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Selective Expression of mRNA Encoding Platelet-Derived Growth Factor B Chain Following Transfection of Foreign Genes Into Cell Lines Derived From Baby Hamster Kidney

Kjell S. Sakariassen, Jerry S. Powell, Elaine W. Raines, and Russell Ross

Departments of Pathology (K.S.S., E.W.R., R.R.) and Medicine (J.S.P.), University of Washington, Seattle, Washington 98195

The genes for platelet-derived growth factor (PDGF) A and PDGF B chains are expressed in a variety of biological situations. Active PDGF consists of two distinct but homologous polypeptide chains, PDGF A and PDGF B, which are found as heterodimers or homodimers. We report a novel situation in which there is selective expression of mRNA encoding PDGF B in cell lines derived from baby hamster kidney (BHK) following transfection with various gene/cDNA constructs and following growth selection with methotrexate. The process of transfection itself, and not expression of the proteins encoded by the transfected genes/cDNAs (hormones, enzymes, and structural proteins), induces expression of PDGF B. No PDGF B mRNA is detectable in control cell lines. Low levels of mRNA encoding PDGF A are constitutively present and are not changed by transfection and/or growth selection. PDGF-like activity is present in the medium whenever PDGF B mRNA is detected. The composition of the secreted PDGF dimer cannot be established from our data, but quantitative analysis of mRNA suggests that the PDGF is a B-B dimer. However, the data show that transcription of the PDGF A and PDGF B genes in BHK cells is regulated independently, similar to that reported for some human tumor cells. Furthermore, the selective expression of PDGF B in response to the introduction of foreign genes and to growth selection may be an important aspect of the reaction of these cells to nonoptimal growth conditions, allowing survival and growth of the cells that express PDGF B.

Key words: PDGF A, PDGF B, BHK cells, gene transfection, growth selection, foreign genes

It has been suggested that platelet-derived growth factor (PDGF) may be involved in fibroproliferative processes such as wound repair, growth and development, atherosclerosis, neoplasia, and myelofibrosis [1]. The responses of cells to the

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PDGF molecule during these events could include cell proliferation [2,3], chemotaxis [4,5], and vasoconstriction [6]. The regulatory mechanisms that control the expression of the two genes encoding PDGF, PDGF A on chromosome 7 [7] and PDGF B on chromosome 22 [8], are not known. The homology between the two mature polypeptide chains is about 60% [7], and biological activity has been demonstrated for heterodimers of A-B and homodimers of either A-A or B-B [9–13].

While conducting experiments on Syrian baby hamster kidney (BHK) cells [14], we were surprised to find induction of significant levels of PDGF B mRNA and concomitant secretion of PDGF-like activity following transfection with various gene/cDNA constructs. A similar response is seen after growth selection with methotrexate. The constitutive expression of PDGF A mRNA remains low and unstimulated. These observations suggest that PDGF B may have a role, at least in BHK cells, in the cellular response to nonoptimal culture conditions.

MATERIALS AND METHODS

Cell Cultures

The mammalian cell line BHK 21-13 (American Type Culture Collection, Rockville, MD), was maintained in Dulbecco's modified essential medium (DMEM, Gibco, New York, NY) containing 10% fetal calf serum (FCS). Cells were passaged and, when 50–70% confluent, were transfected by the calcium phosphate method [15] or the dextran sulfate method [16].

For transient expression of BHK cells in a 100-mm culture dish (Falcon™, Oxford, CA), a total of 20 μg DNA was used: 10 μg of plasmid and 10 μg of carrier salmon sperm DNA. After 48 h, the supernatant was collected, centrifuged at 400g for 10 min to remove cells and debris, and frozen at −20°C.

For stable transfection, the transfection procedure was modified so that the 10 μg of plasmid included a selectable marker gene either as part of the plasmid or on a separate coprecipitated plasmid. After incubation for 18–24 h, varying concentrations of methotrexate (10 nM to 1 μM) or 500 μg/ml neomycin were added to the cultures. Following incubation for several more days, viable colonies resistant to the selection were isolated, passaged, and screened for secretion into the supernatant of the protein product from the transfected gene. Some of the cell lines selected with methotrexate were passaged several times into increasing concentrations of methotrexate (10 nM to 10 μM) in order to amplify the transfected gene.

