Potential Autocrine Role of Insulin-like Growth Factor II during Suramin-induced Differentiation of HT29-D4 Human Colonic Adenocarcinoma Cell Line

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ABSTRACT

Suramin, a drug that binds to several types of growth factors, has been previously shown to induce the enterocyte-like differentiation of HT29-D4 human colonic adenocarcinoma cells, suggesting that growth factors are involved in such a process. Undifferentiated HT29-D4 cells release insulin-like growth factor II (IGF-II) into the culture medium that is totally complexed to heterogeneous IGF binding proteins (IGFBPs) expressing high affinities for this growth factor ($K_{d}$ = 0.02 nM and $K_{d}$ = 1.4 nM). These complexes do not allow IGF-II to bind to HT29-D4 cell surface type I IGF receptors, as evidenced by using $^{125}$I-IGF-II-IGFBP complexes. However, the addition of 40–100 ¿tg/ml suramin, i.e., concentrations identical to the ones that are able to induce HT29-D4 cell differentiation, induces the release of IGF-II from IGF-II-IGFBP complexes, thereby allowing IGF-II to bind to the cell surface receptors. At such concentrations, suramin is indeed unable to alter IGF-II binding to HT29-D4 cells, a capacity that is observed only for concentrations higher than 200 ¿g/ml. Thus, suramin might have the unusual capacity to allow the establishment of an IGF-II autocrine loop involved in HT29-D4 cell differentiation. Consistent with this hypothesis is the fact that exogenously applied IGF-I (2.5 ¿g/ml) or agonist monoclonal antibody αIR-3 (2.5 ¿g/ml), which can bypass IGFBP present in the culture medium, induces part of HT29-D4 cell differentiation that is characterized by an important carcinoembryonic antigen release and the induction of numerous intercellular cysts with microvilli.

INTRODUCTION

Previous reports have shown that the human colonic adenocarcinoma cell line HT29 releases into the culture medium a number of growth factors (1, 2). We have recently reported the release of IGF-II entirely complexed to three forms of IGFBPs (M, 27,000, 28,000, and 31,000 molecular forms) (3), which have been recently identified as isoforms of the IGFBP-4 class (4). In addition, high-affinity type I IGF receptors, but not type II, have been identified at the surface of both HT29 and HT29-D4 cells (5), a clone derived from the parental HT29 cell line (6). Elevated levels of mRNA for IGF-II and/or secretion of elevated amounts of this peptide as compared to control cells or tissues have been reported in a wide variety of tumors (for a review, see Refs. 7 and 8) including human colorectal tumors (9, 10). Moreover, in several cell systems, IGFs, IGFBPs, and type I IGF receptors have been shown to be involved during cell differentiation (11–16), although the mechanisms responsible are not well understood.

HT29-D4 cells constitute an interesting model for studying the relationships that link the endogenous secretion of growth factors and the differentiation state of colon cancer cells. HT29-D4 cells can indeed be induced to differentiate by two ways: (a) the removal of glucose and its replacement by galactose in the culture medium (6); (b) the addition of suramin in the glucose-containing medium in the presence or absence of FCS (17, 18). This latter observation strongly suggests that growth factors secreted by HT29-D4 cells themselves may be involved, at least in part, in the mechanisms that control the process of induction of HT29-D4 cell differentiation. Suramin, a polysulfonated naphthylurea, is indeed known to interfere with growth factor-receptor interaction, primarily by binding to the growth factor itself rather than to its receptor (19–22). In addition, suramin, previously used to treat trypanosomiasis and onchocerciasis (23), has been recently found to have an antineoplastic activity in humans (24, 25).

In order to clarify how suramin could induce HT29-D4 cell differentiation by interacting with cell-released IGF-II complexed to IGFBP, we first characterized the binding parameters of these secreted IGFBPs. Furthermore, we determined how suramin could interfere with IGF-II interaction with either IGFBP in the culture medium or type I IGF receptors at the cell surface. Moreover, we explored the potential autocrine role of this growth factor in the induction of HT29-D4 cell differentiation.

