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DEMONSTRATION OF RECEPTORS FOR A PDGF-LIKE MITOGEN ON HUMAN OSTEOSARCOMA CELLS

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U-2 OS human osteosarcoma cells synthesize, process and secrete a platelet-derived growth factor (PDGF)-like mitogen. Incubation of these cells with 1 mM suramin unmasks PDGF receptor sites which are normally occupied or down regulated by the secreted endogenous PDGF-like mitogen. Partially purified preparations of metabolically labelled U-2 OS conditioned medium binds to U-2 OS cells and binding is inhibited by excess PDGF. These findings suggest that U-2 OS cells are capable of autocrine stimulation.

The v-sis oncogene of the simian sarcoma virus (SSV) codes for a platelet derived growth factor (PDGF)-like protein (1, 2), which is a potent mitogen for mesenchymal cells in culture (3). Messenger RNA for c-sis, the cellular homologue of v-sis (4), has been detected in a number of human tumor derived cell lines (5). The human osteosarcoma-derived cell line, U-2 OS, has been shown to express c-sis RNA (6, 7) and secrete a PDGF-like protein, PDGF-O (6, 8, 9. 10). It has previously been reported that U-2 OS cells exhibit few, if any, cell surface receptors for PDGF (11, 12). Here we report that U-2 OS cells possess PDGF receptors, which can be unmasked by treatment of the cells with the polyanionic compound, suramin. These receptors can bind both human PDGF and PDGF-like mitogen(s) secreted by U-2 OS cells,

ABBREVIATIONS: PDGF, platelet-derived growth factor; MEM, minimum essential medium; PDGF-O, platelet-derived growth factor-like mitogen produced by U-2 OS cells; EGF, epidermal growth factor.

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PDGF-O. These findings demonstrate for the first time that cells which inappropriately synthesize a PDGF-like factor specifically bind the secreted mitogen, which may exert its action through its membrane receptor.

METHODS

PDGF Binding Assays

U-2 OS cells depleted for two days in 0.3% FBS were pre-treated with suramin (0-2.4 mM) for 1.5 hrs at 37°C and rinsed. ^{125}I-PDGF binding was carried out at 4°C as described in (13). For competitive binding, unlabelled PDGF (0-100 ng/ml) was added to binding medium. For Scatchard analysis U-2 OS cells were pre-treated with suramin (1mM) or control buffer and then incubated with ^{125}I-PDGF of constant specific activity. To determine the kinetics of receptor re-occupancy after suramin pre-treatment, U-2 OS cells were pre-treated with suramin (1mM), rinsed and incubated in MEM for 0-24 hrs at 37°C. MEM was then removed, cells rinsed and ^{125}I-PDGF binding proceeded for 1 hr at 37°C. For inhibition of ^{125}I-PDGF binding by PDGF-O, U-2 OS cells were incubated with ^{125}I-PDGF and MEM previously conditioned by U-2 OS cells for 20 hrs. To test for reversibility of decreased ^{125}I-PDGF binding, U-2 OS cells were pre-treated with suramin (1mM), rinsed, incubated for six hours in MEM, rinsed and assayed for ^{125}I-PDGF binding or were treated a second time with suramin and then assayed for ^{125}I-PDGF binding. Counts per minute reported are the mean of duplicate wells.

Preparation of PDGF and PDGF-O

PDGF was prepared from outdated platelets as described in (14). For competition studies PDGF had a specific activity of 500 units/Ug unless otherwise stated. Pure PDGF had a specific activity of 3000 units/Ug. ^{125}I-PDGF was prepared from pure PDGF as described in (13). PDGF-O was prepared by CM-Sepahdex chromatography of ^{35}S-cysteine endogenously labelled U-2 OS conditioned medium as described in (6), except that the labelling time was extended to six hours.

RESULTS

Suramin is capable of competing with low density lipoprotein (15) and PDGF (16) for their respective receptors and to displace bound PDGF from high affinity binding sites (16). By utilizing this property of suramin we were able to unmask previously occupied PDGF receptor sites on U-2 OS cells. Without suramin pre-treatment only low levels of specific PDGF binding to U-2 OS cells can be detected (fig 1A). Without suramin pre-treatment non-specific binding represents a high percentage of total

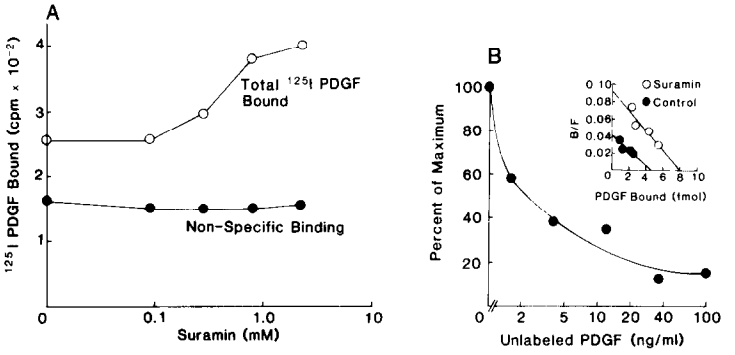

Figure 1A:
^{125}I-PDGF binding to U-2 OS cells pre-treated with suramin (0, 0.08, 0.24, 0.8 or 2.4 mM).