Conditioned media were collected from the cell cultures prior to confluence. Where possible, the cultures were washed twice with serum-free media and cultured in serum-free media for the time specified by the experiment. Transient transfection experiments were performed in DMEM containing 5% FCS.

U-20S cells, a human osteogenic sarcoma cell line (American Type Culture Collection, Rockville, MD), were grown in 150 × 25-mm tissue culture dishes (Falcon™, Oxford, CA) at 37°C in DMEM supplemented with 10% FCS.

Cell counts were performed on the cells used for collection of conditioned media or on parallel plates when cells were harvested for RNA extraction. Adherent BHK cells were suspended in a 1% Versene solution and diluted in a trypan-blue solution, and dye-excluding cells were enumerated by hemocytometer.

Plasmid Constructions and DNA Sequencing

Plasmid constructs were prepared using standard procedures [17] and sequenced by the dideoxy chain termination method [18]. The plasmid expression vector used

for the erythropoietin gene, coagulation factor X cDNA, and the granulocyte macrophage colony-stimulating factor (GM-CSF) gene was derived from a previously described plasmid [19] and contained the simian virus 40 (SV-40) enhancer sequences and origin of replication as well as the adenovirus-2 major late promoter and tripartite leader sequences. The plasmid expression vector (kindly provided by Dr. R. Palmiter, University of Washington, Seattle) used for factor IX cDNA has been described [20]. Isolations of the human genes or cDNAs have been published: erythropoietin gene [21], GM-CSF [22], factor IX [23], factor × (Drs. B. Schach and E.W. Davie, unpublished), and samples were kindly provided by Drs. K. Kaushansky, S. Yoshitake, B Schach, and E.W. Davie, University of Washington, Seattle. Conditioned media from BHK cells stably transfected with a human tissue plasminogen activator cDNA were provided by Dr. I. Molihill of ZymoGenetics, Inc., Seattle.

Northern Blot Analysis

Northern blot analysis of mRNAs encoding PDGF A and PDGF B was performed with ^{32}P-nick-translated cDNA probes following transfer of RNA from agarose/formaldehyde gels to nylon membranes (Gene Screen Plus, NEN Research Products, Boston) [17,24]. Total RNA was extracted from cells lysed by 5.0 M guanidine isothiocyanate and subsequently purified by selective lithium chloride precipitation [25]; 20 μg per lane of purified total cellular RNA was electrophoresed and blotted. The hybridization buffer consisted of 250 mM Na_2HPO_4, 250 mM NaCl, 1 mM EDTA, 7% sodium dodecyl sulfate (SDS), 47% formamide, 10% polyethylene glycol (PEG) (6000), and 250 μg/ml double-stranded salmon sperm DNA. The membranes were hybridized with either a 1,307 base-pair (bp) PDGF A cDNA probe [7] or a 704 bp PDGF B cDNA probe [12] nick-translated with ^{32}P (ICN Radiochemicals, Irvine, CA) to specific activities of about 2×10^9 cpm/μg cDNA. Autoradiography was performed with XAR 5 film (Kodak, Rochester, NY) at −70°C. Prokaryotic and eukaryotic ribosomal RNA (Pharmacia Inc., Piscataway, NJ) were included as standards.

Dot-Blot Analysis of mRNAs Encoding PDGF A and PDGF B

Dot-blot analysis of mRNA encoding PDGF A and PDGF B was performed by means of autoradiography of dot blots of purified total cellular RNA [25] on nylon membranes (Gene Screen Plus, NEN Research Products, Boston) hybridized with appropriate ^{32}P-nick-translated cDNA probes [24]. Total cellular RNA was spotted onto nylon membranes with a 96-well Hybri-Dot™ unit (BRL, Gaithersburg, MD). Amounts of 4 and 2 μg of cellular RNA and amounts of 10, 5, and 1 pg of the respective denatured cDNA probes were bound to the membranes and hybridized with ^{32}P-nick-translated cDNA probes coding for either PDGF A or PDGF B as described for Northern blot analysis.

