Human Neuroblastoma Cells Express α and β Platelet-derived Growth Factor Receptors Coupling with Neurotrophic and Chemotactic Signaling

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Abstract

Both platelet-derived growth factor (PDGF) A- and B-chains are expressed in mammalian neurons, but their precise roles still remain to be clarified. In the present studies, we examined the expression of two PDGF receptor genes in human tumor cell lines derived from neural crest. The expression of α and/or β PDGF receptors was detected in a wide variety of neural crest-derived human tumor cell lines such as neuroblastoma, primitive neuroectodermal tumor, and Ewing's sarcoma by RNA blot analysis, and confirmed by immunoblot analysis. We have also demonstrated that PDGF receptors on the human neuroblastoma cell lines were biologically functional. Accordingly, chemotactic and mitogenic activities were induced by either PDGF-AA or PDGF-BB in serum-free medium. PDGF isoforms as well as nerve growth factor induced morphological changes showing neuronal cell maturation. Moreover, PDGF coordinately increased the levels of the transcript of the midsize neurofilament gene. The neuroblastoma cell lines also expressed the transcripts of PDGF A- and B-chains. These findings suggest that PDGF isoforms are involved not only in the promotion of the neuroblastoma cell growth, but also in neuronal cell migration, growth, and differentiation in human brain development. (*J. Clin. Invest.* 1993. 92:1153–1160.) Key words: autocrine and paracrine • Ewing's sarcoma • nerve growth factor • neural crest • neurofilament gene

Introduction

PDGF is a major connective tissue cell mitogen which is thought to play an important role in normal wound healing (1). The gene for the PDGF B chain has been shown to be the human homologue of the v-*sis* oncogene, and the abnormal expression of PDGF has been implicated in a variety of histopathologic conditions including arteriosclerosis and fibrotic diseases as well as cancer (1–4). Structurally, PDGF consists of two related polypeptide chains (A and B) which are encoded by different genes located on separate chromosomes. They are assembled naturally as a heterodimer (PDGF-AB) and two homodimers (PDGF-AA and PDGF-BB) (1, 2).

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Recently, two PDGF receptor genes whose products show different affinities for the three PDGF isoforms were identified and designated as α and β PDGF receptors (5–8). Each receptor can independently undergo tyrosine autophosphorylation in response to the specific PDGF isoforms, and couple with the signaling pathways for a variety of biological responses including cell proliferation and chemotaxis (9). Thus, the presence of specific PDGF isoforms and the differential expression of each PDGF receptor gene product are thought to be major determinants of the spectrum of known responses of cells to PDGF.

In the nervous system, PDGF is thought to be involved in the growth and differentiation of glial cells, so called gliogenesis and gliosis (10–13). Recently, the expression of PDGF-AA and -BB has been demonstrated in mammalian neuronal cells to be much more abundant than in glial cells (14, 15), although the biological roles of these molecules in brain development still remain to be clarified. The availability of molecular and immunologic probes for each type of human PDGF receptors has now led us to examine receptor expression more precisely (8, 13, 16). Neuronal cells as well as glial cells originally derive from the ectodermal neural crest (17, 18). During neural development, neuroepithelial crest cells migrate from the neural tube to form components of both the peripheral nervous system as well as melanocytes and neuroendocrine cells. A variety of neural tumors are derived from these neural crest precursors. For example, the presumptive stem cell of the neural crest, the sympathogon, differentiates into sympathoblasts, the cells of origin of neuroblastoma, and into its more mature forms, ganglioneuroblastoma and ganglioneuroma, and the chromaffin or nonchromaffin paraganglionic cells, the progenitors of pheochromocytomas and paragangliomas (18, 19). We first examined the expression of PDGF receptors in a wide variety of tumor cell lines derived from neural crest. Surprisingly, all the human neuroblastoma cell lines express the PDGF receptors. We further examine the biological function of the PDGF receptors on the human neuroblastoma cells.

Methods

Cells and growth factors. Human tumor cell lines were obtained from the originators of the lines as well as from the American Type Culture Collection (ATCC) (Rockville, MD), Riken Cell Bank (Tsukuba, Japan), and Japanese Cancer Research Resources Bank (Tokyo, Japan) as described in a previous report (20). The following cell lines were used: human primitive neuroectodermal cell line KU-SN (21), Ewing's sarcoma cell lines RD-ES (ATCC HTB 166) and SK-ES-1 (ATCC HTB 86), and human neuroblastoma cell lines GOTO (22), SK-N-SH (23), SH-SY5Y (a subclone with a neuronal phenotype derived from SK-N-SH), SH-EP-1 (a subclone with an epithelium-like phenotype derived from SK-N-SH) (24, 25), KU-YS (21), SMS-KAN (26), NB-1 (27), CHP-134 (28), and NB-9 (29). The cell lines were routinely grown in RPMI1640 medium containing 10% FCS in a 95% air-5% CO_2 atmosphere at 37°C. Human recombinant PDGF-AA and

PDGF-BB homodimers and mouse natural 2.5 S nerve growth factor (NGF)[1] were purchased from Collaborative Research Inc. (Bedford, MA).

