Role for Membrane and Secreted Insulin-like Growth Factor-binding Protein-2 in the Regulation of Insulin-like Growth Factor Action in Lung Tumors

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ABSTRACT

The insulin-like growth factors (IGFs) have been implicated in the autocrine and/or paracrine growth of a number of tumor types, including lung tumors. Importantly, insulin-like growth factor-binding proteins (IGFBPs), which both enhance and inhibit the physiological and biological actions of the IGFs, have been shown to be secreted in vitro by a wide range of tumors. In particular, IGFBP-2 is frequently produced by human tumor cells, suggesting that this protein may be an important determinant of IGF action in tumors. In the present study, we investigated IGFBP-2 effects in lung tumor cell lines by examining the influence of IGFBP-2 on IGF-receptor interaction and the biological actions of IGF-I and IGF-II.

Affinity cross-linking studies demonstrated expression of type-I and type-II IGF receptors on small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) cells and the presence of abundant membrane-associated IGFBP in SCLC cells but not in NSCLC cells. An antisera specific for IGFBP-2 was used in immunoprecipitation and immunoblotting studies which demonstrated that the membrane-associated IGFBP identified by affinity cross-linking in SCLC cells is IGFBP-2. In NSCLC cells, both IGF-I and IGF-II bound predominantly to IGF-I receptors, whereas in SCLC cells binding was principally to surface-associated IGFBP-2. SCLC cells failed to respond to IGF-I and -II stimulation in a DNA synthesis assay. For NSCLC cells, IGF-II was a more potent stimulator of DNA synthesis than IGF-I. Soluble IGFBP-2 inhibited the binding of radiolabeled IGF-I and -II to both SCLC and NSCLC cells in a concentration-dependent manner and inhibited IGF-stimulated DNA synthesis in NSCLC cells.

These observations indicate that both soluble and membrane-associated IGFBP-2 compete with IGF receptors for ligand binding and, thus, are likely to be important determinants of IGF responsiveness. The findings of the present study also indicate that the type-I receptor on NSCLC cells contains a high-affinity binding site for IGF-II which presumably mediates the biological effects of IGF-II in these cells, thereby implicating IGF-II in the autocrine/paracrine growth of NSCLC.

INTRODUCTION

Several reports have shown that SCLC cells secrete and respond to exogenous IGFs, indicating an autocrine role for these peptides in SCLC cell proliferation (1–6). Although molecular characterization of SCLC-secreted IGFBPs has not been formally addressed, recent reverse transcriptase-polymerase chain reaction studies indicate that approximately 50% of SCLC and 30% of NSCLC lines show IGF-II gene expression (7). In addition to IGF secretion, lung tumor cell lines also secrete an array of IGFBPs (3, 7–9). Six distinct classes of these proteins have been identified to date: IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, and IGFBP-6 (10, 11). These proteins bind the IGFBPs, often with different relative affinities, and in so doing are thought to modulate the physiological and cellular actions of these peptides (12). Our recent studies have shown that coexpression of multiple binding protein genes occurs in several lung tumor cell lines (7). However, whereas IGFBP-1 and IGFBP-3 were variably and often weakly expressed in lung tumor cell lines, all SCLC and NSCLC cell lines showed a high level of IGFBP-2 gene expression. Interestingly, a follow-up study of IGFBP production in a wide variety of tumor types showed IGFBP-2 expression in all cell lines derived from solid tumors (13). These findings raise the possibility that IGFBPs, and particularly IGFBP-2, may influence tumor cell responsiveness to the autocrine and/or paracrine actions of the IGFs. In the present study, we addressed this question in lung tumors by investigating the effect of IGFBP-2 on IGF-receptor interaction and the biological actions of IGF-I and IGF-II.

MATERIALS AND METHODS

Cells.

The SCLC cell line NCI-H69 was donated by Drs. D. Carney and A. Gazdar (National Cancer Institute, Navy Medical Oncology Branch, Bethesda, MD). The derivation and characterization of the SCLC cell lines COR-L88, COR-L32, COR-L42, COR-L47, and COR-L51 and the large cell NSCLC cell line COR-L23 have been described previously (14). These, together with the squamous cell lung carcinoma cell line BEN and the lung adenocarcinoma cell line MOR, were obtained from Dr. P. R. Twentyman, and the squamous cell lung cancer cell line LUDLU-1 was kindly supplied by Dr. P. H. Rabbitts (both from the Clinical Oncology and Radiotherapeutics Unit, Medical Research Council Centre, Cambridge, United Kingdom). All cell lines were routinely grown in RPMI 1640 medium supplemented with 2 mM glutamine, 10% fetal calf serum, 10 μg penicillin/ml, and 10 μg streptomycin/ml (all Gibco BRL, Paisley, Scotland).

