding sites per cell respectively. pRSVUK GEO 5 cells, which were intermediate in

Fig. 3. Cross-linking of radioactive UK *N*-terminal fragment to the cell surface of GEO cells and pRSVUK clones

Near-confluent cells were acid-pretreated and incubated at 4 °C for 3 h with 2.0 nM radioactive *N*-terminal fragment, alone (–), or together with a 50-fold excess of unlabelled like-competitor (+). After the cells were washed with PBS, radioactive *N*-terminal fragment was cross-linked to the cell surface with DSS. The cells were lysed with a solution containing 0.5% CHAPS and the extract was denatured in the presence of 2.3% SDS and 5% β -mercaptoethanol. Samples (25 μ g of protein) of the cell extracts, or radioactive *N*-terminal fragment alone, were electrophoresed in a SDS/12.5%-PAGE gel. The gel was stained with 0.25% Coomassie Blue, dried and exposed to X-ray film (a). The gel was photographed to confirm equalities in protein loading (b). Molecular mass standards are shown to the left. The experiment was repeated twice.

terms of UK secretion rates (Table 1), displayed 7300 UK binding sites per cell. In contrast, pRSVUK GEO 10 cells, characterized as being indistinguishable from non-transfected GEO cells on the basis of UK secretion rates (Table 1), displayed a similar number of UK binding sites (12700 per cell). The dissociation constant values for radioactive UK binding to both GEO cells and their pRSVUK-transfected counterparts were identical (0.8–1.2 nM).

One possible explanation for the above results is a lower level of mRNA encoding the binding site, resulting in the translation of a smaller number of receptors in the pRSVUK-transfected GEO cells. To address this, RNA was extracted from pSV2neo GEO 6 cells and pRSVUK GEO clones 9 and 14 and compared by Northern analysis for steady-state levels of UK receptor transcript (Fig. 5). It is apparent that the increased expression of UK seen in the pRSVUK GEO 9 and 14 transfectants is

Table 2. Binding of radioactive UK to GEO cells transfected with pRSVUK

Near-confluent cultured cells were either exposed to an acid buffer for 3 min (pH 3.0) or washed with PBS ('buffer-washed'). The cells were incubated at 4 °C for 3 h with 2.0 nM radioactive DFP-TC-UK, and after this time washed with PBS containing 1 mg of BSA/ml. The cells were lysed with a solution containing 1% Triton X-100 and the radioactivity was counted. Specific binding of the radioactive UK was calculated by subtracting the binding observed in the presence of a 50-fold excess of unlabelled competitor from that seen in its absence. The data represent means $\pm$ s.d. of three separate experiments.

Clone	Radioactive DFP-UK specifically bound (d.p.m./10 ⁶ cells)	
	Buffer-washed	Acid-pretreated
GEO	10 400 $\pm$ 1200	11 700 $\pm$ 400
pSV2neo GEO 3	10 700 $\pm$ 400	11 900 $\pm$ 300
pSV2neo GEO 6	10 800 $\pm$ 500	12 400 $\pm$ 600
pRSVUK GEO 5	4000 $\pm$ 700	6800 $\pm$ 300
pRSVUK GEO 7/9	1900 $\pm$ 300	4800 $\pm$ 600
pRSVUK GEO 9	2200 $\pm$ 500	3900 $\pm$ 400
pRSVUK GEO 10	10600 $\pm$ 1300	12200 $\pm$ 1100
pRSVUK GEO 14	1300 $\pm$ 200	4200 $\pm$ 300

Fig. 4. Scatchard analysis of UK binding to pRSVUK transfectants

Acid-pretreated cultures of GEO (○), pRSVUK GEO 9 (●) and pRSVUK GEO 14 (▽) were incubated at 4° for 3 h with a range (0.25–12.0 nM) of concentrations of DFP-TC-UK in the presence or the absence of a 50-fold excess of unlabelled competitor. After this time, the cells were washed and lysed. Non-specific binding was subtracted from the total, and the resulting specific binding was plotted as a saturating isotherm (inset) or by the Scatchard method. The experiments were carried out on three separate occasions.

associated with decreased steady-state levels of the receptor transcript. Likewise, a lower level of UK receptor mRNA (results not shown) was also observed for the pRSVUK GEO clone 7/9 when compared with non-transfected GEO cells. These data would suggest that the reduction in the number of binding sites displayed by the UK-overexpressing pRSVUK GEO clones

Fig. 5. Northern blotting of RNA extracted from GEO transfectants for UK receptor mRNA

Electrophoresis and transfer of the RNA was as described in the legend to Fig. 1. The blot was probed with a radiolabelled 0.6 kb cDNA specific for the UK receptor (UK-R) transcript at 42 °C, and subsequently washed at 50 °C in 1.0 × SSC/0.5% SDS. The filter was air-dried and exposed to X-ray film. The filter was re-probed for UK as described in the legend to Fig. 1. Lane 1, pSV2neo GEO 6; lane 2, pRSVUK GEO 9; lane 3, pRSVUK GEO 14. The data are representative of three separate experiments.