Figure 1B:
Competitive ^{125}I-PDGF binding to U-2 OS cells in the presence of unlabelled PDGF (0, 1, 2, 4, 12, 35 or 100 ng/ml). Insert: Scatchard analysis of ^{125}I-PDGF binding to U-2 OS cells with (○) or without (●) suramin pre-treatment.

binding, since few receptor sites are available. Pre-incubation of U-2 OS cells with suramin increases PDGF binding three fold while having no affect on non specific PDGF binding (fig 1A). Scatchard analysis of binding demonstrates that pre-incubation with suramin increases the apparent number of specific PDGF binding sites per cell without changing the dissociation constant (fig 1B). The presence of specific PDGF binding sites on U-2 OS cells was confirmed by competitive inhibition of ^{125}I-PDGF binding with unlabelled PDGF (fig 1B). In contrast to a three fold increase in PDGF binding to U-2 OS cells pre-treated with suramin, suramin pre-treatment increases PDGF binding to non-transformed mesenchymal cells by only 30% (data not presented).

Data presented in Table 1 demonstrate the binding of factors present in medium conditioned by U-2 OS cells to U-2 OS cells. The addition of 1 Ug of partially purified PDGF or 100 ng of pure PDGF decreases the binding of partially purified endogenously labelled U-2 OS conditioned medium by over 50%. In contrast, the addition of excess epidermal growth factor (EGF)

Table 1: PDGF-O Binding to U-2 OS Cells

	Total Counts Bound (CPM)	Inhibition
PCM	11,415	-
PCM + EGF (300 ng)	12,818	0
PCM + PDGF (1Ug)	4,142	63%
PCM + PDGF (100 ng)	5,547	51%

Competitive binding of PDGF-O to U-2 OS cells. Partially purified endogenously labelled U-2 OS conditioned medium (PCM) was incubated with suramin pre-treated U-2 OS cells in 24 well plates for 4 hrs at $4^{o}C$. In some wells EGF (Collaborative Research), partially purified PDGF (1Ug) or purified PDGF (100ng) was also added. Cells were assayed for bound ^{35}S-cysteine.

does not inhibit binding, suggesting that PDGF-O binds specifically to U-2 OS cells through interaction with the PDGF receptor. Counts not displaced by excess PDGF most likely represent endogenously labelled proteins non-specifically bound to U-2 OS cells.

The above experiments indicate that U-2 OS cells have specific binding sites for secreted PDGF-O. Kinetic experiments support this interpretation (Fig 2). Suramin pre-treatment exposes a maximum number of PDGF receptor sites. When U-2 OS cells are first pre-incubated with suramin, rinsed and then allowed to incubate in serum free medium for various time intervals, the number of available PDGF receptor sites decreases. As shown in figure 2A, U-2 OS cells bind approximately 65% less ^{125}I-PDGF after six hours incubation in serum free medium than immediately following suramin pre-treatment, indicating that secreted PDGF-O re-occupies PDGF receptor sites initially made available by suramin pre-treatment. The time dependent decrease in ^{125}I-PDGF binding was found to be reversible, demonstrating that suramin pre-treatment does not inhibit synthesis or re-cycling of PDGF receptors (fig 2B). The kinetic data cannot be

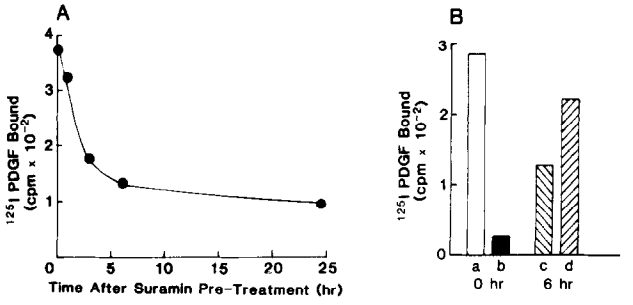

Figure 2A:
Decreased specific ^{125}I-PDGF binding to U-2 OS cells after 1, 3, 6 and 24 hrs incubation in MEM following suramin pre-treatment.

Figure 2B:
U-2 OS conditioned MEM inhibits specific ^{125}I-PDGF binding to U-2 OS cells and the time dependent decrease in specific ^{125}I-PDGF binding is reversible. Bars a and b: 0 hrs following suramin pre-treatment: ^{125}I-PDGF (a), ^{125}I-PDGF and U-2 OS conditioned MEM (b). Bars c and d: 6 hrs following suramin pre-treatment: ^{125}I-PDGF (c), ^{125}I-PDGF following second exposure to suramin (1 mM) (d).

explained by the secretion of a PDGF binding protein or a protease which destroys ^{125}I-PDGF binding capacity since incubation medium was removed and cells rinsed just prior to binding ^{125}I-PDGF. In addition, PDGF-0 secreted by U-2 OS cells blocks ^{125}I-PDGF binding to U-2 OS cells (fig 2B).