Peptides and Radiochemicals.

Recombinant IGF-I and IGF-II were obtained from Calbiochem (Nottingham, United Kingdom) or from Amersham International (Aylesbury, United Kingdom). IGF-I (specific activity, 2000 Ci/mmol), IGF-II (specific activity, 2000 Ci/mmol), [3H]thymidine (specific activity, 27 Ci/mmol), and [methyl-3H]thymidine (specific activity, 57 mCi/mmol) were purchased from Amersham International. The production and characterization of Cys281 rh IGFBP-2 have been described elsewhere (15, 16). The rabbit anti-type-I receptor antisemur (aIRe) (17) was obtained from Cambridge Bioscience (Cambridge, United Kingdom), and rabbit anti-IGFBP-2 antisemur was from Upstate Biotechnology Inc. (Lake Placid, NY).

Characterization of IGF-binding Sites.

Cells in exponential growth were harvested either using a cell scraper for adherent cultures of MOR or by centrifugation for the COR-L88 suspension culture. After three washes in phosphate-buffered saline containing 0.8 mg peptatin/ml, 2 mg aprotinin/ml, and 2 mg leupeptin/ml, cells were resuspended on ice in lysis buffer consisting of 1 mM Tris base containing 0.8 mg pepstatin/ml, 2 mg aprotinin/ml, and 2 mg leupeptin/ml. Cells were disrupted by sequential passage through 21- and 26-gauge syringe needles. The suspension was then centrifuged at 450 × g for 10 min. The resulting supernatant was further centrifuged at 50,000 × g for 1 h. The pellet was resuspended in lysis buffer, and protein determinations were carried out.

Cell membranes (50 μg) were incubated at 25°C with 125I-IGF-I or 125I-IGF-II (1 × 106cpm) in 5 mM Tris buffer with 0.5% BSA in the presence or absence of unlabeled 100 mM IGF-I, 100 mg/ml insulin, or 10 μg/ml of IGF-I. In experiments in which anti-receptor antibody was used, membranes and antibody were preincubated for 1 h at room temperature before the addition of the radiolabeled peptide. The samples were incubated at room temperature in a final volume of 1 ml for 2.5 h. The samples were washed with 1 ml of buffer and centrifuged at 4500 × g for 10 min at 4°C. The pellet was resuspended in 1 ml 5 mM Tris buffer without BSA, and the radiolabeled

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2 The abbreviations used are: SCLC, small cell lung cancer; IGFBPs, insulin-like growth factor-binding proteins; rh, recombinant human; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; Cys, cysteine.
peptide was cross-linked with 10 μl 10 mM disuccinimidyl suberate in 100% dimethyl sulfoxide at 4°C and pH 7.4. The cross-linking reaction was quenched after 15 min by the addition of 200 μl 50 mM Tris-HCl and 5 mM EDTA, followed by a second centrifugation. The samples were then solubilized in SDS sample buffer, boiled for 5 min, and subsequently electrophoresed together with molecular weight markers (Gibco BRL, Paisley, United Kingdom) under nonreducing conditions on a 3–15% acrylamide gel at constant current. The gels were dried and autoradiographed.

**Immunoprecipitation and Immunoblotting of Membrane-associated IGFBP-2.** For immunoprecipitation studies, 200 μg of membrane protein from COR-L88 were cross-linked to radiolabeled IGF-I/II as described above. Pelleted membranes were resuspended in a solution containing 50 mM Tris base (pH 7.4), 150 mM NaCl, 0.01% NaN3 (buffer A), protease inhibitors at the concentration previously given, and 2% Triton X-100 and incubated for 1 h at 4°C. Following addition of 0.5 ml buffer B, solubilized membranes were centrifuged at 30,000 rpm for 30 min at 4°C and 1:1000 dilution of rabbit anti-IGFBP-2 was added to the supernatant. After the membrane-protein/antibody solution was incubated overnight at 4°C, 25 μl of pansorbin (Calbiochem, Nottingham, United Kingdom) were added and incubated for 1 h at 4°C. The pansorbin was pelleted by centrifugation at 6500 rpm and the pellet washed twice with buffer A. The pellet was then incubated in 2× SDS sample buffer for 30 min at 4°C and was subsequently boiled for 30 s. Immunoprecipitated proteins were electrophoresed on a 12.5% SDS-polyacrylamide gel under nonreducing conditions followed by autoradiography.