Fig. 6. Inhibition of radioactive DFP-UK binding to cell surface receptors by anti-(UK A chain) antibody

GEO cells were acid-pretreated and incubated at 4 °C for 3 h with 5 nM radioactive DFP-UK, without or with various concentrations of antibodies #3921 (▲) and #394 (○) to the UK A and B chains respectively. As a control, incubations with radioligand were also conducted in the presence of a 50-fold excess of unlabelled DFP-UK. The cells were washed extensively and lysed with 1% detergent. Cell lysates were counted for radioactivity. The average values and ranges of two separate experiments are shown. The absolute amount of radioligand binding corresponding to 100% was $12\,100 \pm 1500$ d.p.m./ 10^6 cells.

Table 3. Cultured pRSVUK GEO 9 and 14 clones grown in the presence of an anti-(UK A chain) antibody demonstrate augmented binding of radioactive DFP-UK

Subconfluent cultures (10% confluent) were grown for 4 days in the presence or in the absence of monoclonal antibodies to the UK A chain (#3921) or B chain (#394) (each 250 nM). After this time, the near-confluent (90%) cells were assayed for the specific binding of radioactive DFP-UK. The antibodies were without effect on the morphology of the cells, and did not alter their growth rates. The experiments were performed twice and data are shown as means $\pm$ range.

Cells	Antibody...	Radioactive DFP-UK bound specifically (fmol/ 10^6 cells)		
		Absent	A chain	B chain
GEO		11.0 ± 1.0	15.0 ± 1.2	11.5 ± 0.7
pSV2neo GEO 6		11.9 ± 1.2	15.6 ± 0.9	11.3 ± 1.6
pRSVUK GEO 9		4.0 ± 0.4	11.1 ± 0.9	3.5 ± 0.4
pRSVUK GEO 14		3.8 ± 0.3	12.8 ± 1.1	4.1 ± 0.5

findings are similar to the ligand-induced down-regulation of glucocorticoid receptors by dexamethasone, which partly reflected a 75% reduction in steady-state glucocorticoid receptor mRNA levels [34].

Negative regulation of receptors by their respective ligands has been documented for several hormones including EGF, platelet-derived growth factor and dexamethasone [34–36]. This has been shown to be a consequence of the interaction of the ligand with its binding site, which generates a signal culminating in receptor down-regulation. If this mechanism is relevant to the regulation of UK receptor expression by its PA, we argued that blocking the binding of the endogenous UK to its cell surface receptor should culminate in an increase in receptor number. To address this, we selected monoclonal antibodies to the UK A and B chains. In binding studies, antibody #3921, directed at the UK A chain, inhibited the binding of radioactive DFP-UK to GEO cells in a dose-dependent manner (Fig. 6). A concentration of 250 nM of the anti-(A chain) antibody decreased the total amount of radioligand bound by 62%, while a 50-fold excess of unlabelled DFP-UK was only slightly more effective in this regard (78% decrease). In contrast, antibody #394, directed at the catalytic site on the UK B chain, had only a marginal effect (maximum decrease 15%) on the total amount of radioligand bound to GEO cells. Radioactive DFP-UK binding to pRSVUK GEO clones, which display two-thirds fewer receptors than their *neo* counterparts, could be restored to control levels (untreated cells harbouring *neo*) by cultivating them in the presence of an anti-(UK A chain) antibody (#3921), which inhibits the interaction of UK with its receptor. Thus radioligand binding to pRSVUK GEO clones 9 and 14, cultured in the presence of this antibody (11.1 and 12.8 fmol/ 10^6 cells respectively), was indistinguishable from that of untreated GEO or pSV2neo GEO 6 cells (11.0 and 11.9 fmol/ 10^6 cells respectively) (Table 3). In contrast, antibody #394, aimed at the catalytic site on the B chain, was without effect on radioligand binding. Parallel studies with GEO cells and pSV2neo GEO clone 6 showed a modest, although real (30–40%), increase in radioligand binding in response to the anti-(UK A chain) antibody. GEO cells and their *neo*-transfected counterparts secrete low levels of UK, resulting in approx. 10% of the cell surface binding sites being ‘charged’ with endogenous PA [9]. Presumably these results reflect the inability of the UK expressed from the endogenous gene to bind to the receptors, thereby removing a signal for decreased receptor expression.

reflects the translation of a less abundant receptor transcript. Interestingly, pRSVUK GEO clone 14, which had 30–50% less UK receptor mRNA than pRSVUK GEO 9 cells, consistently displayed a similar number of UK receptors (4200 and 4500 per cell respectively). One potential explanation for this is an increased translational efficiency at a threshold level (with pRSVUK GEO 14) of steady-state UK receptor mRNA. Our

On the basis of these results, we propose the existence of an autocrine loop for UK in which the secreted PA binds back to cell surface receptors on GEO colon cancer cells, thereby initiating a series of events (presently undefined) culminating in decreased receptor expression. Future studies should focus on defining the signal transduction pathway(s) by which UK overexpression can result in reduced UK receptor synthesis.

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