MATERIALS AND METHODS

Materials. Recombinant human IGF-I and IGF-II were purchased from Bachem. These peptides were radioiodinated using the chloramine-T method to a specific activity of 150–200 ¿Ci/¿g. αIR-3 was purchased from Oncogene Science. Suramin was a gift from Specia France. Sephadex G-75 and G-200 were purchased from Pharmacia. Bio-Gel P-100 was from Bio-Rad. RPMI containing 20 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, DMEM, Hanks’ balanced salt solution, FCS, and other cell culture reagents were from Flow Laboratories. Tissue culture flasks and multiwell plates were purchased from Falcon. Multitray units were from Nunc. All other reagents were of analytical grade.

Cell Lines and Culture Conditions. The HT29-D4 human colon adenocarcinoma cell line (6) was routinely grown in DMEM containing 25 mM glucose and 10% FCS. Large-scale cultures of HT29-D4 cells were maintained in a 6000-cm² multitray unit as described elsewhere (1).

Collection of HT29-D4 Cell Conditioned Medium. Serum-free HT29-D4 conditioned medium was collected as previously reported (3). Briefly, subconfluent HT29-D4 cell monolayers were washed three times with Hanks’ balanced salt solution and then incubated with serum-free DMEM supplemented with 5 mm glutamine as a maintenance medium. The first collection of conditioned medium, made after 24 h of maintenance culture, was discarded, since FCS-derived growth factors were still expected to be present. Further collections were made, and cultures were refed with fresh DMEM at 48 h intervals for 14 days. Cell debris was removed by centrifugation at 2500 rpm for 20 min; HT29-D4 conditioned medium containing 5 ¿M phenylmethylsulfonyl-fluoride was stored at −20°C.
Preparation of IGFBP from HT29-D4 Conditioned Medium. The conditioned medium (500 ml) was thawed, concentrated fold using an Amicon B15 system (M, cutoff, 15,000), then passed through a 25 x 90 cm Sephadex G-200 column, equilibrated with 0.05 M NH4HCO3 (pH 8.0) at 4°C, and calibrated with appropriate standards. Fractions (2.7 ml) were assayed for binding of IGF-I or IGF-II as described below. Active fractions corresponding to an apparent molecular mass range of 30-50 kilodaltons (IGFBP) were pooled, lyophilized, and reconstituted in 7 ml 1 M acetic acid. After clarification by ultracentrifugation at 100,000 x g for 120 min, the supernatant was applied to a 1.6 x 60 cm Bio-Gel P-100 column and eluted with 1 M acetic acid in order to dissociate bound endogenous IGF-II from IGFBP. Fractions from 30 kilodaltons to the F0 were pooled, lyophilized, and then frozen and stored at -50°C for further analysis.

Preparation of 125I-IGF-IGFBP Complexes. Aliquots of IGFBP (about 200 g protein) were incubated overnight with either 125I-IGF-I or 125I-IGF-II (10 ng, about 4.5 x 10^3 cpm) in PBS containing 0.1% BSA and passed over a Sephadex G-75 column (1.6 x 50 cm) eluted with the same buffer. The radioactivity of each fraction was measured in a gamma counter. Fractions corresponding to the first eluted radioactive peak were pooled and used in the further experiments as radioactive IGF-IGFBP complexes. To assess the absence of free 125I-IGF in these preparations they were routinely checked, by the binding assay described in the next paragraph, to see that they were unable to induce specific 125I-IGF binding on HT29-D4 cells.

IGF Binding Assay to HT29-D4 Cell Surface Receptors. This was done as described elsewhere. Briefly, HT29-D4 cells were cultured in 16-mm tissue culture wells as described above. At confluent density, cell culture trays were placed on ice 30 min before and throughout the assay. Cell monolayers were washed three times in serum-free DMEM at 4°C and then incubated in a total volume of 0.2 ml of binding medium (RPMI containing 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 0.1% BSA, and 0.1 mM potassium iodide, and adjusted to pH 7.0) for 2 h at 4°C (equilibrium binding conditions) with 125I-IGF-I or 125I-IGF-II (2.5 x 10^4 cpm) with or without various concentrations of suramin. At the end of the incubation, cells were washed three times with cold PBS containing 0.1% BSA and then lysed in the same buffer containing 1% Triton X-100 and 10% glycerol, and cell-associated radioactivity was counted in a gamma counter. Nonspecific binding, determined as the radioactivity bound in the presence of 2 mg/ml of unlabeled IGF-I or IGF-II, was subtracted from total binding to obtain specific binding. Experiment points were estimated in triplicate. SD was always less than 5%.

