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AUTOCRINE REGULATION OF GROWTH: II. GLUCOCORTICOIDS INHIBIT TRANSCRIPTION OF c-sis ONCOGENE-SPECIFIC RNA TRANSCRIPTS

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The ductus deferens smooth muscle tumor cell line (DDT_1MF-2) expresses c-sis proto-oncogene poly A^+ RNA transcripts which are thought to encode at least one subunit of the potent mitogen platelet derived growth factor (PDGF). We have previously demonstrated that glucocorticoids block DDT_1MF-2 cells in G_0/G_1 stage of the cell cycle, and that exogenously applied PDGF reinitiates cell cycle progression. In this paper we document that glucocorticoids act to inhibit cell cycle progression by inhibiting the expression of c-sis poly A^+ transcripts, which we suggest are encoding a PDGF-like molecule for DDT_1MF-2 cells.

We have previously reported (1-3) that androgens stimulate and glucocorticoids inhibit progression of DDT_1MF-2 cells through the cell cycle. Since these cells contain both androgen and glucocorticoid receptors (4), it is reasonable to speculate that the mechanisms involved in growth regulation may be receptor mediated. Furthermore, it is known that many tissues of mesenchymal origin require protein growth factors which act through membrane receptors to sustain growth, and that tumor tissues might produce their own growth factors (5,6). This led us to carry out the studies presented in a previous paper (7) in which we documented that exogenously applied PDGF (but not EGF or insulin) induced glucocorticoid-blocked cells to re-enter the cell cycle. Since PDGF has been shown to share extensive amino acid sequence homology with the simian sarcoma virus oncogene (v-sis) product (8,9), we initiated experiments to analyze the role of steroid hormones in the regulation of c-sis oncogene expression using a v-sis specific probe cloned from simian sarcoma virus (10).

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MATERIALS AND METHODS

Materials: Tissue culture reagents were obtained from Grand Island Biologicals, Grand Island, NY. Platelet derived growth factor was receptor grade from Seragen, Inc., Boston, MA. [^{32}P]-dCTP, [^{32}P]-dATP and [^{35}S]-methionine were obtained from New England Nuclear, Boston, MA. Micrococcal nuclease-treated rabbit reticulocyte lysate was obtained from Promega Biotec, Madison, WI. Oligo dT cellulose was obtained from Collaborative Research, Waltham, MA. All other chemicals which were of reagent grade or better were obtained from Sigma Chemical Co., St. Louis, MO, or Bethesda Research Laboratories, Gaithersburg, MD.

Cell Culture Conditions: Cells were cultured as previously described (11,12).

RNA Isolation and Dot Blot Analysis: Poly A$^+$ RNA was isolated by oligo dT cellulose chromatography (13) from total cellular RNA prepared by centrifugation through CsCl in the presence of guanidine-isothiocyanate (14). Dot blot analysis was performed as follows: poly A$^+$ RNA, dissolved in distilled H_2O, was adjusted to 2% formaldehyde and heated at 60°C for 15 minutes. The sample was cooled and diluted 5-fold with 20 x SSC prior to filtration. Serial dilutions were carried out with 15 x SSC. RNA at the indicated concentrations were applied to pre-wetted .45 µm nitrocellulose filters (Schleicher and Scheull, BA-85). The filters were dried at 80°C under vacuum for 2 hours and stored at 4°C.

Prehybridization and hybridization were carried out essentially as described by Thomas et al. (15). Filters were probed with nick translated DNA ($1x10^6$ cpm/ml, specific activity > $5x10^7$ cpm/µg) for 30-35 hours at 42°C, washed at the final stringency of 3 x SSC at 64°C for 1 hour, and autoradiographed at -70°C for 5 days. The v-sis probe used was a Pst 1 1.1 kb fragment cloned in pBR322 (10). The 1.1 kb sis insert was isolated and nick translated as described by Maniatis et al. (16).