RNA blot analysis. Total cellular RNAs were extracted, electrophoresed on a 1% agarose/formaldehyde gel, transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH), and cross-linked by UV-Stratalinker (Stratagene, Inc., La Jolla, CA) as described (30). The filters were hybridized at 42°C in a buffer (50% [wt/vol] formamide/5 × SSC (1 × SSC is 0.15 M sodium chloride/0.015 M sodium citrate), pH 7.0/1 × Denhardt's solution [0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrolidone]/5 mM Sodium phosphate/0.1% SDS/salmon sperm DNA [100 μg/ml]) containing ^{32}P-labeled cDNA probes prepared with a random-primed labeling kit (Megaprime, Amersham International, Amersham, Bucks, UK; 2 × 10^9 cpm/μg of DNA). The cDNA probes used were human α and β PDGF receptors (8, 31), human midsize neurofilament (32), and human β-actin (Clontech, Palo Alto, CA). After 16 h of hybridization, filters were washed twice for 20 min in 2 × SSC at room temperature followed by 30 min in 0.1 × SSC/0.1% SDS at 55°C and subjected to autoradiography at −70°C with XAR-5 film (Eastman Kodak Co., Rochester, NY) and intensifying screens.

Immunoblot analysis. Cells were washed twice with PBS and lysed with Staph-A buffer (10 mM sodium phosphate, pH 7.5, 100 mM sodium chloride, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate, 0.1% aprotinin, 1 μg/ml leupeptine, 1 μg/ml pepstatine, and 1 mM PMSF) and clarified by centrifugation at 10,000 *g* for 30 min. Proteins (150 μg per lane) were resolved by electrophoresis in 7% SDS-polyacrylamide gels, transferred to nitrocellulose filters, and proved by immunoblot analysis using anti-α PDGF receptor or anti-β PDGF receptor peptide antiserum (with or without the respective immunizing peptide) (9) and ^{125}I-protein A (1,369 MBq/mg, Amersham International). The filters were subjected to autoradiography. α and β PDGF receptor-specific peptide antisera were obtained after immunization of rabbits with the peptides KKSYEKIHIDFLKSD and KKKYQQVDEEFLRSD, respectively. The peptides were coupled to keyhole limpet hemocyanin by using glutaraldehyde (Takara Shuzo, Shiga, Japan).

DNA synthesis. The mitogenic effect of human recombinant PDGF on neuroblastoma cells was measured by [^{3}H]thymidine incorporation. Confluent cells in 24-well plates were washed twice with PBS and then incubated for 48 h in the absence of FCS. After serum starvation, cells were further incubated in the presence of PDGF isoforms, NGF or 10% FCS for 20 h followed by a 4-h incubation with [*methyl*-^{3}H]thymidine (0.5 μCi/ml; 25 mCi/mmol, Amersham International). The radioactivity of incorporated [^{3}H]thymidine in 5% trichloroacetic acid–insoluble precipitates was counted by using a LS5000 scintillation counter (Beckman Instruments, Inc., Fullerton, CA) after lysing the cells in 20 mM NaOH and 1% SDS (30).

Chemotaxis assay. Cell migration was assayed by means of a modified Boyden chamber technique using 5-μm pore size filters (Nucleopore Corp., Pleasanton, CA), as described previously (30). Cells were washed twice, suspended in serum-free RPMI1640 medium, and added to the upper chamber. Human recombinant PDGF isoforms were diluted in the same medium and added in the lower chamber. After 6 h of incubation at 37°C, cells that had migrated to the opposite side of the filters were fixed with methanol, and stained. The number of migrating cells was determined by a light microscopy (×100).

Morphological examination. The human neuroblastoma cell lines SK-N-SH, SH-SY5Y and SH-EP-1 were grown in serum-free RPMI1640 medium with or without human recombinant PDGF or mouse natural NGF for 72 h. Changes in morphology were observed every 24 h and photographed by using a phase-contrast microscopy (Nikon, Tokyo) and Palapan 3200B film (Polaroid Corporation, UK).

Reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was performed to detect small quantities of PDGF A- or B-chain mRNA. First-strand complementary DNA was prepared using total cellular RNA (5 μg) and a SuperScript preamplification system (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD). PCR was carried out using a GeneAmp PCR kit (Perkin-Elmer Cetus, Norwalk, CT). The primers used were 5′-CCATTCGGAGGAAGAGAAGCAT-3′ (sense strand) and 5′-ATAATCCGGATTCAGGCTTGTG-3′ (antisense strand) specific to the human PDGF A-chain (33), and 5′-GCCTGGGTTCCCTGACCATTGC-3′ (sense strand) and 5′-CACAGGCCGTGCAGCTGCCACT-3′ (antisense strand) specific to the human PDGF B-chain (34), both of which yielded 323-bp products. The reaction conditions were 30 s at 95°C, 60 s at 55°C, and 60 s at 72°C for 35 cycles in a GeneAmp PCR system 9600 (Perkin-Elmer Cetus). To confirm amplification of the appropriate DNA fragments, the RT-PCR products (8 μl of the 50-μl reaction mixture) were electrophoresed in a 1.5% agarose gel and transferred to nitrocellulose filters. Then, the filters were hybridized at 42°C in a buffer containing 50% (wt/vol) formamide and ^{32}P-labeled PDGF A- or B-chain cDNA, as described (8, 33, 34).