For immunoblotting studies, 100 μg of membrane proteins from SCLC cell lines COR-L88, NCI-H69, COR-L42, COR-L47, and COR-L51 and from NSCLC cell lines MOR, COR-L23, LUDLU-1, and BEN were electrophoresed on a 12.5% SDS-polyacrylamide gel under nonreducing conditions. Proteins were transferred to nitrocellulose paper as described elsewhere (18). After transfer, additional protein-binding sites on the nitrocellulose paper were blocked by incubation overnight in 5 mM EDTA, 0.25% gelatin, 0.01 M NaN3, 0.15 M NaCl, 0.05% Nonidet P-40, and 0.05% Nonidet P-40. The paper was then incubated overnight at 4°C with rabbit anti-IGFBP-2 antibody (1:1000 in the previously described buffer). After the paper was washed, affinity-purified 125I-protein A was used to visualize anti-IGFBP2 antibody binding.

**Ligand-binding Assays.** For IGF receptor assays, membrane samples (100 μg protein) were incubated overnight at 4°C with 125I-labeled IGF-I/IGF-II (50 pm) and appropriate dilutions of cold peptide in a final volume of 0.25 ml of 20 mM Tris-HCl, 10 mM MCl2 (pH 7.5) containing 0.2% bovine serum albumin (buffer B). Nonspecific binding was determined by adding an excess of 100 nM IGF-I/IGF-II. Membrane-bound and free peptide were separated by centrifugation followed by a single wash in ice-cold buffer B. The radioactivity associated with pelleted membranes was determined in a gamma counter. Determination of binding parameters was performed using the nonlinear least squares fitting program Enzfitter (Biosoft, Cambridge, United Kingdom).

To investigate the influence of soluble IGFBP-2 on IGF-cell interaction, L88 and MOR cells seeded into 24-well plates (2 × 105 cells/well) were washed twice in phosphate-buffered saline and incubated at 4°C for 90 min in 0.5 ml binding buffer containing 0.1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-0.12·m NaCl-5·m KCl-1·2·m MgSO4-8·m glucose-25 mg BSA/ml (pH 7.6), 108·cpm (50 pm) 125I-IGF for 125I-IGF-II in the presence or absence of 100 nM cold IGF-I, 100 nM cold IGF-II, or 0.1 nM-100 nM IGFBP-2. Cells were subsequently washed briefly in ice-cold binding buffer, and cell-associated radioactivity was determined on a gamma counter.

**DNA Synthesis Assay.** Prior to IGF/IGFBP-2 treatment, cells were prelabeled with 0.25 μCi/ml [3H]thymidine in RPMI 1640 medium containing 10% fetal calf serum to provide an internal standard to control for potential variation in cell number per well in the DNA synthesis assay. Cells were incubated in serum-free RPMI 1640 medium for 96 h to effect growth arrest. To determine whether cells had become growth arrested, cultures were then treated with 60 ng colcemid/ml for 24 h and subjected to flow cytometric cell cycle analysis to detect the presence or absence of G2 arrest. Cultures showing no evidence of G2 accumulation were considered growth arrested; those showing G2 accumulation were considered actively cycling.

Cells were removed from the tissue culture flasks by incubation in 0.02% EDTA for 10 min (NSCLC), or by centrifugation (SCLC), followed by washing in serum-free RPMI 1640 medium. Cells (2 × 103/well) were then seeded into 96-well plates in RPMI 1640 medium supplemented with 10 μg transferrin/ml and 0.8 ng/ml sodium selenite (1) and treated with either 0.1–100 nM IGF-I or 0.1–100 nM IGF-II in the presence or absence of equimolar concentrations of IGFBP-2. After the cells were incubated at 37°C for 20 h, 0.1 μCi [3H]thymidine was added per well, and the cells were incubated for a further 20 h. Cells were then lysed in 0.1% SDS followed by treatment with 10% trichloroacetic acid. Trichloroacetic acid-precipitable material was then harvested onto Whatman GF filters (Searl Technical Ltd., St. Albans, United Kingdom).