Cell-Free Translation: One µg of poly A$^+$ RNA from control or glucocorticoid-treated cells (same RNA as used in Figure 1, lanes A and B) was translated in a micrococcal nuclease-treated rabbit reticulocyte lysate exactly as described by the suppliers (Promega Biotec). The reaction was stopped at 30 minutes and 10 µl of translation products were analyzed on a 10.5% polyacrylamide slab gel under reducing conditions (17).

RESULTS AND DISCUSSION

The mechanism of the growth regulation of DDT$_1$MF-2 cells by PDGF and glucocorticoids was investigated by measuring c-sis poly A$^+$ RNA transcripts utilizing a homologous v-sis probe (10). Total cellular RNA was prepared, poly A$^+$ RNA transcripts were isolated and dot blotted as described in Methods. As shown in Figure 1 (lane A), RNA from control cells hybridize the nick translated v-sis probe. Conversely, triamcinolone acetonide treatment for 18 hours dramatically reduces the levels of c-sis RNA transcripts (Figure 1, lane B). If cells treated for 24 hours with triamcinolone acetonide were then grown for an additional 18 hours in the absence of steroid, the c-sis transcripts start to reappear (Figure 1, lane D) and approach the levels found in untreated cells (Figure 1, lane C). However, if cells were treated for 24 hours with triamcinolone acetonide and then for an additional 18 hours with

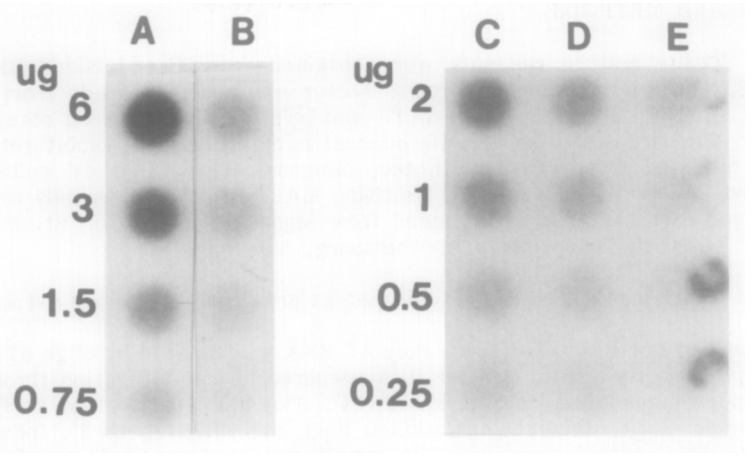

Figure 1. Dot blot analysis of c-sis proto-oncogene expression in DDT_1MF-2 cells. Cells growing in 100 mm Falcon tissue culture dishes were treated with ethanol (lanes A, C) or 1 x 10^{-7}M triamcinolone acetonide (lanes B, D, E) for 18-24 hours. At this time, RNA was prepared from cells representing lane A (control) or lane B (1 x 10^{-7}M triamcinolone acetonide). At 24 hours, lanes C, D, and E underwent a media change. Ethanol (< 0.1%) was added to lanes C and D; 1 x 10^{-7}M triamcinolone acetonide and 50 ng/ml PDGF (human receptor grade from Seragen) were added to cells representing lane E. Following 18 hours of incubation, transcripts were isolated as described in Methods, blotted and probed with nick translated v-sis DNA.

both the steroid and 50 ng/ml platelet derived growth factor (Figure 1, lane E), a treatment documented to stimulate the re-entry of DDT_1MF-2 cells to the cell cycle (7), there was no effect on restoration of c-sis RNA transcripts. The latter demonstrates that depletion of c-sis RNA is not the result of DDT_1MF-2 cell blockade in G_0/G_1.

