Interaction of Secreted Insulin-like Growth Factor-I (IGF-I) with Cell Surface Receptors Is the Dominant Mechanism of IGF-I's Autocrine Actions*

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The insulin-like growth factors (IGFs), like other growth factors, are thought to exert many of their growth and differentiation promoting actions through autocrine pathways (1-3). The findings that both IGFs and IGF receptors often are expressed in cells known to respond to IGFs and that monoclonal antibodies directed against IGFs often abolish growth-dependent effects in the absence of exogenous IGFs provide cogent arguments for invoking autocrine modalities of action (4-6). The precise mechanisms of IGF-stimulated autocrine actions, however, remain largely unknown. Among the questions relevant to the autocrine mechanisms of IGF actions is the cellular location of the interaction between the IGFs and IGF receptors. Concurrent synthesis of both IGF and its receptor molecules raises the possibility that autocrine actions may be stimulated intracellularly prior to IGF secretion. As a first step toward answering such questions, we created an autocrine model of IGF-I action in FRTL-5 cells by generating cell lines that express a hIGF-IA fusion gene driven either by the mouse metallothionein-I (MT-I) or IGF-II 5' (5/11) genomic regulatory regions (7). FRTL-5 cells are a diploid, non-transformed line of rat thyroid follicular cells (8) whose growth in culture is dependent upon the presence of TSH and IGFs or insulin (9-11). Compared to wild type FRTL-5 cells, these IGF-I-expressing FRTL-5 cells exhibit high basal DNA synthesis and their mitogenic response to TSH is indistinguishable from the response of wild type cells to TSH when IGF-I is added exogenously (7).

We now extend these investigations to ask whether secretion of IGF-I is necessary for its autocrine actions. Taking advantage of known capacity of specific amino acid sequences to cause retention of proteins in the endoplasmic reticulum (12, 13), we have created a new line of FRTL-5 cells that express a mutant IGF-IA precursor containing the amino acid sequence, Lys-Asp-Glu-Leu (KDEL), at its carboxyl terminus. As predicted, the mutant IGF-IA/KDEL precursor expressed by stably transfected FRTL-5 cells is retained intracellularly. While this IGF-IA/KDEL mutant was shown to possess biologic activity comparable to mature IGF-I, its expression in FRTL-5 cells does not replicate the autocrine actions previously demonstrated for IGF-I in transfected FRTL-5 cells bearing IGF-IA transgenes. This observation, taken together with the findings that a monoclonal antibody against IGF-1 blocks IGF-I actions in IGF-I-secreting transfected FRTL-5 cells, provides strong evidence that IGF-I secretion and interaction with cell surface receptors is necessary for its autocrine actions.

**Experimental Procedures**

Materials—Coon's modified Ham's F-12 medium (Coon's F-12), newborn calf serum, cysteine-free Eagle's minimum essential medium were purchased from GIBCO. Bovine insulin, human transferrin, mouse γ globulin (mlG), protein A-Sepharose, and G418 were purchased from Sigma. Bovine TSH (1.23 units/mg) for cell culture was purchased from Armour (Kankakee, IL), while purified bovine TSH...
Constructs of a transgene containing a KDEL sequence—A polymerase chain reaction (PCR)-directed mutagenesis strategy was used to make a mutant human IGF-IA fusion gene containing the endoplasmic reticulum retention sequence KDEL (see Fig. 1 and its legend for details of the methods used). The hIGF-IA expression vector pMIG (14, 15) was used as a template for mutagenesis. PCR amplification was performed with Taq polymerase according to the specifications of the manufacturer (Perkin-Elmer Cetus) using a Perkin Elmer DNA thermal cycler. The resultant plasmid, called pNEO/IA/KDEL, was subjected to DNA sequencing (16) to confirm the appropriate insertion of the KDEL encoding sequence and to exclude unintended alterations in the coding sequence.

Partial Purification of IGF-I from Cell Lysates—Cells were collected from 3 near-confluent T-150 flasks, washed twice with PBS, resuspended in 30 mM of 1 M acetic acid, and lysed by repeated freezing and thawing. Lysates then were centrifuged for 15 min, the supernatant collected and neutralized. An IGF-I polyclonal antibody was eluted from the cells by incubation with 2% SDS, 0.05 M Tris-HCl, pH 7.4, and, after two PBS washes, were reincubated for another 24 h. After removing nonspecific binding, the monolayers were washed four times with cold PBS containing 0.5% BSA, then solubilized in 0.5 ml of 0.1% SDS, 0.2 M NaOH, and radioactivity determined in a Packard Auto Gamma 5650.