Results

Expression of α and β PDGF receptors in human neuroblastoma cell lines. First, we examined whether α and β PDGF receptors are expressed in a variety of human tumor cell lines originating from the neural crest. Total RNAs extracted from a human primitive neuroectodermal cell line KU-SN, Ewing's sarcoma cell lines RD-ES and SK-ES-1, and human neuroblastoma cell lines GOTO, SK-N-SH, KU-YS, SMS-KAN, NB-1, CHP-134, and NB-9 grown in the medium containing 10% FCS were subjected to RNA blot analysis. The filter was first hybridized with human full-length β PDGF receptor cDNA probe (Fig. 1, *right panel*), and then the same filter was rehybridized with human full-length α PDGF receptor cDNA probe (Fig. 1, *left panel*). The 6.4-kb α receptor and/or 5.3-kb β receptor–specific transcripts were detected not only in Ewing's sarcoma (lanes *2* and *3*) and the bipotential neuroblastoma cell line, GOTO (lane *4*), which can be induced to neuronal or schwannian cells in vitro (35), but also in human primitive neuroectodermal tumor (lane *1*) and human neuroblastoma cell lines (lanes *5–10*) under the stringent hybridization conditions. All the human neural crest-derived tumor cell lines examined in this study expressed α and/or β PDGF receptors to various extents. The expression level of each type receptor seemed to be differentially regulated in each cell line. The transcript sizes were the same as those of human normal fibroblast PDGF receptors (data not shown). We further studied the expression of PDGF receptors in GOTO, SK-N-SH, SMS-KAN, and NB-1 cells with the specific antisera for each type of PDGF receptor protein species. As shown in Fig. 2, mature 180-kD and immature 160-kD α PDGF receptors (8) were detected by anti-α PDGF receptor peptide serum. Moreover, anti-β PDGF receptor peptide serum could detect both 180-kD (mature form) and 165-kD (immature form) β PDGF receptors. The respective sizes of mature and immature forms of the two receptors were the same as those in different cell types (8). Moreover, these proteins were specifically competed by the immunizing peptide (data not shown). These data confirmed that two types of PDGF receptors were differentially expressed in human neuroblastoma cell lines.

PDGF-induced mitogenic signal transduction in human neuroblastoma cells. We next focused on the biological functions of PDGF receptors on SK-N-SH cells for further studies,

1. *Abbreviations used in this paper:* NGF, nerve growth factor; RT, reverse transcription.