Free available IGF-I present in the culture medium of HT29-D4 cells treated by IGF-I in excess for 24 h was assayed after molecular sieving of the conditioned medium on Sephadex G-75 as previously described.

IGF Binding Assay to IGFBP. These assays were performed essentially as previously described using an albumin (2%)-treated charcoal (5%) suspension in PBS for separating free from bound tracer. In binding studies, IGFBP (about 10 pg protein for IGF-I and 1 pg protein for IGF-II tracer binding) was incubated in triplicate with various amounts of either 125I-IGF-I or 125I-IGF-II in a total volume of 0.4 ml of PBS, pH 7.4, containing 0.2% BSA for 16 h at 4°C. Nonspecific binding (2 mg/ml of either cold IGF-I or IGF-II) was subtracted from total data. The SD was never greater than 7%. Binding data were analyzed by using the EBDA/LIGAND computer program (27, 28) distributed by Biosoft. The model which best described the results was determined by F test comparison of the sum of squares of the various models examined and was used to derive the values for binding capacities and dissociation constants (Kd) of IGFBP. In suramin-induced dissociation experiments, 125I-IGF-IGFBP complexes (6.0 x 10^4 cpm) were incubated in triplicate with or without various amounts of suramin in a total volume of 0.4 ml of PBS, pH 7.4, containing 0.2% BSA for 2 h at 4°C. Results were expressed as the percentage of 125I-IGF remaining bound to IGFBP, i.e., the radioactivity measured in the supernatant, with respect to the bound radioactivity measured in the absence of suramin.

CEA Measurement. HT29-D4 cells were plated (1.5-2.0 x 10^6 cells) in 25-cm² flasks and cultured as described above. Two days later, IGF-I (2.5 pg/ml) or αIR-3 (2.5 pg/ml) was added in the culture medium. This supplemented medium was renewed every day. The daily collected cell culture medium was centrifuged for 10 min at 2500 rpm and stored at -20°C. The amount of CEA was determined with the commercially available Abbott CEA-EIA Monoclonal One-Step kit.

Electron Microscopy. HT29-D4 cells, treated or not by IGF-I as described above, were fixed in situ with 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4, for 2 h, washed overnight using the same buffer containing 7.5% saccharose, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon and observed with a Jeol 100C electron microscope.

RESULTS

Binding Parameters of IGFBP. The binding characteristics of IGFBP released into the culture medium by the undifferentiated HT29-D4 cells were examined in saturation binding experiments with either 125I-IGF-I or 125I-IGF-II as a ligand. The Scatchard plot derived from 125I-IGF-I binding data analyzed by the LIGAND computer program is shown in Fig. 1. The best fit of the data was consistent with a single class of binding site (Kd = 3.6 nM; Bmax = 6 fmol bound/µg IGFBP). When 125I-IGF-II was used as a ligand, the Scatchard plot was curvilinear (Fig. 2). Computer analysis of the binding data by LIGAND predicted that they could be resolved into a specific high-affinity, low-capacity IGF-II binding site (Kd = 0.02 nM; Bmax = 1.2 fmol/µg IGFBP) and a specific low-affinity, high-capacity IGF-II binding component (Kd = 1.4 nM; Bmax = 80 fmol/µg IGFBP). Although these values probably represent the resultant of composite binding parameters, it follows that IGFBP isolated from HT29-D4 conditioned medium had an affinity for IGF-II manyfold higher than for IGF-I.

Effect of Suramin on IGF Binding. To determine whether suramin could induce HT29-D4 cell differentiation by interfering with the binding of endogenous released IGF-II to cell-associated type I IGF receptors, we first analyzed the drug effect on IGFs binding to HT29-D4 cells. Fig. 3 shows that suramin inhibited the specific binding of both human recombinant 125I-IGF-I and 125I-IGF-II with a 50% inhibitory concentration of approximately 0.15 mM, i.e., ~200 µg/ml. In addition, Fig. 4 shows that suramin was also able to efficiently dissociate either 125I-IGF-I or 125I-IGF-II from preformed 125I-IGF-I.
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Fig. 2. Scatchard analysis of 125I-IGF-II binding to IGFBP secreted by HT29-D4 cells. IGFBP was incubated with various concentrations (1.0 x 10^{-11} to 2.7 x 10^{-8} M) of 125I-IGF-II for 16 h at 4°C and then analyzed for specific IGFBP-associated radioactive binding as described in "Materials and Methods." The data plotted represent the result of a representative experiment made in triplicate and analyzed by LIGAND. —- computer-generated best fit for a two-site binding model; —- —- —- two binding sites.