RESULTS

The Expression of the pNEO/IA/KDEL Construct in FRTL-5 Cells Results in Intracellular Accumulation of the IGF-IA/KDEL Precursor—In a previous report we described the creation of autocrine models of IGF-I action in FRTL-5 cells (7). Two plasmids encoding a hiGF-I fusion gene, driven either by mouse MT-I or IGF-II 5' genomic regulatory regions (designated pNEOMIG and p5'II/IA, respectively), were transfected. With either construct, stable lines of transfected FRTL-5 cells secreted IGF-I into 24-h conditioned medium in concentrations ranging between 0.5 and 3 ng/ml. These lines exhibited high basal DNA synthesis and had a DNA synthetic response to TSH that was indistinguishable from that induced by TSH and IGF-I in wild type or control transfected cells (p5'II/NEO). In the present study a mutant hIGF-IA expression vector containing a sequence encoding the KDEL endoplasmic reticulum retention signal, designated pNEO/IA/KDEL, was constructed and transfected into FRTL-5 cells (Fig. 1). After transfection and selection, single G418-resistant colonies were screened for IGF-I expression by RIA. As anticipated, immunoreactive IGF-I was detectable in cell lysates of transfected, but not in their conditioned media. In the two colonies chosen for detailed characteriza-
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**FIG. 1. Construction of the hIGF-IA/KDEL expression vector, pNEO/IA/KDEL.** Panel A, schematic of the strategy to create pNEO/IA/KDEL. Initially, two DNA fragments were amplified from the hIGF-IA expression vector, pMIG (14, 15), one of 482 bp using primers A and B and one of 474 bp using primers C and D. Each amplification used a primer containing a 5' tail complementary to the 5' tail of a primer used to amplify the other fragment (B and C). The 5' tails of these primers (B and C) contained the KDEL encoding sequence (C) or its complement (B), as well as additional complementary sequence. Consequently, the single-stranded DNA resulting after melting each PCR product contained complementary ends (including the KDEL sequence) that hybridize, and thus it allowed amplification of a single DNA fragment using the other primers (A and D) used to amplify the fragments. The four oligonucleotide primers used were as follows: oligonucleotide A = TACTCCG TAGCTCCAGCTTC (identical to bases 41-60 of the mouse metallothionein-I sense strand regulatory sequence (57), i.e. this sequence hybridizes to the sense strand); oligonucleotide B = GGTTTTCTCCCTAAGCTCTGTCCTCATCTGTTACTG (sequence that is complementary to the bases 478-504 of the sense strand at the 3' end of the hIGF-IA cDNA, and containing sequences complementary to the KDEL encoding sequence inserted immediately before the stop codon); oligonucleotide C = ACCTACAGGATGAAAGK(G)GAC(D)GAG(E)CTTTL TAG(stop)GAGACCCTCTCG (sequence identical to the 3' end of the hIGF-IA cDNA sense strand sequence, bases 483-509, and contained the KDEL sequence); and oligonucleotide D = ATTAGGAGTTGGAGACCCTCTCG (sequence complementary to the 3' end of the human growth hormone gene sense strand, bases 2412-2433; Ref. 38). After purification of the first PCR products, the two fragments were combined, denatured and annealed, and then subjected to PCR with primers A and D to amplify a 956-bp fragment with the KDEL sequence inserted. This fragment was cut at internal BglII and SphI sites, and then subcloned into pNEONUT at the same restriction sites (7).

**Panel B**, graphic of the pNEO/IA/KDEL, showing that the KDEL sequence is inserted immediately before the stop codon, as indicated in enlarged box. mMT-I, mouse metallothionein-I promoter; rSS, rat somatostatin signal sequence; 3'hGH, 3' end of hGH gene containing polyadenylation signal sequence; Neo R, neomycin resistance gene.