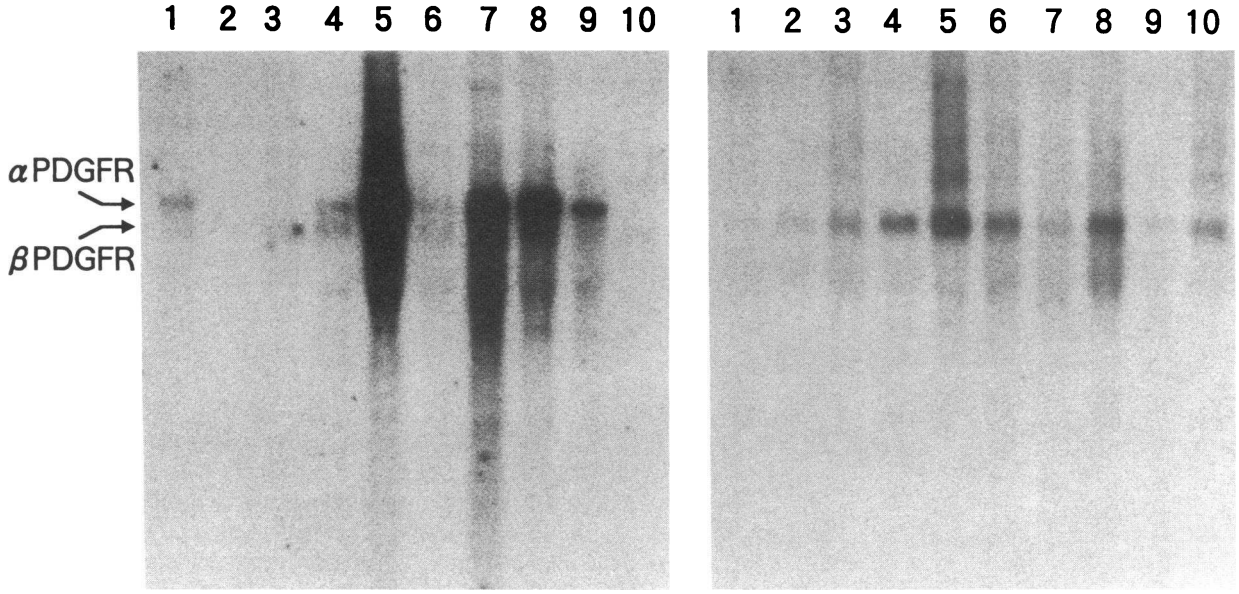

Figure 1. Expression of α and/or β PDGF receptor transcripts in human neural crest-derived tumor cell lines. Total RNAs (20 μg) from human primitive neuroectodermal cell line (lane *1*, KU-SN), Ewing's sarcoma cell lines (lane *2*, RD-ES; lane *3*, SK-ES-1), and human neuroblastoma cell lines (lane *4*, GOTO; lane *5*, SK-N-SH, lane *6*, KU-YS; lane *7*, SMS-KAN; lane *8*, NB-1; lane *9*, CHP-134; lane *10*, NB-9) were electrophoresed in a 1% agarose gel. The RNA blot filter was first hybridized with ^{32}P-labeled human β PDGF receptor (*βPDGFR*) cDNA (*right panel*) and then rehybridized with α PDGF receptor (*αPDGFR*) cDNA (*left panel*). Arrows indicate the α and β PDGF receptor transcripts.

because this cell line showed the most prominent expression of α and β PDGF receptors among the cell lines examined above. SK-N-SH consists of two phenotypically different cells. Therefore, we also used two subclones, SH-SY5Y and SH-EP-1, which showed neurogenic and neuroepithelial phenotypes, respectively (24, 25). The expression of both α and β PDGF receptor genes on SH-SY5Y and SH-EP-1 were also confirmed by the RNA- and immunoblot analysis as described above (data not shown).

In order to determine whether the PDGF receptors on human neuroblastoma cells couple with intracellular mitogenic signaling pathways, we analyzed the abilities of recombinant PDGF homodimers to induce DNA synthesis in serum deprived condition. As shown in Fig. 3, human recombinant PDGF-BB significantly increased the [^{3}H]thymidine incorporation into all three cell lines in a dose-dependent manner. PDGF-AA also stimulated the DNA synthesis of these cells significantly, although the mitogenic potency of PDGF-AA was less than that of PDGF-BB. Whereas 25 ng/ml of PDGF-BB resulted in at best a 1.5-fold increase of [^{3}H]thymidine incorporation into these neuroblastoma cell lines, 10% FCS could induce 2- to 3-fold increases above the control level (Fig. 3). However, NGF had no effect on the DNA synthesis of the cells (data not shown). This observation was consistent with the previous report (36).

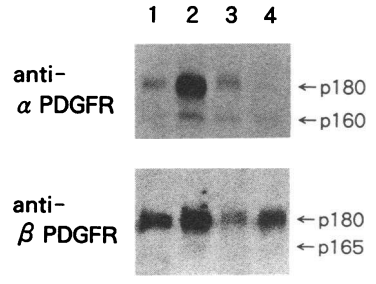

Figure 2. Expression two types of PDGF receptor gene products on human neuroblastoma cell lines. For analysis of α and β PDGF receptor proteins, total cell lysates of human neuroblastoma cell lines (lane *1*, GOTO; lane *2*, SK-N-SH; lane *3*, SMS-KAN; and lane *4*, NB-1) were prepared and subjected to immunoblot analysis using peptide antisera specific for each human PDGF receptor (8). Arrows indicate the α and β PDGF receptor protein species, confirmed by blocking with the immunizing peptides (data not shown).

PDGF-induced chemotactic activities in human neuroblastoma cells. Chemotaxis is another major biological activity of PDGF on a variety of mesenchymal cells such as fibroblasts, smooth muscle cells, and osteoblasts (1, 37, 38). Thus, we sought to investigate whether pathways for PDGF-mediated chemotaxis were coupled with the PDGF receptors in the neuroblastoma cells. We devised a chemotaxis assay using a modified Boyden chamber technique (9). SH-SY5Y, SK-N-SH and SH-EP-1 cells migrated towards recombinant human PDGF-AA and -BB homodimers in the serum-free medium (Fig. 4). Checkerboard analysis revealed that the cell migration induced by PDGF was due to direct cell migration (chemotaxis), but not due to random migration (chemokinesis) (data not shown) (38). The chemotactic as well as mitogenic activities of PDGF-BB were more potent on these neuroblastoma cells expressing two types of PDGF receptors than those of PDGF-AA. The different isoforms of PDGF can bind to two different receptors with different affinities (8, 39, 40). Moreover, there is no difference between the biological activities of PDGF-AA and -BB on cell expressing comparable amounts of each receptor (9). Based on the data described above, the differential activities of PDGF-AA and -BB on the neuroblastoma cells are probably due to the fact that PDGF-BB could trigger both α and β PDGF receptors on the cells but PDGF-AA could only activate α receptors.