IGF-IGFBP complexes using IGFBP isolated from HT29-D4 conditioned medium. The 50% inhibitory concentration was 0.04 and 0.05 mM, i.e., about 60 and 75 µg/ml, respectively.

These experiments show that the amount of suramin required for the dissociation of IGF-IGFBP complexes was about 3 times lower than the amount able to inhibit IGF binding to cell surface receptors. We therefore attempted to determine whether this differential effect of suramin could allow the specific binding of radiolabeled IGF-II, complexed to IGFBP, to the HT29-D4 cell surface type I IGF receptors. HT29-D4 cells were therefore incubated at 4°C with 125I-IGF-II-IGFBP complexes and various amounts of suramin, and then specific 125I-IGF-II cell binding was determined. In agreement with our previous results (3) showing that IGFBP bound all secreted IGF-II thus preventing its binding to cell surface type I IGF receptors, no specific 125I-IGF-II binding to cells was observed when HT29-D4 cells were incubated with 125I-IGF-II-IGFBP alone (Fig. 5). However, the addition of suramin allowed the specific binding of 125I-IGF-II from the radiolabeled complexes to the cell surface (Fig. 5). This was observed only in a definite range of suramin concentrations between 0.02 and 0.1 mM with an optimum at 0.04 mM, i.e., ~60 µg/ml. These suramin concentrations are identical to the ones found to be able to dissociate IGF-II from IGF-II-IGFBP complexes without preventing IGF binding to cell surface type I IGF receptors (compare Figs. 3 and 4). At this optimum suramin concentration, about 50% of 125I-IGF-II prebound to IGFBP was released, i.e., ~30,000 cpm. Thus, the percentage of 125I-IGF-II bound to cells in these experimental conditions (~8% of released free IGF-II) was of the same order of magnitude as the one obtained when about 0.1 ng 125I-IGF-II (~30,000 cpm) was mixed with HT29 cells.

Fig. 3. Suramin inhibition of 125I-IGFs binding to HT29-D4 cell surface type I IGF receptors. HT29-D4 cell monolayers were incubated with suramin at the indicated concentrations with either 125I-IGF-I (■) or 125I-IGF-II (□) for 2 h at 4°C. Suramin was added immediately prior to adding the labeled material. Specific cell-associated binding was determined as described in "Materials and Methods" and expressed as a percentage of binding achieved in the absence of the drug. Data represent the mean of three duplicate experiments. SD was always less than 5%.

Fig. 4. Effect of suramin on the dissociation of 125I-IGFs from IGFBP isolated from HT29-D4 conditioned medium. Complexes of 125I-IGF-IGFBP prepared as described in "Materials and Methods" were incubated with suramin at the indicated concentrations for 2 h at 4°C and then analyzed for 125I-IGF-I (■) or 125I-IGF-II (□) specific binding which remained bound to IGFBP as described in "Materials and Methods." Data expressed as a percentage of the binding achieved in the absence of the drug represent the mean of three duplicate experiments. SD was always less than 5%.

Fig. 5. Induction of 125I-IGF-II binding from 125I-IGF-II-IGFBP complexes to HT29-D4 cell surface. HT29-D4 cell monolayers were incubated with 125I-IGF-II-IGFBP complexes (about 6.0 x 10^6 cpm ) and suramin at the indicated concentrations for 2 h at 4°C. Specific cell-associated binding was determined as indicated in "Materials and Methods" and expressed as a percentage of the total radioactivity added. Data represent the mean of three triplicate experiments. SD was always less than 5%.

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It was also verified that HT29-D4 cells did not release IGFBP into the medium in the binding experimental conditions used, i.e., 2 h at 4°C. A repartitioning of the labeled IGF between released IGFBP and the HT29-D4 cell surface can therefore be excluded.