**Statistical Analyses.** The concentration dependence of IGF-I, IGF-II, and IGFBP-2 effects on DNA synthesis, either alone or combined as described above, was investigated using analysis of variance. No significant concentration dependence was observed for any of the treatments. On the basis of this finding all data pertaining to a particular treatment group were pooled. A 2-tailed, unpaired T test on pooled data (n = 15) was used to compare stimulation of DNA synthesis by IGF-I and IGF-II. A single group T test on pooled data was used to compare changes in DNA synthesis for each treatment group relative to control, untreated cells with a group mean value of 1. Analyses were carried out using the Statworks 512 program on a Macintosh SE/30 computer.

**RESULTS**

**Characterization of IGF Binding to NSCLC.** Under nonreducing conditions in the absence of competing peptide 125I-IGF-I was cross-linked to proteins which migrated with estimated M<,> >300,000 (Fig. 1A, lane 1). Cross-linking of 125I-IGF-I was markedly inhibited by both IGF-I (lane 2) and IGF-II (lane 3) and was weakly inhibited by insulin (lane 4). Preincubation of membrane proteins with antibody to the type-I receptor (a1R3) markedly inhibited cross-linking of radiolabeled IGF-I (lane 5). These findings are consistent with 125I-IGF-I binding to the IGF-I receptor.

Under nonreducing conditions, in the absence of competing peptide, 125I-IGF-II was cross-linked to proteins which migrated with...
estimated $M_r > 300,000$ and $210,000$ (lane 6). It can be seen that in the absence of competing peptide $^{125}$I-IGF-II bound preferentially to protein with estimated $M_r > 300,000$ and that relatively little $M_r 210,000$ complex formation is detected. Formation of IGF-II-protein complexes with $M_r > 300,000$ was markedly inhibited by unlabeled IGF-I (lane 7) and IGF-II (lane 8), partially inhibited by unlabeled insulin (lane 9), and slightly decreased by preincubation with antibody to the type-I receptor (lane 10). Cross-linking of radiolabeled IGF-II to the $M_r 210,000$ protein was potentiated in the presence of unlabeled IGF-I (lane 9) and IGF-II (lane 8), partially inhibited by unlabeled insulin (lane 9), and slightly decreased by preincubation with antibody to the type-I receptor (lane 10). Taken together, these findings demonstrate that IGF-II interacts predominantly with the IGF-I receptor in the absence of excess IGF-I and with the type-II receptor in the presence of competing IGF-I.

**Characterization of IGF Binding to SCLC Cells.** Under nonreducing conditions in the absence of competing peptide $^{125}$I-IGF-I was cross-linked predominately to a protein which migrated as a complex with apparent $M_r 37,000$ (Fig. 1B, lane 1). Cross-linking of $^{125}$I-IGF-I was markedly inhibited by unlabeled IGF-I (lane 2) and by unlabeled IGF-II (lane 3). Insulin had no effect on the formation of the $M_r 37,000$ complex (lane 4). Preincubation of membranes with antibody to the IGF-I receptor had a slight inhibitory effect on formation of the $M_r 37,000$ IGF-I-protein complex (lane 5). Taken together, these findings demonstrate that IGF-I interacts predominantly with low molecular weight IGF-binding protein.

Under nonreducing conditions in the absence of competing peptide, $^{125}$I-IGF-II was cross-linked predominately to a protein which migrated as a complex with apparent $M_r 37,000$ (Fig. 1B, lane 6). Cross-linking in the presence of unlabeled IGF-I markedly inhibited formation of the $M_r 37,000$ complex but enhanced formation of an $M_r 210,000$ IGF-II-protein complex (lane 7). In contrast, cross-linking in the presence of unlabeled IGF-II abolished the formation of both the $M_r 210,000$ and $37,000$ IGF-II-protein complexes (lane 8). In the presence of insulin (lane 9) or antibody to the type-I receptor (lane 10), cross-linking of $^{125}$I-IGF-II was predominately to the protein which migrated as a complex with apparent $M_r 37,000$, although the formation of the $M_r 37,000$ IGF-II-protein complex was consistently partially inhibited by antibody to the type-I receptor. Taken together, these findings demonstrate that IGF-II interacts predominantly with low molecular weight IGF-binding protein and with the type-II receptor in the presence of IGF-I.