Quantitation of PDGF-Like Activity

PDGF-like activity was measured by radioreceptor assay (RRA), utilizing subconfluent cultures of human adult fibroblasts or juvenile human skin fibroblasts (American Type Culture Collection, Rockville, MD) as recently described in detail by Bowen-Pope and Ross [26]. The lower limit of detection is 40 pg.

RESULTS

Northern Blot Analyses of PDGF A and PDGF B in BHK Cells

Total cellular mRNA was extracted from selected BHK cell lines and the U-20S cell line and analyzed by Northern blot techniques for mRNA encoding PDGF A and PDGF B (Fig. 1). None of the tested BHK cell lines had significant levels of PDGF A mRNA detectable by these Northern blot analyses. However, autoradiography for longer exposure times revealed faint bands corresponding to PDGF A mRNAs. Message sizes for PDGF A of 2.8, 2.3, and 1.7 kb are observed in the U-20S cells. However, all cell lines, with the exception of the control BHK cell lines, have PDGF B mRNA, characteristically of only one message size, 3.4 kb.

Dot-Blot Analysis of mRNAs Encoding PDGF A and PDGF B in BHK Cells

Constitutive expression of PDGF A, as detected by dot-blot analyses, occurs in all BHK cell lines, independently of transfection and/or growth selection (Table I). No PDGF B is detected in normal growing BHK cells. However, transfection with a variety of gene/cDNA constructs and/or growth selection with methotrexate or neomycin induce selective expression of PDGF B. Combination of transfection and growth selection, transfection alone, or growth selection alone causes selective expression of PDGF B. U-20S cells, which are known to have mRNAs encoding

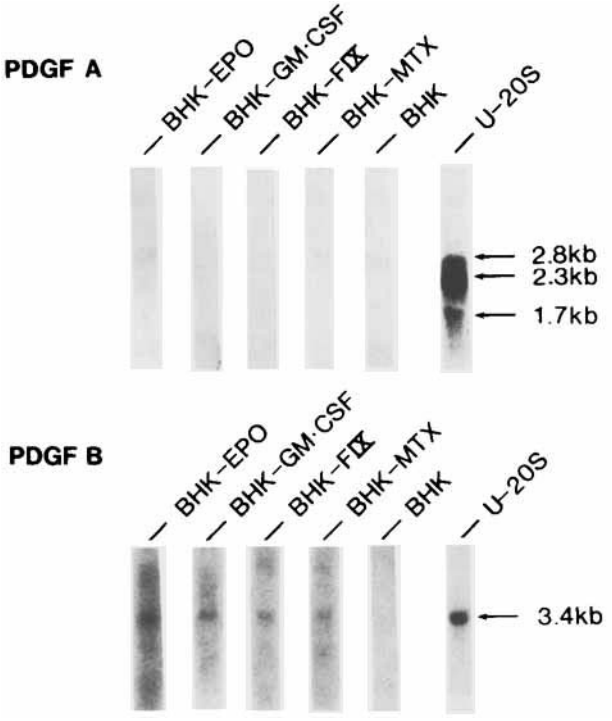

Fig. 1. Northern blot analysis of mRNAs encoding **PDGF A** and **PDGF B**. Twenty micrograms of total cellular RNA was loaded per well. No PDGF A could be detected in any of the BHK cell lines by this method. RNA from U-20S cells had three messages with approximate sizes of 2.8, 2.3, and 1.7 kb. PDGF B mRNA of about 3.4 kb was detected in all cell lines with the exception of the control BHK cell line.

TABLE I. Effect of Gene/cDNA Transfection and Growth Selection on Production of PDGF-Like Activity (RRA) by BHK Cells

	PDGF A (mRNA)[a]	PDGF B (mRNA)[a]	PDGF-like activity (pg/10^6 cells)
No transfection or selection			
U-20 S	+	+	1,004
BHK cell lines (5)	+	−	Not detected
Human skin fibroblasts	−	−	Not detected
Transfection followed by selection with methotrexate (0.05–5 μM)			
Erythropoietin gene	+	+	313
GM-CSF gene	+	+	416
Factor IX cDNA	+	+	123
Factor X cDNA	+	+	252
Gap junction protein cDNA	+	+	Not determined
Anti-sense gap junct.Prot. cDNA	+	+	Not determined
Transfection followed by selection with neomycin (500 μg/mL)			
Tissue PA cDNA	+	+	200
Transfection without selection			
Erythropoietin gene	+	+	118
GM-CSF gene	+	+	52