**FIG. 2. Characterization of [35S]cysteine-labeled IGF-I from either conditioned media or cell lysates of pNEO/IA/KDEL-, p5'II/IA-, or p5'II/NEO-transfected FRTL-5 cells.** Conditioned media or cell lysates were incubated with a polyclonal hIGF-I antiserum, and the immunocomplexes were harvested by further incubation with protein A-Sepharose, as described under "Materials and Methods." These immunocomplexes were analyzed by denaturing 15% SDS-PAGE. The arrows indicate the location of the bands representing the IGF-I transgene product (14-15 kDa) and mature (7-8 kDa) peptides, respectively. Aliquots of conditioned media and/or cell lysates from pNEO/IA/KDEL- and p5'II/IA-transfected FRTL-5 cells also were incubated with 100 ng of unlabeled recombinant hIGF-I prior to immunoreaction (the lanes designated +IGF-I). The absence of a radiolabeled band in these lanes indicates that the antibody is reacting with authentic IGF-I.
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TABLE I  
Biological activity of cell lysate preparations

<table>
<thead>
<tr>
<th>Lysate preparations</th>
<th>Immunoreactive IGF-I a</th>
<th>Thymidine incorporation b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coon’s F-12 with 0.1% BSA</td>
<td>ND c</td>
<td>185 ± 41</td>
</tr>
<tr>
<td>p5'II/NEO</td>
<td>ND</td>
<td>177 ± 12</td>
</tr>
<tr>
<td>pNEOMIG</td>
<td>0.25</td>
<td>1,053 ± 69</td>
</tr>
<tr>
<td>pNEO/IA/KDEL</td>
<td>3.90</td>
<td>3,186 ± 267</td>
</tr>
<tr>
<td>rhIGF-I d</td>
<td>10.00</td>
<td>1,684 ± 253</td>
</tr>
</tbody>
</table>

a Immunoreactive IGF-I concentrations are derived from the assay of each cell lysate preparation at multiple concentrations in duplicate. 
b ng/30 ul dpm/well. 
c ND, not detectable. 
d Recombinant hIGF-I.

Resistance gene (IGFBP-5) were subjected to the same purification procedures. This preparation exhibited neither detectable immunoreactive IGF-I nor the capacity to stimulate thymidine incorporation in wild type cells. Compared with recombinant hIGF-I, both IGF-I precursor preparations exhibited greater bioactivity than would be predicted based upon their immunoreassayable IGF-I, suggesting the possibility that the IGF-IA precursors are more potent mitogens than is mature IGF-I.

FRTL-5 Cells Expressing the IGF-IA KDEL Mutant Do Not Exhibit the DNA Synthetic Activity of IGF-I-secreting Transfected FRTL-5 Cells—To determine whether the intracellular IGF-IA expression created in pNEO/IA/KDEL-transfected FRTL-5 cells results in IGF-I autocrine actions, we compared TSH-stimulated DNA synthesis in p5'II/NEO (control-transfected cells), pNEOMIG, and pNEO/IA/KDEL-transfected FRTL-5 cells (Fig. 3, top panel). As previously demonstrated in IGF-I-secreting transfected FRTL-5 cells (pNEOMIG and p5'II/IA; Ref. 7), TSH stimulated thymidine incorporation with a peak response occurring at a concentration of 1 nM (see pNEOMIG in Fig. 3, top panel). In contrast, cells expressing the IGF-IA KDEL mutant exhibited only a minimal increase in thymidine incorporation in response to TSH. This TSH response was comparable with that of control-transfected (p5'II/NEO) and wild type (not shown) FRTL-5 cells. Furthermore, pNEO/IA/KDEL-transfected cells responded to the addition of TSH with and without exogenous IGF-I in a fashion similar to control-transfected and wild type FRTL-5 cell (Fig. 3, bottom panel). The response of the pNEO/IA/KDEL cell lines to IGF-I alone, however, was blunted, consistently being about one-third of that observed in non-IGF-expressing cells. In the experiment shown in Fig. 3 (bottom panel), the IGF-I-secretting line, pNEOMIG, exhibited a modest increase in DNA synthesis in response to exogenous IGF-I and a marked increase to response to TSH, which is not appreciably augmented by exogenous IGF-I. Therefore, while the IGF-IA/KDEL mutant has the capacity to produce substances with substantial mitogenic activity (see above), its expression in transfected FRTL-5 cells does not replicate the autocrine actions of IGF-I observed in IGF-I-secreting transfected cell lines.