**Immunodetection of Membrane-associated IGFBP-2.** Incubation of $^{125}$I-IGF-II cross-linked membrane proteins from COR-L88 with a rabbit antiserum to IGFBP-2 immunoprecipitated the $M_r 37,000$ $^{125}$I-IGF-II-protein complex as shown in Fig. 2. Immunoprecipitated IGFBP-2 was made up of a protein doublet.

SDS-polyacrylamide gel electrophoresis of membrane proteins followed by immunoblotting with anti-IGFBP-2 antiserum (Fig. 3) demonstrated the presence of membrane-associated immunoreactive protein in COR-L88 and MOR which again appeared as a doublet. It can be seen that more membrane-associated immunoreactive IGFBP-2 is present in SCLC cell line COR-L88 than in NSCLC cell line MOR. Fig. 4 shows immunodetection of membrane-associated IGFBP-2 in a panel of SCLC and NCLC cell lines. It can be seen that all SCLC cell lines possess relatively abundant membrane-associated IGFBP-2 compared to NSCLC cell lines.

**Influence of Cys$^{281}$ rh IGFBP-2 on IGF Receptor Binding.** Fig. 5 shows that IGFBP-2 inhibited the binding of $^{125}$I-IGF-I and $^{125}$I-IGF-II to both NSCLC cell line MOR (A) and to SCLC cell line COR-L88 (B) in a concentration-dependent manner. It can be seen that IGFBP-2 inhibited IGF-II binding to MOR cells more effectively than to L88 cells: the concentrations of IGFBP-2 giving 50% inhibition of $^{125}$I-IGF-I and $^{125}$I-IGF-II binding to MOR cells being 3 and 0.1 nM, respectively, whereas the concentrations IGFBP-2 giving 50% inhibition of $^{125}$I-IGF-I and $^{125}$I-IGF-II binding to L88 cells were 2 and 3 nM, respectively.

**Effect of IGFBP-2 on DNA Synthesis in IGF-I- and IGF-II-responsive Cells.** Flow cytometric cell cycle analysis of COR-L88 cells incubated for 96 h in serum-free RPMI 1640 showed that these cells were not growth arrested under these conditions, as evidenced by the accumulation of cells in the G2 phase of the cell cycle in the presence of colcemid. In contrast, adenocarcinoma cell line MOR was growth arrested following incubation in serum-free RPMI 1640 as evidenced by the failure of these cells to undergo G2 arrest following subsequent treatment with colcemid.

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**Fig. 2.** Immunoprecipitation of $M_r 37,000$ IGFBP complex from COR-L88 membrane proteins by rabbit anti-IGFBP-2 antiserum. Track 1, membrane proteins cross-linked to $^{125}$I-IGF-II; track 2, membrane proteins cross-linked to $^{125}$I-IGF-II followed by immunoprecipitation.

**Fig. 3.** Immunoblotting by rabbit anti-IGFBP-2 antiserum of membrane-associated IGFBP-2 in SCLC cell line COR-L88 and NSCLC cell line MOR. Arrow, $M_r$ in thousands.

**Fig. 4.** Immunoblotting of membrane-associated IGFBP-2 in a panel of SCLC and NSCLC cell lines by rabbit anti-IGFBP-2 antiserum. Arrow, $M_r$ in thousands.
and C, respectively. It can be seen that the relative competition curves for both $^{125}$I-IGF-I and $^{125}$I-IGF-II binding are very similar with half-maximal inhibition of $^{125}$I-IGF-I binding occurring at concentrations of 0.9 nM IGF-I and 0.9 nM IGF-II and of $^{125}$I-IGF-II binding at 0.4 nM IGF-I and 0.9 nM IGF-II.

DISCUSSION

Previous studies have demonstrated the secretion of IGF-binding proteins by SCLC and NSCLC cells both in vitro and in vivo (3, 7–9). Indeed, the synthesis and secretion of IGFBPs appear to be a property common to many different tumor cells, with IGFBP-2 being produced most frequently (13). An important finding of the present study was the detection of abundant membrane-associated IGFBP-2 in some, but not all, lung tumor cell lines. Interestingly, membrane-associated IGFBP-2 was readily detected by immunoblotting in SCLC cell line COR-L88 and in all other SCLC cell lines studied but was present at much lower levels in NSCLC cell line MOR and three other NSCLC cell lines examined. Importantly, immunoblotting of IGFBP-2 in lung tumor cell-conditioned media showed no consistent difference in the amount of IGFBP-2 secreted by SCLC and NSCLC cells, indicating that the differences in membrane-associated IGFBP-2 in SCLC and NSCLC cells are not a consequence of differences in IGFBP-2 secretion. Studies are in progress to investigate the nature of IGFBP-2-membrane interaction.