At the present time, our studies have not established at what level glucocorticoids inhibit c-sis oncogene expression. However, we have shown that the rate of ^3H-uridine incorporation into control or triamcinolone acetonide-treated cells is quantitatively similar; furthermore, glucocorticoids do not cause a general decrease in the qualitative activity of poly A^+ RNA isolated from DDT_1MF-2 cells. The latter is demonstrated in Figure 2, where 1 µg samples of poly A^+ RNA from control and glucocorticoid-treated cells were analyzed in a cell-free translation system. There is a similar distribution of translation products in both treatment groups. In addition, a novel protein of approximate molecular weight of 29,000 appears in the glucocorticoid-treated group. We speculate that this protein is intimately involved in glucocorticoid regulation of growth, since a DDT_1MF-2 variant that is insensitive to glucocorticoid treatment lacks this protein (in preparation).

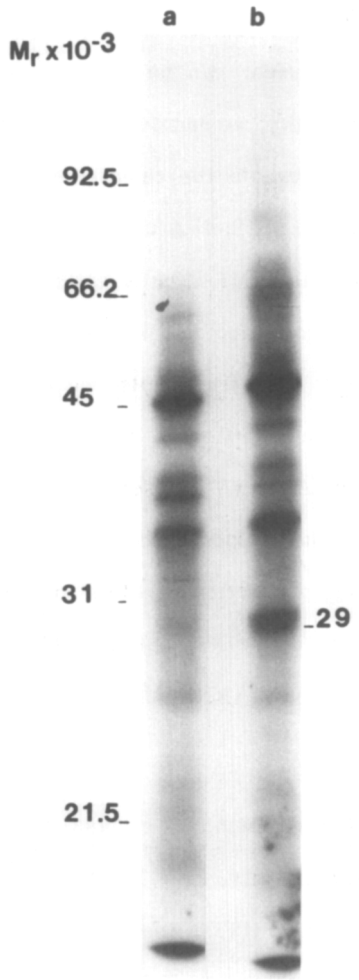

Figure 2. Analysis of poly A^+ RNA from control and glucocorticoid-treated cells by cell-free translation. RNA was prepared as described in Methods and Materials by two passes over oligo dT cellulose (13). Control cells were grown in < 0.1% ethanol and harvested at 80% confluency (lane A). A similar number of replica plated cells were treated for 18 hours with 1 x 10^{-7}M triamcinolone acetonide prior to harvesting (lane B). Cell-free translation was carried out using 1 µg of poly A^+ RNA in a micrococcal nuclease-treated rabbit reticulocyte lysate translation assay exactly as described by the supplier (Promega Biotec, Madison, WI). Ten µl of the total incubation mixture was analyzed on a 10.5% acrylamide gel under reducing conditions (17).

The recent discovery that platelet derived growth factor has close homology to the predicted amino acid sequence of a protein encoded by the c-sis oncogene (8,9) lends credence to the hypothesis that autocrine regulation of growth may be an important factor in the ontogeny of cancer. However, documentation of autocrine regulated growth of cancer cells requires a system where growth ceases when production of the autocrine ceases. Furthermore, reinitiation of cell cycling must

result by exogenous application of the missing autocrine. This paper (and see ref. 7) reports that just such a requirement can be met utilizing the hamster DDT_1MF-2 smooth muscle cell line. Specifically, we can demonstrate that triamcinolone acetonide treatment of DDT_1MF-2 cells prevents the release into the cytoplasm of c-sis poly A^+ transcripts and the subsequent production of a PDGF-like molecule. In a previous paper (7) we documented that DDT_1MF-2 cells enter a G_0/G_1-like state following 18 hours of treatment with glucocorticoids. We have now shown that under similar conditions a reduction of c-sis RNA transcripts occurs. When PDGF is applied to the cells in the presence of glucocorticoids, however, the cells re-enter the cell cycle, even though c-sis poly A^+ RNA transcripts remain at a low level. These findings are consistent with the proposal that glucocorticoids inhibit growth of DDT_1MF-2 cells by attenuating production of PDGF by reducing c-sis mRNA.

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