Induction of Differentiation Markers in HT29-D4 Cells Cultured in the Presence of Exogenously Added IGF-I or αIR-3. The results reported above suggest that suramin could be able to dissociate IGF-II-IGFBP complexes, thus allowing free IGF-II to interact with HT29-D4 cell surface type I IGF receptors to create an autocrine loop. If such a mechanism is involved, at least in part, in the ability of suramin to induce HT29-D4 cell differentiation (17, 18), a similar triggering must be obtained by adding IGF-II to the culture medium. However, we have shown above that HT29-D4 cell-secreted IGFBP had both a high binding capacity and a high affinity for IGF-II. In contrast, IGF-I, which was poorly bound by IGFBP, should have a better capacity than IGF-II to deliver a biological action via the cell surface type I IGF receptors. Moreover, αIR-3, a monoclonal antibody against the type I IGF receptor (29), might induce a similar triggering, since it was shown to be an agonist antibody especially able to activate the type I IGF receptor-associated tyrosine kinase (30).

HT29-D4 cells growing in standard culture conditions were therefore exposed to an excess of either IGF-I (2.5 ng/ml) or αIR-3 (2.5 ng/ml) 48 h after seeding; this supplemented medium was then renewed daily in order to maintain high concentrations of both glucose and either IGF-I or αIR-3. Since the HT29-D4 cell differentiation process was found to be highly correlated with CEA apical cell surface expression and release (31), the amount of CEA released into the culture medium was quantified every day. As shown in Fig. 6, this amount increased sharply 3 days after the cells had reached confluency. At the optimum (day 13), a 10- and 3-fold CEA concentration increase was measured in the culture medium of IGF-I-treated (63.6 ng/ml/24 h) and αIR-3-treated (20.8 ng/ml/24 h) cells, respectively, as compared to untreated control cells (6.5 ng/ml/24 h). CEA release strongly decreased after two successive passages. The most important fall was observed after the first passage (day 24) (P < 0.01) (Fig. 6). It was routinely verified that the culture medium of IGF-I-treated cells still contained free IGF-I after a 24-h culture period. At the light microscope level, semithin sections of confluent HT29-D4 cells (day 13) showed multilayers 2–4 cells thick (Fig. 7). At this time, we observed numerous vacuoles in IGF-I-treated cells (Fig. 7B) which were absent in control cells (Fig. 7A). These vacuoles are in fact intercellular cysts and were shown by electron microscopy to be small foci of differentiation. The cellular membranes facing the lumen of the cysts indeed displayed well-organized microvilli with typical cytoskeletal rootlets related to the axis of the microvilli (Fig. 8). At day 13, the ultrastructural observation did not evidence a polarized monolayer with a well-organized apical brush border as previously reported for either suramin- or galactose-differentiated HT29-D4 cells (6, 17, 18). However, at day 24, semithin sections of IGF-I-treated cultures exhibited rare areas which were a cell monolayer, whereas few intercellular cysts were seen. By transmission electron microscopy, such cells were polarized with an apical brush border facing the medium and tight junctions (not shown).

DISCUSSION

We have demonstrated recently that the HT29 human colonic carcinoma cell line releases IGF-II into the culture medium (3). This growth factor is not free but totally complexed to several molecular forms of IGFBP (3) that have been identified as isoforms of the IGFBP-4 class (4). In addition, low amounts of IGFBP were detectable (5). It is generally acknowledged that neither membrane type II IGF receptors nor membrane-linked IGFBP are detectable (5). It is generally acknowledged that both IGF-I and IGF-II signaling is mediated by the type I IGF receptor (7, 32). Moreover, IGFs have been shown to play an essential role in cell differentiation in several cell models (11–16). We have therefore searched for a central role for autocrine secretion of IGF-II during the HT29-D4 cell differentiation process. Moreover, the previous finding that such a process can be induced by suramin in the presence or absence of FCS (17, 18) also suggests that this drug might act at the level of autocrine growth factors. In recent years, suramin has been used as a useful laboratory
In this study, we investigate what could be the mechanisms that account for HT29-D4 cell differentiation induced by suramin. An attractive hypothesis is that IGF-II secreted by HT29-D4 cells may be involved in the state of cell differentiation through its interaction with the type I IGF receptors displayed at the cell surface. However, free IGF-II is not available for signaling via these receptors, since this growth factor is tightly bound to IGFBP, preventing its binding to receptors (3). Also, neither IGF-II nor IGFBP was found to be bound to the surface of HT29-D4 cells (5), suggesting that an IGF autocrine loop is not operative in these cells. In the same way, we show here that preformed $^{125}$I-IGF-II-IGFBP complexes do not allow $^{125}$I-IGF-II binding to the HT29-D4 cell surface (Fig. 5). Such a pattern appears to be due to the sequestration of IGF-II in the culture medium by high amounts of IGFBP expressing a high affinity for IGF-II, and this in contrast to its affinity for IGF-I (Figs. 1 and 2).