The importance of membrane-associated binding protein is indicated by membrane cross-linking studies which show that in the presence of low levels of membrane-associated IGFBP-2, as in NSCLC cell line MOR, the IGFs bind to the type-I and type-II receptors, whereas in the presence of abundant membrane-associated binding protein, as in SCLC cell line COR-L88, the IGFs bind predominantly to membrane IGFBP-2, with relatively little binding to IGF receptors. The importance of membrane-associated IGFBP-2 in determining IGF-receptor interaction is further exemplified by the observation that, in SCLC cell line COR-L88, binding of $^{125}$I-IGF-II to the type-II receptor can only be detected in the presence of excess IGF-I, which presumably displaces $^{125}$I-IGF-II from surface IGFBP, allowing binding to the type-II receptor which has a lower affinity for IGF-I than has IGFBP-2. These findings suggest that cell surface-associated IGFBP-2 acts as a reservoir of IGF-binding sites which effectively compete with IGF receptors for ligand binding. This contention is supported by the findings of others (6) which indicate that...
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The inhibitory action of soluble IGFBP-2 on ligand-receptor interaction and IGF-induced DNA synthesis in NSCLC cell line MOR is consistent with the contention that secreted/exogenous IGFBPs can act as blocking agents preventing IGF association with cell surface receptors. Similar observations have been made for IGFBP-1 and IGFBP-3 which block the cell surface association of radiolabeled IGF-I and which can inhibit either cell proliferation and/or cell function in a variety of different cell types (21-24).

The marked difference in the amount of surface-associated IGFBP-2 in SCLC and NSCLC cells and the influence this appears to have on IGF-receptor interaction suggest that the responsiveness of the two different histological types of lung cancer to IGF stimulation may be very different. The finding that the SCLC cell line COR-L88 failed to respond to IGF-I and IGF-II stimulation, whereas the NSCLC cell line MOR, having relatively little surface-associated IGFBP-2, did show increased DNA synthesis particularly following stimulation with IGF-II, may be supportive of a role for surface IGFBP-2 in the regulation of IGF responsiveness. However, the nonresponsiveness of COR-L88 to IGF stimulation may be related to the finding that these cells could not be growth arrested and were actively cycling at the time of IGF treatment. Further studies are needed to clarify the relationship between membrane IGFBPs and IGF responsiveness. Although others have reported convincing increases in DNA synthesis in only 2 of 7 SCLC cell lines (1) using a similar DNA synthesis assay as a measure of IGF responsiveness, in vitro cell proliferation (2) and clonogenic assays (6), both of which involve prolonged exposure to IGF stimulation, do indicate that IGF-I and -II promote SCLC cell proliferation in the majority of cell lines studied under these conditions. Such findings may indicate that exogenous IGFs are released eventually from surface IGFBP-2 and then interact with IGF receptors to stimulate cell proliferation. Importantly, bound IGFs have been shown to be released from membrane-associated IGFBP-3 via a time-dependent process which decreases the affinity of IGFBP-3 for bound IGFs (20). Thus, while surface (and secreted) IGFBP-2 and other IGFBPs may be perceived as reservoirs for the IGFs which initially compete with IGF receptors for ligand binding, they may function to maintain high concentrations of IGFs in the tumor cell environment by releasing the IGFs in a time-dependent manner.

An unexpected finding in the present study; as demonstrated by cross-linking studies is that 125I-IGF-II preferentially binds to the IGF-I receptor and not to the type-II receptor in the NSCLC cells. 125I-IGF-II binding to the type-II receptor was only detected in the presence of saturating concentrations of IGF-I which displaced IGF-II from the type-I receptor, allowing it then to bind to the type-II receptor. We believe that the data obtained from ligand-binding and competition studies can best be interpreted by postulating that both IGF-I and IGF-II bind to the type-I receptor with almost equal affinity, an observation also reported for the IGF-I receptor from human placenta (26). Interestingly, IGF-II proved to be a more potent mitogen than IGF-I for MOR, an observation which cannot be explained.

lung tumor-derived IGFBPs have higher affinities for the IGFs ($K_d$ 0.1-0.2 nm) compared with those of IGF-I receptors on a wide range of lung tumor cell lines ($K_d$ 0.69-5.21 nm) (8).

