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ANTAGONISTIC EFFECT OF PDGF AND NGF ON TRANSCRIPTION OF RIBOSOMAL DNA AND TUMOR CELL PROLIFERATION

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SUMMARY: The molecular mechanism by which NGF and PDGF affect growth of tumor cells was tested in human melanoma WM 266-4 and colorectal carcinoma SW 707 cell lines. We present evidence that NGF translocated to the nucleus and bound to the chromatin of SW 707 cells, which express the cell surface and the chromatin receptor for NGF, inhibits ribosomal RNA synthesis which in consequence leads to inhibition of cell proliferation. In WM 266-4 cells, which do not express NGF receptor, NGF does not affect cell proliferation. In contrast, PDGF translocated to the nucleus of both SW 707 and WM 266-4 cells activates ribosomal RNA synthesis. We report here that NGF abolishes PDGF-activated ribosomal RNA synthesis and PDGF-stimulated growth of tumor cells.

Growth of neoplastic cells and maintenance of their neoplastic state is regulated by endogenous and exogenous growth factors which interact with appropriate cell surface receptors (1-4). The molecular mechanism of interaction between growth factors and cells still remains in the realm of speculation. We and others have found that platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and nerve growth factor (NGF) internalized by cells are translocated to the nucleus and bound to chromatin proteins (5-14). The nuclear translocation of insulin (15,16) and of vasoactive intestinal peptide (17) have also been described. Bouche et al. (18) described nuclear localization of the fibroblast growth factor and its activatory effect on ribosomal RNA synthesis. In this paper we analyze the effect of NGF and PDGF on transcription. NGF is essential for the survival and differentiation of sympathetic and sensory neurons (19,20). It stimulates mitotic response in adrenal chromaffin cells (21), but inhibits proliferation of rat pheochromocytoma (22), and human melanoma and colorectal carcinoma tumor

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Abbreviations used in this paper: EGF, epidermal growth factor; NGF, nerve growth factor; PDGF, platelet-derived growth factor; PBS, phosphate buffered saline.

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cells (7,23). PDGF, on the other hand, is a mitogen for connective tissue cells *in vitro* and is synthesized by several tumor cell lines (24). We present evidence that NGF and PDGF translocated to the nucleus of tumor cells exert opposite effects on ribosomal RNA synthesis. The PDGF-stimulated growth of tumor cells is abolished by NGF.

MATERIALS AND METHODS

Tumor cell lines

WM 266-4 melanoma and SW 707 colorectal carcinoma cells were grown in Eagle's minimal essential medium/L15(3:1) supplemented with 10% bovine serum as described (5,7).

Incubation of intact cells with NGF or PDGF

Cells were incubated for 24 h with ^{125}I-PDGF (1 ng/ml, spec. act. 52 cpm/pg, PDGF AB from human platelets, Collaborative Research) or with ^{125}I-NGF (10 ng/ml, mouse, Sigma) labeled by the lactoperoxidase method (7). Cells were washed with PBS and fractionated into cytoplasm, nucleoplasm, nuclear membranes and chromatin as described (5-7).

Incubation of isolated nuclei with PDGF and/or NGF

Nuclei used in a cell-free-system were isolated by homogenization in 0.25 M sucrose, 10 mM KCl, 1.5 mM $MgCl_2$, 100 mM Tris-HCl (pH 7.6), 12 mM 2-mercaptoethanol, 0.02% Triton X-100 and centrifugation at 600*g* for 10 min followed by ultracentrifugation through 2.2 M sucrose, 10 mM Tris-HCl (pH 7.9), 1.5 mM $MgCl_2$ (90,000*g* for 60 min) (7). Nuclei (2-3 x 10^6) were incubated with ^{125}I-PDGF (1 ng/ml human PDGF AB, spec. act. 50 cpm/pg, Collaborative Research) in an incubation medium containing 0.25 M sucrose, 20 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$ and 500 ng/ml bovine serum albumin (BSA). Incubation was performed for 1 h at room temperature. After incubation, nuclei were centrifuged (600*g* for 10 min), washed 3 times with 50 mM Tris-HCl (pH 7.5), 12.5 mM NaCl, 12.5 mM $MgCl_2$, homogenized in 1 mM Tris-HCl (pH 7.6), and centrifuged through 1.7 M sucrose, 10 mM Tris-HCl (pH 7.9). Nucleoplasm was taken from the top, nuclear membranes from the interface, and chromatin from the bottom of the tube as described (7). Electrophoretic analysis of native and chromatin bound ^{125}I-PDGF was performed according to Laemmli (25).