The data presented here show that suramin is able to dissociate IGF-II-IGFBP complexes at concentrations between 40 and 100 $\mu$g/ml (Fig. 4), thereby allowing the release of free IGF-II into the culture medium. It is quite possible that suramin is also able to induce an IGF-II release from soluble type II IGF receptors. These drug concentrations are identical to the ones previously found to induce an optimum enterocyte-like differentiation of HT29-D4 cells (17, 18). In contrast, they are significantly lower than the ones required to antagonize free IGF-II binding to HT29-D4 cell surface type I IGF receptors, i.e., 200-400 $\mu$g/ml (Fig. 3). Thus, it is possible that free IGF-II released from IGFBP under well-defined suramin concentrations interacts with the membrane type I IGF receptors to trigger a potential autocrine biological effect, e.g., the induction of a cell differentiation pathway. If such a hypothesis is correct, it would be possible to induce HT29-D4 cell differentiation by adding a ligand able to directly interact with the cell surface type I IGF receptors, i.e., bypassing IGF-II high-affinity IGFBP in the culture medium. This was attempted by adding an excess of recombinant IGF-I, since secreted IGFBPs are found to express a low affinity for this ligand. Under such an experimental design, HT29-D4 cells release high amounts of CEA (about 2.3 ng/10^6 cells/24 h at the optimum), a recognized marker of the tumor phenotype, since they confer a partial or complete autonomy on the cell (33, 34). However, several recent observations lead to the emerging picture that autocrine growth factor stimulation is not necessarily a transforming event and that autocrine/paracrine stimulation may be a physiological mechanism used to exercise control over growth and differentiation of normal cells in many tissues, especially in the intestinal epithelium (35-38). This may be consistent with a model in which the disappearance of a biological response of cells to a specific growth factor they normally release might lead to defects in the regulation of the delicate balance between cell proliferation and cell differentiation.

In this study, we investigate what could be the mechanisms that account for HT29-D4 cell differentiation induced by suramin. An attractive hypothesis is that IGF-II secreted by HT29-D4 cells may be involved in the state of cell differentiation through its interaction with the type I IGF receptors displayed at the cell surface. However, free IGF-II is not available for signaling via these receptors, since this growth factor is tightly bound to IGFBP, preventing its binding to receptors (3). Also, neither IGF-II nor IGFBP was found to be bound to the surface
ing. In particular, the roles of the other growth factors also secreted by HT29-D4 cells, e.g., transforming growth factor α and β (2), may be addressed, since they may act by either stimulating or inhibiting cell proliferation and/or cell differentiation.

Finally, several classes of IGFBP have been identified that modulate IGF actions in a positive or negative fashion. These IGFBPs express relative differences in affinity for IGF-I and IGF-II that may determine the bioavailability of the IGFs to their target cells (42–45). Thus, the factors that control the level and type of IGFBP secreted into the conditioned medium in vitro as well as into the extracellular spaces in vivo may be important modulators of the balance between inhibitory or enhancing IGF effects on the colonic epithelial cells. Whether the molecular forms of IGFBP secreted by cancer colon epithelial cells and by their nonmalignant counterparts are distinct is yet to be determined.

In summary, suramin, a drug that has been widely used as a laboratory tool to shunt growth factor autocrine loops, exhibits here an unusual opposite property allowing such a self-triggering. This potential is linked to the differential effects delivered by this drug on IGF-II interactions with either the secreted IGFBP or the cell-associated type I IGF receptors. Furthermore, the treatment of undifferentiated HT29-D4 cells with an excess of either IGF-I or aIrr-3 to mimic the putative autocrine loop results in the induction of an intermediate stage of cell differentiation that is associated with the appearance of typical luminal membranes with microvilli and an important CEA release, a known marker of HT29-D4 cell differentiation.

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