Then, we also examined the chemotactic activities of PDGF-BB in other human neuroblastoma cell lines KU-YS, SMS-KAN, NB-1, CHP-134, and NB-9. All of these cell lines

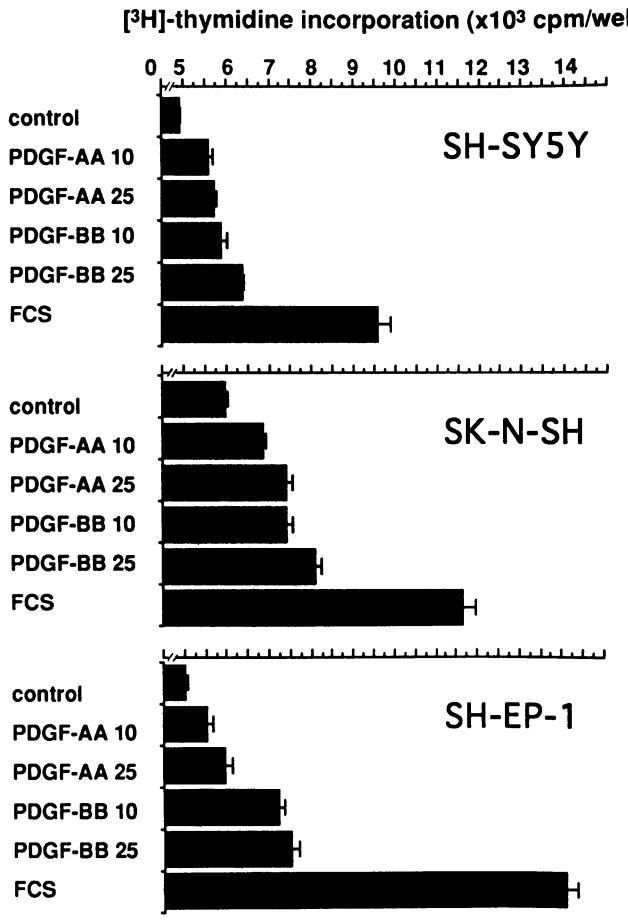

Figure 3. Mitogenic response of human neuroblastoma cells by PDGF isoforms. DNA synthesis by human neuroblastoma cell lines (SH-SY5Y, SK-N-SH, and SH-EP-1) in the presence of PDGF-AA or -BB (10 or 25 ng/ml) or 10% FCS was measured by [^{3}H]thymidine incorporation in serum-free medium as described in Methods. Controls are shown as [^{3}H]thymidine incorporation in the serum-free medium. Results represent the mean±SD ($\times 10^3$ cpm per well) of six replicates and are representative for three independent experiments.

migrated toward a solution of 25 ng/ml PDGF-BB, although they were less sensitive than SK-N-SH (Fig. 5). The chemotactic responses seemed to be correlated to the expression levels of PDGF receptor mRNAs.

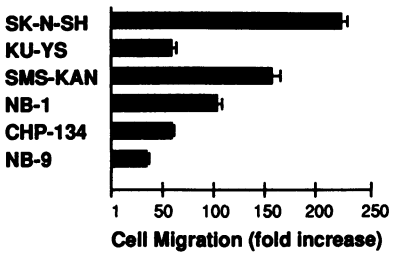

Figure 5. The chemotactic response of various human neuroblastoma cell lines to recombinant PDGF-BB. The migration toward 25 ng/ml of human recombinant PDGF-BB was assayed as described in Methods. Results are expressed as described in Fig. 4. Results represent the mean±SD values of triplicate samples and are representative for three independent experiments.

Morphological and neurotrophic changes induced by PDGF on SK-N-SH and SH-SY5Y cells. SH-SY5Y cells were spindle shaped with short neurite-like processes in serum-free medium (Fig. 6 *a*). The processes began to elongate 12 h after the PDGF treatment, and the cells had a decreased tendency to aggregate (Fig. 6 *b*). The reticular net formations between the cytoplasms were remarkably enhanced by PDGF. PDGF increased the proportion of neurite-bearing cells and increased neurite length as did NGF (Fig. 6, *b* and *c*). These morphological changes by PDGF were also seen in SK-N-SH cells. The neurite-like processes of SK-N-SH cells cultured in the medium without PDGF were very short (Fig. 6 *d*). SK-N-SH cells with long neurites began to increase 12 h after PDGF or NGF addition. The morphological alteration by PDGF as well as NGF occurred in almost all cells by 24 h (Fig. 6, *e* and *f*). Although PDGF did not significantly change the shape of flat epithelial SH-EP-1 cells within 24 h (Fig. 6, *g* and *h*), it decreased adhesiveness to a culture dish by 48 h (data not shown). In contrast to PDGF, NGF made SH-EP-1 cells more flattened and increased the adhesiveness (Fig. 6 *i*).

To elucidate whether these morphological changes induced by PDGF accompany neuronal differentiation, we further examined neurofilament gene expression before and after PDGF treatment. The expression of a 3.5-kb midsize neurofilament specific transcript could be detected in SK-N-SH and SH-SY5Y cells cultured in the absence of PDGF, but not in SH-EP-1 cells (Fig. 7). PDGF treatment for 24 h significantly increased the expression in both SK-N-SH and SH-SY5Y cells, while it did not change the expression level of a β-actin mRNA. The augmentation of neurofilament mRNA in PDGF-treated