Transcription in isolated nuclei, analysis of RNA

[^{32}P]UTP incorporation into nuclear RNA and ribosomal RNA synthesis was tested in isolated nuclei incubated 1 h with NGF and/or PDGF (7,23). Nuclei (5 x 10^6 nuclei in 1 ml volume) were incubated for 1 h at room temperature in 0.25 M sucrose, 20 mM Tris-HCl (pH 7.8), 10 mM MgCl2, 500 ng/ml BSA, 0.3 mM each ATP, GTP, CTP, 20 µCi/ml [^{32}P]UTP (Amersham, spec. act. 3000 mCi/mmol), NGF (10 ng/ml), and/or PDGF (1/2 maximal unit/ml), washed 3 times with 50 mM Tris-HCl (pH 7.5), 12.5 mM NaCl, 12.5 mM $MgCl_2$, homogenized in 1 mM Tris-HCl (pH 7.6), centrifuged through 1.7 M sucrose and fractionated into chromatin, nuclear membrane and chromatin as described above. The amount of synthesized RNA was determined by trichloroacetic acid precipitation. RNA was isolated from nuclei incubated with [^{32}P]UTP in the absence or presence of NGF by proteinase K digestion, phenol-chloroform extraction and ethanol precipitation and tested by dot-blot hybridization with plasmid pBR322 containing *Eco*RI fragments of human rDNA (26), kindly provided by Dr. R. D. Schmickel.

Effect of NGF and PDGF on cell proliferation

Cells were incubated 3 d with [6-^{3}H]thymidine (10 μCi/ml, spec. act. 24 Ci/mmol; Amersham) in the absence or presence of NGF (10 ng/ml) and/or PDGF (1/2 maximal unit/ml, Collaborative Research).

RESULTS

Effect of NGF and/or PDGF on cell proliferation

We have selected two cell lines: colorectal carcinoma SW 707 which expresses both surface and chromatin NGF receptors (7) and is growth-inhibited by NGF (Table 1); and as a control, melanoma WM 266-4 which does not express cell surface or chromatin NGF receptor (7). Growth of the WM 266-4 melanoma cell line was not affected by NGF (Table 1).

In contrast, SW 707 and WM 266-4 cells, both of which secrete PDGF, when exposed for 3 d to exogenous PDGF (Table 1) showed a three-fold increase of [^{3}H]thymidine uptake which suggests that PDGF activates growth of these cells.

In order to investigate whether NGF might influence the activatory effect of PDGF on proliferation, cells were exposed for 3 d to NGF and PDGF simultaneously. Proliferation of SW 707 cells exposed to both growth factors was inhibited by 60%, i.e., to a similar level as in the presence of NGF alone (Table 1). Thus in SW 707 cells expressing the NGF receptors, NGF may abolish PDGF-activated growth of tumor cells. In control WM 266-4 cells, NGF had no inhibitory effect on PDGF activated-proliferation.

Table 1
Effect of NGF and PDGF on DNA and RNA synthesis

Cell Line	Growth factor	[^{3}H]thymidine incorporation into chromatin		[^{3}H]uridine incorporation into cytoplasmic, nucleoplasmic and chromatin RNA	
		cpm	%	cpm	%
SW 707	–	9,000	100	18,200	100
	NGF	2,800	31	8,350	46
	PDGF	27,400	304	54,600	300
	PDGF + NGF	3,600	40	8,750	48
WM266-4	–	8,100	100	18,000	100
	NGF	8,100	100	18,000	100
	PDGF	26,200	284	54,290	301
	PDGF + NGF	26,280	284	54,180	301

Data show a representative series of experiments; SD of the mean value from 4 independent series of experiments did not exceed 10%.