Because the IGF-I-secreting FRTL-5 cell lines pNEOMIG and p5'II/IA also express the IGF-IA precursor intracellularly, we used a monoclonal mouse-derived antibody raised against hIGF-I (Sm 1.2) to neutralize media IGF-I as a means of determining whether the IGF-I secreted by these cells was responsible for the increased TSH-stimulated DNA synthesis. Increasing concentrations of Sm 1.2 or a control preparation of mlgG were incubated with pNEOMIG (Fig. 4, top panel) and p5'II/IA (Fig. 4, bottom panel) transfected FRTL-5 cells, and TSH-stimulated thymidine incorporation was measured. In both cell lines, Sm 1.2 decreased TSH-stimulated thymidine incorporation in a dose-dependent fashion, with a 90% decrease occurring at 20 μg/ml and a near-complete abolition occurring at 100 μg/ml. Similar concentrations of mlgG had only a minimal effects on TSH-stimulated thymidine incorporation.

**IGF-I-secreting FRTL-5 Cells Exhibit Increased IGF-binding Protein-5 (IGFBP-5) Synthesis, an Activity That Is Not Replicated in IGF-IA/KDEL Mutant Expressing FRTL-5 Cells**—Because IGF-I is known to stimulate the synthesis of some IGFBPs (26, 27), we collected serum-free, conditioned media from IGF-I-expressing and control-transfected FRTL-5 cells and utilized Western ligand blots to determine if they contained IGFBPs. As shown in Fig. 5, a single band migrating at an apparent size of 30 kDa was seen in concentrated (125-fold) conditioned media from each cell line. This protein was identified as IGFBP-5 by demonstrating that the IGF-binding bands on the same ligand blot were immunostained with a specific IGFBP-5 antibody (the generous gift of Dr. D.R. Cleemans, Department of Medicine, University of North Carolina; Ref. 28) and by the identification of a specific IGFBP-5 transcript of the expected size (23) in total RNA extracted from FRTL-5 cells (see below). The abundance of IGFBP-5 was low in conditioned medium from control p5'II/NEO-transfected FRTL-5 cells, as it was in medium from...
ever, was comparable with that in control-transfected FRTL-5 cells. These findings indicate that IGF-I stimulates IGFBP-5 synthesis and that secreted IGF-I is primarily responsible for this IGF-I action, as it is for IGF-I stimulation of DNA synthesis.

Northern analysis of total RNA prepared from wild type and control, p5'II/NEO-transfected FRTL-5 cells demonstrated a low abundance 6.0-kilobase transcript that was increased about 10-fold when the cells were incubated with 100 ng/ml IGF-I (Fig. 6), findings that confirm IGF-I's capacity to stimulate IGFBP-5 synthesis in FRTL-5 cells. On the other hand, IGF-I-secreting FRTL-5 cells transfected with pNEOMIG and p5'II/A exhibited an abundance of IGFBP-5 transcripts comparable with that observed in IGF-I-stimulated wild type and control-transfected FRTL-5 cells. Furthermore, incubation of these cells with Sm 1.2 (50 μg/ml) reduced IGFBP-5 transcription to that found in wild type and control-transfected FRTL-5 cells not exposed to IGF-I. These findings indicate that secreted IGF-I is responsible for the stimulation of IGFBP-5 expression. Consistent with this interpretation is the finding that IGF-IA/KDEL expressing cells only exhibit appreciable IGFBP-5 mRNA expression when incubated with exogenous IGF-I. Their response to exogenous IGF-I, however, was only about one-third that of wild type or control-transfected cells. In addition, the finding that incubation of IGF-IA/KDEL-expressing cells with Sm 1.2 abolishes the low abundance IGFBP-5 transcript observed under basal conditions suggests that small amounts of IGF-IA/KDEL or mature IGF-I may be secreted.

The Influence of IGF-I and Sm 1.2 on IGFBP-5 mRNA abundance in wild type and IGF-I-expressing FRTL-5 cells. Northern analysis of IGFBP-5 transcripts was performed with total RNA (20 μg/lane) using a 32P-labeled rat IGFBP-5 cDNA probe. Total RNA was prepared from quiescent wild type, p5'II/IA-, p5'II/NEO-, pNEOMIG-, and pNEO/IA/KDEL-transfected FRTL-5 cells that were cultured for 24 h in Coon's modified Ham's F-12 containing 0.1% BSA with or without IGF-I (100 ng/ml) and/or Sm 1.2 (μg/ml), as indicated.