[a]Dot-blot analysis

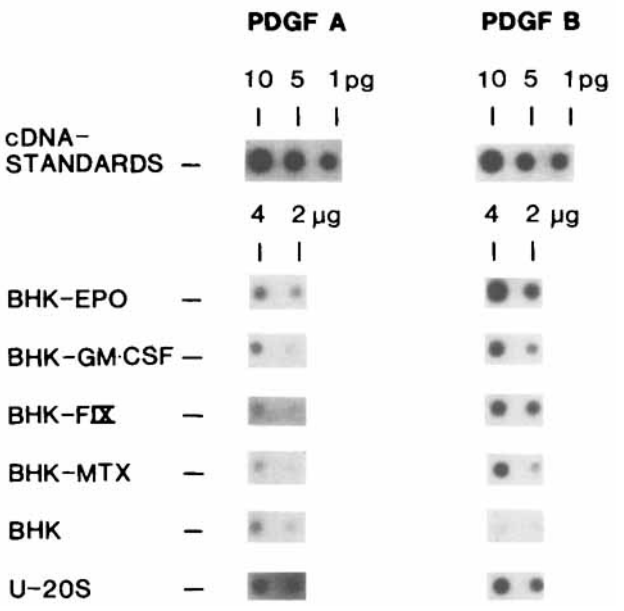

Fig. 2. Dot-blot analysis for mRNA encoding **PDGF A** and **PDGF B.** Four and 2 μg of total cellular RNA from U-20S cells and the various BHK cell lines were spotted. Ten, 5, and 1 pg of denatured cDNA coding for PDGF A and PDGF B were included.

PDGF A and PDGF B [7], were included as controls. No PDGF mRNAs were detected in cultured human skin fibroblasts. Figure 2 shows some representative dot blots of total cellular RNA hybridized with the PDGF A cDNA probe and with the PDGF B cDNA probe.

Coordinate Induction of Exogenous PDGF-Like Activity

Exogenous PDGF-like activity was measured by RRA in media conditioned by a number of BHK cell lines and compared with the U-20S cell line (Table I). No PDGF-like activity is detected in five normal cultured cell lines. However, significant amounts of PDGF-like activity ranging from 123 to 416 pg/10^6 cells are present following transfection with various gene/cDNA constructs and growth selection with methotrexate and neomycin. Transfection without growth selection results in PDGF-like activity, but lower amounts than those detected after a combination of transfection and growth selection. Thus, growth selection with methotrexate alone without any preceding transfection results in PDGF levels, which increase in parallel with increasing concentrations of methotrexate (Fig. 3).

DISCUSSION

BHK cell lines are descendents of cells derived from kidney cells passaged in vitro [14]. These cell lines have been used extensively for a variety of studies, including transfection and expression of cloned genes. BHK cells have been characterized as fibroblastoid with some epithelial phenotypic traits [14]. Our somewhat serendipitous observations that stably transfected BHK cell lines selectively express mRNA encoding PDGF B and that PDGF-like activity is detected in the corresponding media prompted us to investigate this phenomenon in more detail.

In all cell lines in which PDGF B mRNA was detected by Northern and/or dot-blot hybridization, PDGF-like activity was also observed in the respective media. PDGF-like activity was measured by a RRA that was specific for PDGF [26]. However, this assay is insufficient to determine the composition of PDGF, since heterodimers of A and B chain and homodimers of either chain compete for binding with radiolabeled PDGF, a heterodimer purified from human platelets [27,28]. The induction of relatively high levels of PDGF B mRNA suggests that the PDGF detected