Table 2
Total RNA and ribosomal RNA synthesis in isolated nuclei exposed to NGF and/or PDGF

Cell	Growth factor	[^{32}P]UTP uptake into RNA (cpm/10^6 nuclei)	% total RNA synthesis	[^{32}P]UTP RNA hybridization to rDNA (cpm)	% synthesis
SW 707	control	38,000	100	15,000	100
	NGF	17,000	45	4,000	30
	PDGF	109,500	288	45,900	306
	NGF + PDGF	18,250	48	5,250	35
WM 266-4	control	27,500	100	11,500	100
	NGF	27,400	100	11,600	100
	PDGF	79,500	289	35,000	304
	NGF + PDGF	80,300	292	35,650	310
A431	control	40,000	100	17,000	100
	PDGF	39,950	100	16,900	100
	PDGF*	40,200	100	17,100	100

Data shows a representative series of experiments; SD of the mean value from 4 independent series of experiments did not exceed 15%.

*Nuclei of A431 cells were incubated with PDGF in the presence of membrane-containing cytoplasm from SW 707 cells prepared as described previously (23, 27).

Effect of NGF and PDGF on transcription in intact cells and a cell-free system

To establish whether NGF and PDGF exert an effect on transcription, cells were exposed to each of the growth factors. In SW 707 cells exposed to NGF, incorporation of [^{3}H]uridine into RNA decreased by 54%, indicating that NGF inhibited RNA synthesis. In control WM 266-4 cells NGF did not affect RNA synthesis (Table 1). Incubation of the same cells with PDGF resulted in 3-fold higher RNA synthesis compared with control cells. When SW 707 cells were simultaneously exposed to NGF and PDGF, RNA synthesis decreased by approximately 52%, i.e., to the same level of RNA synthesis observed in cells exposed to NGF alone. In control WM 266-4 cells activated by PDGF, RNA synthesis was not affected by NGF (Table 1).

To determine whether the effect of NGF and PDGF on RNA synthesis is mediated by surface receptors (induction of the second messengers) or through direct interaction with cell nucleus, further experiments were performed on isolated nuclei (Table 2).

In SW 707 cell nuclei incubated with NGF, total RNA synthesis, measured as [^{32}P]UTP incorporation into RNA, decreased by 55% and ribosomal RNA (rRNA)

synthesis decreased by 70%. In control WM 266-4 cell nuclei synthesis of RNA was not affected by NGF (Table 1). In contrast to the results obtained with NGF, exposing the isolated nuclei of both cell lines to PDGF activated rRNA synthesis 3-fold (Table 2).

Nuclei isolated from SW 707 cells and simultaneously exposed to NGF and PDGF showed an inhibition of rRNA synthesis. In control WM 266-4 cell nuclei, NGF did not affect rRNA synthesis activated by PDGF (Table 2).

The results we have presented suggest that by direct interaction with the cell nucleus NGF and PDGF exert opposite effects on ribosomal RNA synthesis.

To eliminate a possibility that the activatory action of PDGF on rRNA synthesis is mediated by the plasma-membrane receptor, nuclei isolated from A431 cells, which do not express the PDGF receptor on either cell surface or in chromatin (27), were incubated with PDGF in the presence of membrane-containing cytoplasm from SW 707 cells (Table 2). In this reconstituted system rRNA synthesis was not affected by PDGF which suggests that activation of rRNA synthesis occurs after PDGF binds to the chromatin of the target cells and the process does not require binding to the plasma-membrane receptor.

In control experiments with other growth factors (EGF, insulin and IGF-I incubated with nuclei from SW 707 or WM 266-4 cells which express receptors for these growth factors) rRNA synthesis was not affected (not shown) indicating specific NGF and PDGF interactions with ribosomal DNA.

Interaction of NGF and PDGF with chromatin

As shown in Fig. 1A, chromatin-bound PDGF in both cell lines shows electrophoretic mobility similar to that of native PDGF which suggests that PDGF is translocated to the nucleus in nondegraded form. NGF was bound only to the

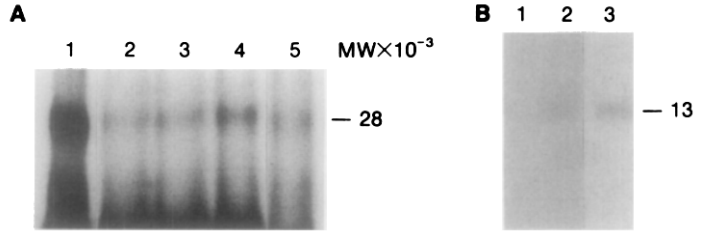

Figure 1.
Autoradiogram of SDS-PAGE (15% polyacrylamide). A: free ^{125}I-PDGF (lane 1) and chromatin-bound ^{125}I-PDGF (lanes 2-5) after 24 h incubation of intact SW707 (lane 2) and WM 266-4 (lane 3) cells and after 1 h incubation of SW 707 (lane 4) and WM 266-4 (lane 5) nuclei with ^{125}I-PDGF. B: chromatin-bound ^{125}I-NGF after 24 h incubation of SW 707 cells (lane 2) and after 1 h incubation of SW 707 cell nuclei (lane 3) with ^{125}I-NGF. ^{125}I-NGF is undetectable in chromatin of WM266-4 cells (lane 1).