FIG. 6. The influence of IGF-I and Sm 1.2 on IGFBP-5 mRNA abundance in wild type and IGF-I-expressing FRTL-5 cells. Northern analysis of IGFBP-5 transcripts was performed with total RNA (20 μg/lane) using a 32P-labeled rat IGFBP-5 cDNA probe. Total RNA was prepared from quiescent wild type, p5'II/IA-, p5'II/NEO-, pNEOMIG-, and pNEO/IA/KDEL-transfected FRTL-5 cells that were cultured for 24 h in Coon's modified Ham's F-12 containing 0.1% BSA with or without IGF-I (100 ng/ml) and/or Sm 1.2 (μg/ml), as indicated.

FIG. 4. The inhibitory effect of Sm 1.2, a monoclonal antibody to hIGF-I, on TSH-stimulated DNA synthesis in IGF-I-secreting FRTL-5 cells. Quiescent pNEOMIG (top panel) or p5'II/IA (bottom panel) transfected FRTL-5 cells were incubated in Coon's modified Ham's F-12 containing 0.1% BSA and TSH (1 nM) plus Sm 1.2 (solid lines) or mouse IgG (control; broken lines) at the concentrations indicated on the x axis, and [methyl-3H]thymidine incorporation was measured. The results are expressed as means ± S.E. of triplicate wells. Note that the scale of the x axis in both panels is not continuous. In addition, error bars that are not shown are too small to be depicted with the markers used.

FIG. 5. Autoradiogram of a ligand blot showing IGFBP-5 in the media conditioned by control-transfected (p5'II/NEO, lane 1), IGF-I-expressing (p5'II/IA and pNEOMIG, lanes 2 and 4, respectively), and pNEO/IA/KDEL-transfected (pNEO/IA/KDEL, lane 3) FRTL-5 cells. Lane 5 represents 2 μl of normal rat serum. Lanes 1-4 represent the equivalent of 1 ml of original conditioned media that was concentrated to 8 μl. The ligand blot was exposed to x-ray film for 9 h. The migration of molecular weight markers is shown on the right.
receptors. Wild type, p5'II/NEO-transfected, and pNEO/IA/KDEL-transfected FRTL-5 cells were incubated with $^{125}$I-IGF-I in presence or absence of IGF-I (200 ng/ml) or insulin (100 µg/ml), as indicated, and cross-linked with diaminobenzidine substrate, as described. Solubilized samples were reduced, analyzed by 7.5% SDS-PAGE, and autoradiographed. Molecular weight markers are shown in the right lane. The band migrating at about 130 kDa is characteristic of the α subunit of type I IGF receptor. This interpretation is confirmed by the dramatic reduction of the intensity of this band by 200 ng/ml IGF-I and its diminution by a 500-fold higher concentration of insulin (100 µg/ml).

FIG. 7. Affinity cross-linking of $^{125}$I-IGF-I to cell surface receptors. Wild type, p5'II/NEO-transfected, and pNEO/IA/KDEL-transfected FRTL-5 cells were incubated with $^{125}$I-IGF-I in presence or absence of IGF-I (200 ng/ml) or insulin (100 µg/ml), as indicated, and cross-linked with diaminobenzidine substrate, as described. Solubilized samples were reduced, analyzed by 7.5% SDS-PAGE, and autoradiographed. Molecular weight markers are shown in the right lane. The band migrating at about 130 kDa is characteristic of the α subunit of type I IGF receptor. This interpretation is confirmed by the dramatic reduction of the intensity of this band by 200 ng/ml IGF-I and its diminution by a 500-fold higher concentration of insulin (100 µg/ml).

FIG. 8. Scatchard plot of $^{125}$I-IGF-I binding to wild type (closed circles) and to pNEO/IA/KDEL (crosses), pNEOMIG (open squares), and p5'II/IA (closed squares) transfected FRTL-5 cells. All FRTL-5 cells were incubated with $^{125}$I-IGF-I in Coon's F-12, 0.1% BSA for 24 h, as described. Cell number was similar, as judged by protein content, in each cell line (range 191.4 ± 4 µg/well to 218.3 ± 20.3 µg/well; mean ± S.D.). Each data point represents the mean of duplicate experiments.

The results of this study provide strong evidence that the dominant mechanism of IGF-I autocrine actions is effected by secreted IGF-I and its subsequent interaction with cell surface receptors. Specifically, we have shown that IGF-I expression in FRTL-5 cells results in an autocrine phenotype when expression results in IGF-I secretion, while this phenotype does not occur when IGF-I expression is predominately intracellular. Furthermore, in FRTL-5 cell lines exhibiting IGF-I autocrine actions, exposure to an IGF-I antibody blocks IGF-I-stimulated activity, again indicating that the activities observed are exerted by the media-secreted IGF-I. Using in vitro radiolabeling, we found that mature IGF-I is the major form of secreted IGF-I, while the IGF-IA or IGF-IA/KDEL precursor forms predominates intracellularly. The molecular form of the IGF-I expressed, however, does not account for the differing phenotypes in these transfected FRTL-5 cell lines, because we have shown that each of the IGF-I precursors expresses biological potency that is at least comparable with that of mature recombinant hIGF-I.