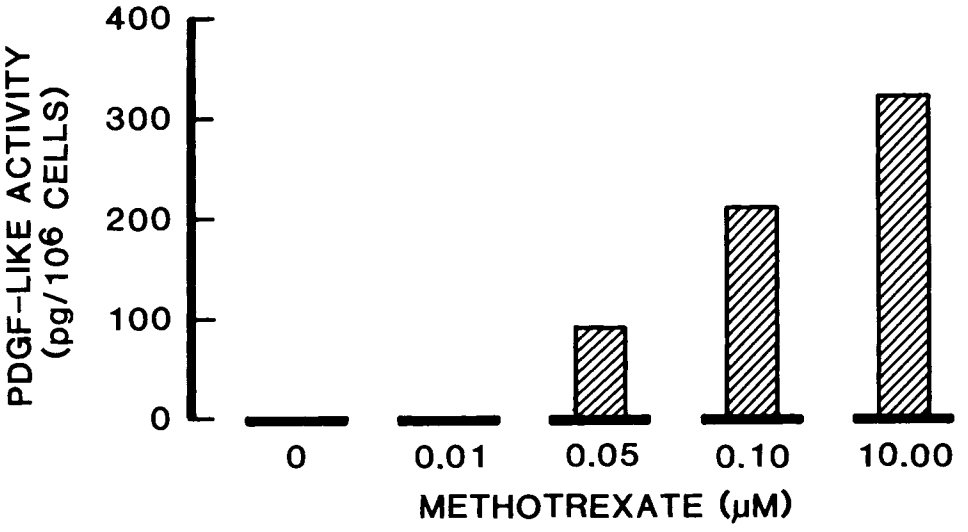

Fig. 3. Effect of methotrexate concentration (0, 0.01, 0.05, 0.10, and 10.00 μM) on exogenous PDGF-like activity in media conditioned for 24 h by BHK cells. PDGF was measured by radioreceptor assay.

in the media is a homodimer of the B-chain. A homodimer of the B chain was recently found in porcine platelets [10]. The size of the PDGF B mRNA of 3.4 kb is similar to the sizes reported for PDGF B mRNA from a variety of other cell types [7,29,30]. No other crosshybridizing material was observed at high stringency, including mRNAs for the A-chain, which has an approximately 60% homology with the B-chain [7]. Thus, induction of PDGF B mRNA occurred whenever PDGF was detected in the medium. The specificity of the expression of PDGF B mRNA was further tested by dot-blot and Northern blot analyses using cDNA probes for epidermal growth factor and transforming growth factor-alpha. No hybridization signal was detected for these growth factors (data not shown). In these experiments PDGF B mRNA was not detected in any of the control BHK cell lines.

The cellular response that results in selective expression of PDGF B mRNA appears to involve more basic mechanisms than simply transfection of BHK cells with particular plasmid or gene/cDNA constructs. This effect was observed after expression of several different genes or cDNAs encoding hormones, enzymes, and structural proteins. Perhaps more importantly, the effect was observed in transfections in which various important components of the expression plasmids were changed, demonstrating that PDGF B mRNA levels were not a function of a particular promoter or enhancer used in the expression constructs. Similarly, growth selection of control BHK cell lines with antibiotics and transfection without growth selection were sufficient to induce PDGF B mRNA. However, the combination of transfection and growth selection resulted in additive effects on the expression levels of exogenous PDGF-like activity.

Our data extend previous findings of independent expression of PDGF A and PDGF B mRNAs in other cell lines [7,31–33]. In the situations described in this paper, BHK cell lines selectively express PDGF B mRNA with no detectable increase in the constitutive low levels of expressed PDGF A mRNA. In addition to confirmation of independent expression of PDGF A and PDGF B, the lack of coordinate expression of PDGF A and PDGF B suggests that these gene products may have independent functions in their roles in fibroproliferative responses.

A common denominator in the varied situations in which we have observed selective PDGF B mRNA expression may be a requirement for PDGF B protein formation as part of the response of BHK cells to transfection with foreign genes/cDNAs or response to the selective pressure of methotrexate. Expression of PDGF B may be a part of the response of BHK cells to these situations and may confer a growth advantage to cells that express PDGF B. Of interest in this regard, the so-called "stress-induced genes," heat-shock genes [34] and ubiquitin genes [35–37], both appear in response to nonoptimal growth conditions and as part of selective degradation of certain intracellular proteins during growth [38]. The intriguing possibility arises that PDGF B mRNA may be selectively expressed in similar situations involving the response of certain cell types. Whether transcription of the PDGF B gene is linked to the stress-induced genes encoding heat-shock proteins and ubiquitin proteins remains to be determined.

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