chromatin of SW 707 cells (not to that from WM266-4 cells) and also showed electrophilic mobility characteristic for the native NGF (Fig. 1B).

To test whether the antagonistic effect of NGF and PDGF on transcription of rDNA is exerted by binding of these growth factors to the same or to the different chromatin regions, chromatin isolated from SW707 cells was incubated with NGF (1 h) followed by incubation with ^{125}I-PDGF. Incubation with NGF did not decrease binding of PDGF (data not shown) which suggests that NGF and PDGF recognize different binding sites of the chromatin.

DISCUSSION

The obtained results show that NGF and PDGF taken up by nucleus and bound to the chromatin exert direct effect on transcription of ribosomal DNA. The effect on transcription is exerted by these growth factors only after they have bound to the chromatin of target cells but not of not-target cells. Thus NGF and PDGF probably specifically affect transcription of rDNA by binding to the chromatin proteins specific for the target cells rather than interacting with polymerase I, which is present in the nuclei of all cell lines. This finding is consistent with our observation that the chromatin of target cells contains specific receptors which bind growth factors which have been translocated to the nucleus (5-8,23). Since NGF and PDGF bind to different chromatin regions and NGF may abolish activatory effect of PDGF, a simple model of ribosomal RNA synthesis regulation by these growth factors may be suggested, as shown in Figure 2.

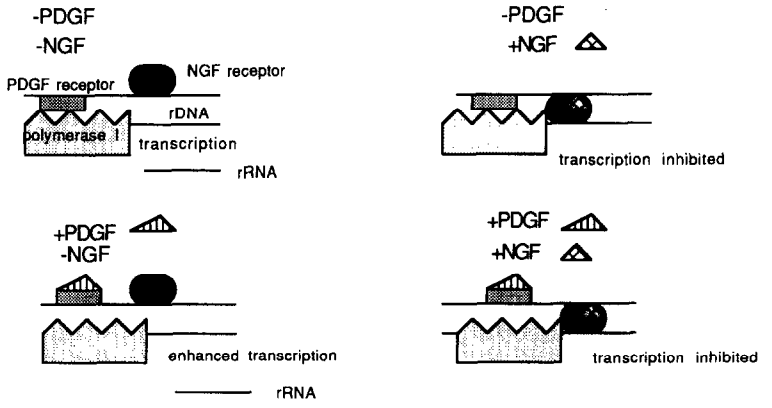

Figure 2.
Hypothetical mechanism of PDGF and NGF effect on transcription of ribosomal DNA. Binding of PDGF to its chromatin receptor induces conformational changes which enhance transcription of ribosomal DNA by polymerase I. Binding of NGF to its chromatin receptor blocks binding of polymerase I to DNA which inhibits transcription of ribosomal genes independently of the presence or absence of PDGF.

Since ribosomal RNA synthesis occurs efficiently in the absence of PDGF we suggest that PDGF, after binding to specific chromatin proteins, induces conformational changes which only enhance transcription of rDNA (Fig. 2). In contrast, NGF bound to its chromatin receptor induces conformational changes which make polymerase I unable to bind to the DNA.

Bouche *et al.* (18) also described the activation of rRNA synthesis by fibroblast growth factor translocated to the nucleolus. Since both PDGF and fibroblast growth factor stimulate cell competence to enter $G_0 \rightarrow G_1$ phase of the cell cycle (28), we suggest that activation of rRNA synthesis, critical for reassembly of ribosomes, may represent a common mechanism of action for these factors. EGF, insulin and IGF-I which do not induce cell competence to enter the $G_0 \rightarrow G_1$ phase of the cell cycle (28) seem not modulate RNA synthesis or to interfere with PDGF action.

As shown in this paper, some growth factors may activate the growth of tumor cells by exerting a direct effect on gene regulation, while others, by a similar mechanism, may act directly as growth inhibitors.

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