The present study demonstrates that soluble IGFBP-2 also competes with IGF receptors for ligand binding as evidenced by the concentration-dependent inhibition of IGF-I and IGF-II binding to MOR cells by soluble IGFBP-2. Soluble IGFBP-2 more effectively inhibited the binding of IGF-II than of IGF-I to MOR cells, reflecting the higher affinity of IGFBP-2 for IGF-II than for IGF-I (15). Soluble IGFBP-2 less effectively inhibited IGF-II binding to COR-L88 than to MOR cells, a manifestation of the observed differences in the nature of the IGF membrane-binding sites on the two cell types, i.e., IGF receptors on MOR cells and surface-associated IGFBP-2 in the SCLC cell line. The latter finding does not appear to be consistent with reports demonstrating that secreted IGFBPs have a higher affinity for ligand when in solution than when adherent to cell surfaces (19, 20), since concentrations of IGFBP-2 in excess of 10 nm were required to make a significant impact on 125I-IGF-I and -II binding to COR-L88.

Reports demonstrating that secreted IGFBPs have a higher affinity for IGFs (20). Thus, while surface (and secreted) IGFBP-2 and other IGFBPs may be perceived as reservoirs for the IGFs which initially compete with IGF receptors for ligand binding, they may function to maintain high concentrations of IGFs in the tumor cell environment by releasing the IGFs in a time-dependent manner.

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Fig. 7. A, Binding of 125I-IGF-I (2) and 125I-IGF-II (2) to MOR membranes. Competitive displacement curves for 125I-IGF-I (B) and for 125I-IGF-II (C). O, IGF-I as displacing (DISP) peptide; 2, IGF-II as displacing peptide. % Bound is defined as % of maximum specific binding in the absence of competing peptide.
by simple cross-reaction with the type-I receptor, and it may be that interaction with a discrete, high-affinity site on the type-I receptor for IGF-II binding mediates the biological effects of IGF-II in these lung tumor cells. We reported previously the ubiquitous expression of the IGF-II gene in lung tumor cell lines. In contrast, expression of the IGF-I gene was detected in only approximately 30% of lines studied (7). This led to speculation that IGF-II may be more widely involved in the autocrine growth of lung tumors. The findings of the present study lend support to this contention and, further, identify the likely involvement of high-affinity IGF-II binding sites on IGF-I receptors in the growth-promoting action of IGF-II.

The present study indicates that, in lung tumor cells, regulation of the cellular action of the IGFs depends on a complex interplay among surface-associated IGFBP-2, extracellular IGFBP-2, and IGF receptors and possibly IGF receptor subtypes. Since we and others have shown the coexpression of multiple different IGFBPs by lung tumor cell lines, the scenario is likely complicated further by the actions of other membrane and secreted IGFBPs. The various forms of IGFBP may play distinct roles in controlling the availability of IGFs to tumor cells and their receptors, in regulating the half-life of circulating IGF, or in modulating the biological activity of the IGFs. The rate of secretion of a particular form of IGFBP and its degree of cell surface association may all contribute to the control of IGF action: in the case of IGFBP-3, it has been shown that association with the cell membrane is an important factor in the potentiation of IGF action by this IGFBP (27). Indeed, we recently detected IGFBP-3 in the membranes of MOR and other NSCLC cell lines and are currently investigating the action of this protein in these cells. 3 The finding that the association of IGFBP-3 with the cell membrane potentiates IGF action raises the possibility that other membrane-associated IGFBPs have similar actions. Hence, in COR-L88 membrane-associated IGFBP-2 may potentiate the mitogenic action of endogenously produced IGFs to such an extent that exogenous IGF has no additional effect in these cells. In this regard, purified IGFBP-2 has been shown to potentiate the mitogenic activity of IGF-I on porcine smooth muscle cells (28), perhaps by enhancing receptor activation or by a receptor-independent effect. Such findings together with those of the present study indicate the potential growth-promoting and -inhibiting actions of the IGFBPs and highlight the need for further information regarding the roles of individual IGFBPs in controlling growth of a particular tumor type before these agents are considered as pharmacological neutralizers of IGF action for tumor growth control.

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