In this study we assessed IGF-I actions using two independent measures, thymidine incorporation and IGFBP-5 gene expression. IGF-I's stimulation of these activities probably involves differing mechanisms because IGF-I's major effects on DNA synthesis in FRTL-5 cells are exerted in concert with TSH, while its capacity to increase IGFBP-5 expression does not require additional exogenous regulatory factors. Nonetheless, our data show that both of these IGF-I actions is similarly dependent upon IGF-I secretion. Although expression may be exerted through differing ultimate pathways, IGF-I's signaling mechanism appears to be shared and to result from the interaction of secreted IGF-I and the cell surface type I IGF receptor. The affinity cross-linking studies confirm the expression of the type I receptor on the FRTL-5 cell surface. They also suggest that the type I receptor is the major, if not the only, FRTL-5 cell surface protein capable of binding IGF-I. This interpretation is supported by the Scatchard analyses of FRTL-5 cell IGF-I binding data, because the linear plots derived from these analyses indicate a single class of receptors. The Scatchard analyses also indicate that receptor concentration is reduced in IGF-I-secreting FRTL-5 cells, a finding that can be taken as further evidence of secreted IGF-I's interaction with the cell surface type I receptor.

As anticipated, expression of the IGF-IA/IA/KDEL mutant in FRTL-5 cells resulted in the intracellular accumulation of the IGF-IA/KDEL precursor. The KDEL amino acid sequence is recognized by specific binding sites in the endoplasmic reticulum, and, thus, proteins containing this and similar sequences are retained intracellularly as they are cycled through the endoplasmic reticulum and Golgi apparatus (12, 13). Such binding of KDEL-containing proteins, however, does not exclude protein processing or secretion. We were unable to detect secreted IGF-I in the IGF-IA/KDEL-expressing FRTL-5 cells. Two findings in IGF-IA/KDEL-expressing cell lines, however, suggest that small amounts of IGF-I were secreted. 1) The low abundance IGFBP-5 mRNA observed in
IGF-I/IA/KDEL-expressing cells under basal conditions could not be visualized when cells were exposed to the antibody to IGF-I. 2) 125I-IGF-I specific binding and the calculated receptor concentration is modestly reduced in these cells. Of potential importance is the finding that IGF-IA/KDEL-expressing cells exhibited a diminished response to exogenous IGF-I, being about one-third that of wild type and control-transfected cells for both thymidine incorporation and IGFBP-5 expression. The possible reduction in receptor number exhibited by the IGF-IA/KDEL-expressing cells does not seem responsible for this blunted IGF-I response, because the IGF-I-secret ing FRTL-5 cell lines have a greater reduction in responsiveness to IGF-I.

While our findings strongly argue that secreted IGF-I is a major effectors of IGF-I’s autocrine actions, they do not exclude actions of intracellular IGF-I or its precursors. The IGF-I-secret ing FRTL-5 cell lines express the IGF-I precursor and mature IGF-I intracellularly. Either of these proteins could exert intracellular actions that make the effects of secreted IGF-I possible. In addition, our findings suggest a desensitization of IGF-IA/KDEL-expressing cells to exogenous IGF-I also, yet they respond exuberantly to the IGF-I receptor number, yet they respond exuberantly to the IGF-I.

Evidence that growth factors can act intracellularly has been reported. For example, expression of interleukin-3 possessing a carboxyl-terminal KDEL signal has been shown to make 32D cells independent of their requirement for this growth factor (32). Evidence for intracellular autocrine actions of the v-sis protein product, the viral homolog of the platelet-derived growth factor B chain, also have been reported (33, 34). Other reports, however, provide evidence that v-sis protein actions require interaction with the platelet-derived growth factor cell surface receptor (35, 36). Whether growth factors can exert their autocrine action intracellularly, therefore, remains controversial. It seems likely that the mechanisms of growth factor autocrine actions will prove to differ with different growth factors and to depend upon nature of the expressing cell.

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