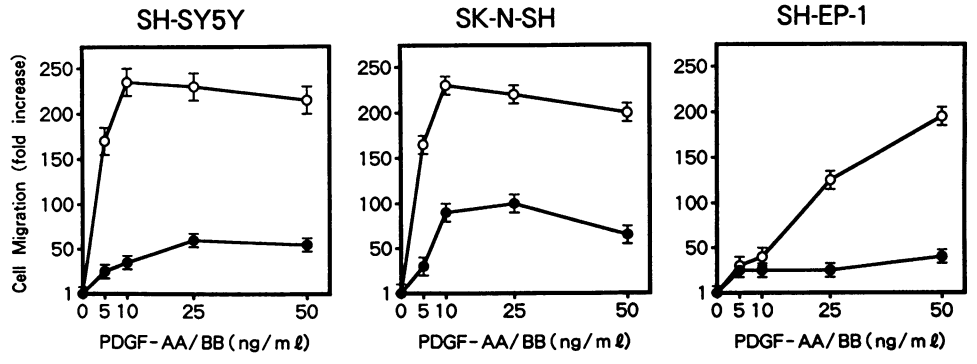

Figure 4. Dose-response curves of PDGF-mediated chemotaxis in SH-SY5Y, SK-N-SH, and SH-EP-1 cells. Cell migration of SH-SY5Y (*left*), SK-N-SH (*middle*), and SH-EP-1 cells (*right*) was assayed by means of a modified Boyden chamber technique using Nucleopore filters as described in the methods. Recombinant human PDGF-AA (●) or PDGF-BB (○) was added in the lower chambers. Results are expressed as the -fold increase in migrated cell numbers compared to that observed using RPMI1640 medium without PDGF. Results represent the mean±SD values of triplicate samples and are representative for three independent experiments.

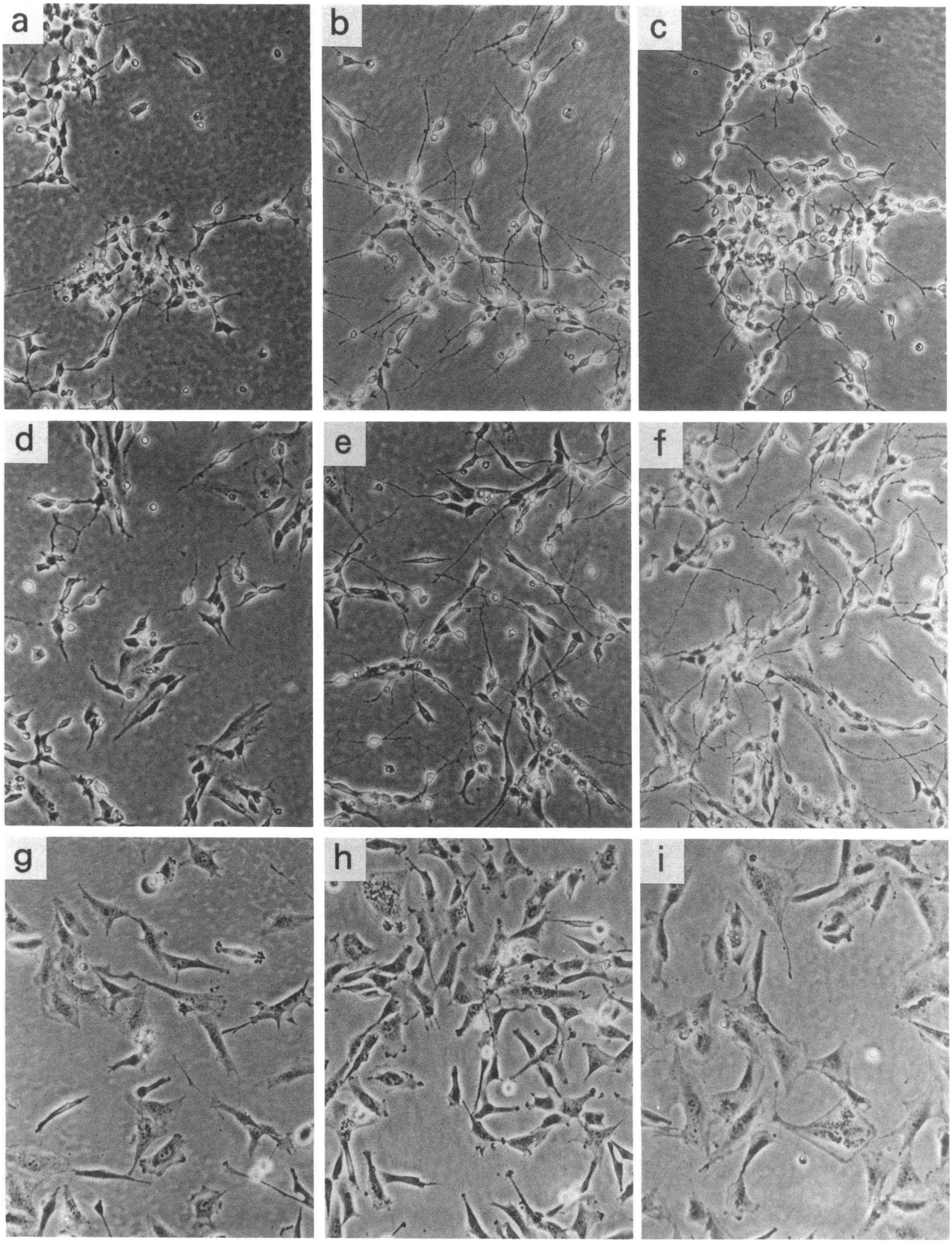

Figure 6. Changes in cell morphology induced by PDGF and NGF. SH-SY5Y (*a–c*), SK-N-SH (*d–f*) and SH-EP-1 (*g–i*) cells were cultured in serum-free medium without (*a, d, g*) or with 25 ng/ml of human recombinant PDGF (*b, e, h*) or 50 ng/ml of mouse natural NGF (*c, f, i*). Shown are phase-contrast photomicrographs (×200) of the living cells at 24 h.