DISCUSSION

Heldin, et al. (11) reported that U-2 OS cells, which secrete a PDGF-like protein, PDGF-O, lack demonstrable PDGF receptors. Betsholtz, et al. (12) recently reported that a clone of U-2 OS has specific PDGF binding sites. They found that pre-treatment of this clone with low pH increased ^{125}I-PDGF binding, suggesting that low pH causes the endogenous PDGF-like mitogen to dissociate from its receptor. Findings presented here demonstrate that suramin is capable of unmasking PDGF binding sites on the U-2 OS parent cell line. Indirect evidence that the PDGF-like mitogen produced by U-2 OS cells binds to specific binding sites on U-2 OS cells is provided by the following: i) U-2 OS conditioned medium contains a factor which blocks ^{125}I-PDGF

binding to U-2 OS cells and ii) kinetic data indicate that PDGF receptors unmasked by suramin pre-treatment are largely re-occupied six hours after incubation in serum free medium. Blockage of endogenously labelled PDGF-O to U-2 OS cells with purified PDGF directly establishes that PDGF-O binds specifically to U-2 OS cells. These data suggest that PDGF-O may act on U-2 OS cells through binding to specific cellular receptors, in a fashion similar to the action of PDGF on target cells. They also suggest that secretion of PDGF-O and subsequent receptor occupiation and/or down regulation is responsible for the previously observed PDGF independence of U-2 OS cells (9). The recent finding that a hydrophobic leader sequence is needed for transformation by the v-sis gene product also suggests that secretion is a necessary step for the activity of PDGF-like mitogens produced by transformed cells (17).

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REFERENCES

1. Doolittle, R.F., Hunkapiller, M.W., Hood, L.E., DeVare, S.G., Robbins, K.C., Aaronson, S.A., and Antoniades, H.N. (1983) Science 221, 275-277.
2. Waterfield, M.D., Scrace, G.T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, A., Westermark, B., Heldin, C.H., Huang, J.S. and Deuel, T.F. (1983) Nature 304, 35-39
3. Antoniades, H.N. (1984) Biochem. Pharm. 33, 2823-2828.
4. Dalla-Favera, R., Gelmann, E.R., Gallo, R.C. and Wong-Staal, F. (1981) Nature 292, 31-35.
5. Eva, A., Robbins, K.C., Anderson. P.R., Scrinivasan, A., Tronick, S.R., Reddy, E.P., Ellmore, N.W., Gallen, A.T., Lautenberger, J.A., Papas, T.S., Westin, E.H., Wong-Staal, F., Gallo, R.C. and Aaronson, S.A. (1982) Nature 295, 116-119.
6. Graves, D.T., Owen, A.J., Barth, R.K., Tempst, P., Winoto, A., Fors, L., Hood, L.E. and Antoniades, H.N. (1984) Science 226, 972-974.
7. Johnsson, A., Heldin, C.H., Wasteson, A., Westermark, B., Deuel, T.F., Huang, J.S., Seeburg, P.H., Gray, A., Ullrich, A., Scrace, G., Stroobant, P., and Waterfield, M.D. (1984) EMBO, J. 3, 921-928.

8. Heldin, C.H., Westermark, B. and Wasteson, A. J. Cell. Physiol. (1980) 105, 235-246.
9. Graves, D.T., Owen, A.J. and Antoniades, H.N. Cancer Research (1983) 43, 83-87.
10. Betsholtz, Heldin, C.H., Nister, M., Ek, B., Wasteson, A. and Westermark, B. (1983) Biochem. Biophys. Res. Com. 117, 176-182
11. Heldin, C.H., Westermark, B. and Wasteson, A. (1981) Proc. Natl. Acad. Sci USA 78, 3664-3668.
12. Betsholtz, C., Westermark, B., Ek B., Heldin, C.H. (1984) Cell 39, 447-457.
13. Graves, D.T., Antoniades, H.N., Williams, S.R., and Owen, A.J. (1984) Cancer Res. 44, 2966-2970.
14. Antoniades, H.N., (1981) Proc. Natl. Acad. Sci. (USA) 78, 7314-7317.
15. Schneider, W.J., Beisiegel, U., Goldstein, J.L. and Brown, M.S. (1982) J. Biol. Chem 257, 2664-2673.
16. Williams, L.T., Tremble, P.M., Lavin, M.F. and Sunday, M.E. (1984) J. Biol. Chem. 259, 5287-5294.
17. Hannink, M. and Donoghue, D.J. (1984) Science, 226, 1197-1199.