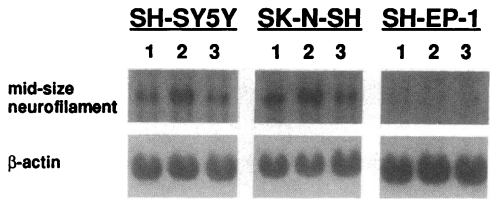

Figure 7. Effect of PDGF treatment on the expression of neurofilament mRNA in the human neuroblastoma cells. Total RNA extracted from the SH-SY5Y, SK-N-SH, and SH-EP-1 cultured in the serum-free medium without (lane *1*) or with PDGF-BB (25 ng/ml; lane *2*) or NGF (50 ng/ml; lane *3*) for 24 h were subjected to RNA blot analysis. The RNA blot filter was hybridized with ^{32}P-labeled human midsize neurofilament cDNA (32). The same filter was rehybridized with human β-actin cDNA probe.

cells in serum-free medium confirmed the neurotrophic effect of PDGF. The expression of a midsize neurofilament mRNA was not significantly enhanced by NGF, although the neurite outgrowth was enhanced by NGF as well as by PDGF. NGF might affect the posttranslational processing of the neurofilament. The absence of neurofilament mRNA in SH-EP-1 cells is consistent with the phenotype as described (24, 25).

Expression of PDGF A- and B-chains in human neuroblastoma cell lines. The mitogenic activity of PDGF on neuroblastoma cells appeared to be markedly less compared with the chemotactic activity (Figs. 3 and 4). We, therefore, examined the expression of PDGF which could rise the possibility of an autocrine or paracrine stimulation in the cell proliferation. Relatively high levels of PDGF A-chain transcripts were found in total RNA from SH-EP-1 by RNA blot analysis (Fig. 8 *A*). SK-N-SH and SH-SY5Y demonstrated barely detectable transcripts of PDGF A-chain. And a PDGF B-chain transcript was undetectable in any cell lines by RNA blot analysis (data not shown). In order to detect small quantities of transcripts, we used RT-PCR methods (Fig. 8 *B*). Southern blot analysis of RT-PCR products demonstrated the expression of PDGF A-chain not only in SH-EP-1 but also in SK-N-SH and SH-SY5Y. PDGF B-chain mRNA was also found in all three cell lines. No signal was observed in the RT-PCR products without

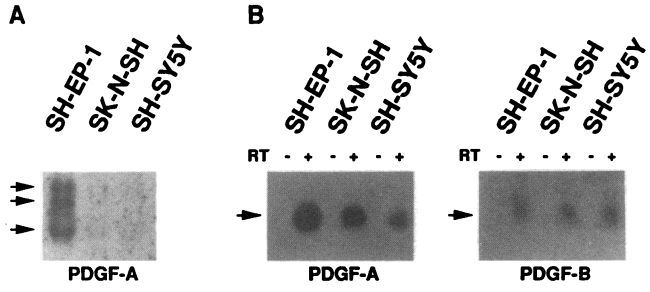

Figure 8. Expression of PDGF A- and B-chain transcripts in human neuroblastoma cell lines. (*A*) Total RNAs (20 μg per lane) extracted from the SH-EP-1, SK-N-SH, and SH-SY5Y were subjected to RNA blot analysis using ^{32}P-labeled human PDGF A-chain cDNA. (*B*) PCR products of reverse-transcribed RNA extracted from the cell lines were confirmed by Southern analysis using ^{32}P-labeled PDGF A- or B-chain cDNA. Reverse transcription (*RT*) was performed on each RNA sample with (+) or without (−) reverse transcriptase. Arrows indicate specific signals.

reverse transcriptase (Fig. 8 *B*). It confirmed that the observed signals were due to the presence of specific transcripts and not contaminating DNA.

Discussion

We demonstrate here the expression of PDGF receptor genes in a wide variety of human tumor cell lines originating from the neural crest. Not only Ewing's sarcoma, but also human primitive neuroectodermal tumor and neuroblastoma cell lines express α and/or β PDGF receptors. Moreover, the PDGF receptors on neuroblastoma cell lines functionally couple with chemotactic and neurotrophic signal transduction pathways.

The presence of specific PDGF isoforms and the relative expression of each PDGF receptor gene product are major determinants of the response of cells to PDGF. Receptors for PDGF have previously been found only on cells of mesenchymal and glial origin. Whereas mesenchymal cells are derived from the mesoderm, glia cells develop from neuroectoderm as well as do neurons and melanocytes (17, 41). Aberrant expression of the PDGF receptors was reported in one anaplastic thyroid carcinoma cell line and in primary human lung carcinomas (42, 43). The appearance of these transcripts is thought to be aberrant, because the normal epithelial counterpart of these cells that give rise to the tumor lack PDGF receptors and do not respond to PDGF. Even though the receptors are aberrantly expressed on human neuroblastoma cells, the cells possess the signal-transduction pathways which could couple with the activated PDGF receptors resulting in neuronal cell growth and migration. Thus, the receptor expression seems to be crucial in tumor development by conferring a selective growth advantage to the cancer cells (16).

The ability of simian sarcoma virus to induce fibrosarcoma in experimental animals argues that at least the overexpression of PDGF-BB may constitute one important event in malignant transformation of certain cell types (1, 2). The expression of specific receptors is necessary for the ability to transform in response to PDGF. Thus, the constitutive production of PDGF isoforms in tumor cells contributes to tumor cell growth through an autocrine mechanism (44–47). Actually, a variety of neural crest-derived tumor cell lines including neuroblastomas, gliomas, and melanomas have been reported to express specific transcripts of PDGF genes and to produce PDGF-like molecules (33, 48, 49). Coordinate expression of PDGF and its receptor genes has been reported in human malignant glioma cell lines (50). Recently, we have demonstrated the autocrine loop in several human sarcoma cell lines including glioma (16). Our findings that human neuroblastoma cell lines SK-N-SH, SH-SY5Y, and SH-EP-1 express PDGF A- and B-chain transcripts and functional PDGF receptors could raise the possibility that a PDGF autocrine loop would be involved in the transformation of human neuroblastoma. In that there is no direct evidence in the present studies for an autocrine mechanism, it is important to confirm whether the PDGF autocrine loop could contribute to the tumor cell growth.

In vivo, PDGF isoforms are produced by normal surrounding tissues or cells present in tumors, such as macrophages and vascular endothelial cells (2, 51, 52). Moreover, normal mammalian nervous tissue has been shown to express PDGF-AA and -BB (14, 15). Therefore, the PDGF receptors coupling with a mitogenic signaling pathways in human neuroblastoma

cells are beneficial for the tumor growth in part through a paracrine mechanism in nervous system.

In addition to autocrine or paracrine activation, other mechanisms such as receptor gene amplification, overexpression, or structural alteration are likely to play a role in the malignant process (4, 53, 54). The sizes of PDGF receptor transcripts on human neuroblastoma cell lines were the same as those of normal human fibroblasts, although the presence of point mutations is not excluded. Moreover, the levels of their expression were lower than in normal fibroblasts.

Molecular and cytogenetic studies of Ewing's sarcoma have produced important clues regarding the molecular pathogenesis of this tumor (20, 55). Although Ewing's sarcoma was originally thought to arise from the endothelial cell, recent evidence suggests that it is probably derived from primitive neural tissue. The expression of PDGF receptors in Ewing's sarcoma does not exclude the hypothesis of neuroectodermal origin, because all the neural crest-derived tumor cells examined here express the receptors. The expression of PDGF receptor genes in primitive neuroectodermal tumor cells as well as in Ewing's sarcoma would suggest that these receptors are expressed during an early stage of differentiation of neural crest in human embryogenesis (17, 41, 56, 57).

Cell migration is an important function for the brain development (18, 19). Neural crest cells undergo an extensive migration through the embryo during early development, and differentiate into many diverse cell types (17, 41). PDGF-AA is readily secreted and thus is ideally suited to serve as a chemoattractant (38). PDGF A chain mRNA is developmentally expressed at very high levels in both the peripheral and central nervous systems of the mouse (14). By embryonic day 15, higher levels of PDGF A mRNA expression have been demonstrated in spinal cord and in dorsal root ganglia than in brain. Sympathetic ganglia also express the transcript in embryonic animals (14). The chemotactic properties of PDGF for neuronal cells suggest that PDGF may serve an inductive role to organize nervous system (57).

Studies of PDGF in nervous tissue have principally focused upon its role in gliogenesis (10–13, 17) and in the pathogenesis of glioma (16, 50), because PDGF receptors have been thought to be found only in mesenchymal and glial cells. The present studies show that a wide range of neural crest-derived tumor cells express PDGF receptors, and that PDGF has neurotrophic effects on human neuroblastoma cells. The expression of two types of PDGF receptor genes is thought to be differentially regulated (30, 50, 57). It is less likely that both types of receptor genes located on distinct chromosomes are simultaneously and aberrantly expressed in a single tumor cell. These findings invite speculation that the PDGF system has important roles in neuronal cell migration, growth and differentiation in vivo and that PDGF isoforms possibly act as neurotrophic factors in early brain development. The most convincing evidence for this view has been derived from the recent studies by Smits et al. (58). They showed expression of functional β PDGF receptor in newborn rat brain neurons in vivo as well as in vitro. Our studies are the first demonstration of the expression of α and β PDGF receptor genes in human cells showing neurogenic phenotype. To gain further insights for the new action of PDGF as a neurotrophic factor in the human development (14, 15, 59), further studies are required on the developmental expression of the PDGF isoforms and their receptors on the normal human nervous systems.

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