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EXPRESSION OF BIOLOGICALLY ACTIVE HUMAN PLATELET-DERIVED GROWTH FACTOR (PDGF) B-RECEPTOR IN XENOPUS LAEVIS OOCYTES

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SUMMARY Platelet-derived growth factor (PDGF) consists of three different isoforms, PDGF-AA, PDGF-AB and PDGF-BB, which bind to at least two types of receptors: the B-receptor, to which only PDGF-BB binds, and the A/B receptor, to which all three isoforms bind. Microinjection of synthetic mRNA in Xenopus laevis oocytes was used to obtain cell-surface expression of the human PDGF B-receptor. The production of receptor molecules of correct size (190 kd) was demonstrated by specific immunoprecipitation; the binding properties of the membrane- associated PDGF B-receptor were investigated with highly purified recombinant [^{125}I]labeled human PDGF-BB and -AA. Unlike Swiss mouse 3T3 cells, which possess both B- and A/B-receptors and, therefore, bind both isoforms with high affinity, the mRNA-injected oocytes bound only the BB isoform. Mock-injected oocytes showed no specific binding.

Platelet-derived growth factor (PDGF) is a potent mitogen and chemoattractant for various cells of mesenchymal origin in culture (see [1] for review). PDGF consists of dimers of two distinct, but highly similar polypeptide chains termed A-chain and B-chain [2,3]. While the PDGF-AB heterodimer is the predominant isoform present in human platelets, PDGF-AA homodimers have been isolated from U2-OS osteosarcoma cell-conditioned media [4] and PDGF-BB homodimers have been isolated from porcine platelets [5].

Earlier studies involving platelet PDGF have presumed the existence of only one type of PDGF receptor. Using affinity crosslinking methodology this receptor has been described as a 160-185 kDa membrane glycoprotein [6,7] that has an extracellular

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Abbreviations: PDGF, platelet-derived growth factor; RNA, ribonucleic acid; mRNA, messenger RNA; cDNA, complementary deoxyribonucleic acid; bp, base pair.

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ligand binding domain [8], and an intracellular split tyrosine kinase domain [9-11].

Recently, however, evidence has been obtained that there may exist at least two classes of PDGF receptors in human dermal fibroblasts. Binding experiments suggested that one class (termed the "B-receptor") binds only PDGF-BB and the other (termed "A/B-receptor") binds all three isoforms of PDGF (AA, AB and BB) [12,13]. Furthermore, BHK cells transfected with the cDNA for one type of human PDGF receptor bind only PDGF-BB with high affinity [14], which is consistent with the predicted "B-receptor" class.

Microinjection of mRNA into Xenopus laevis oocytes is a useful tool for the expression of a wide variety of cytoplasmic and cell surface proteins. Various types of receptor molecules including the acetylcholine receptor [15], the sodium channel [16], the insulin-like growth factor II receptor [17], the human nerve growth factor receptor [18] and the murine interferon-γ receptor [19] have been expressed and characterized in this system.

In the present study we demonstrate the expression of human PDGF B-receptor in Xenopus laevis oocytes. This receptor is functional and, in agreement with previous data [14], binds only PDGF-BB with high affinity.

MATERIALS AND METHODS

Synthesis of receptor mRNA Capped 3.8 kb transcripts, containing the complete coding region of the PDGF B-receptor mRNA, were generated in a coupled transcription-capping reaction from plasmid pT-PDGF-BR(Fig.1) linearized with HindIII. A reaction mixture containing 40 mM Tris pH 7.5, 6 mM MgCl, 2 mM spermidine, 10 mM NaCl, 10 mM dithiotreitol, 1 unit/µl RNasin (Pharmacia), 500 µM UTP, CTP, ATP, 25 µM GTP, 250 µM m7G(5')ppp(5')G (Pharmacia), 20-50 µg/ml linearized DNA template and 1 unit T7 RNA polymerase (Pharmacia) was incubated at 37°C for 10 min. The GTP concentration was then raised to 500 µM and incubation was continued for 1 h. RNAs were freed from DNA by RNase-free DNase treatment and subsequent phenol/chloroform (1:1) extraction (twice) and ethanol precipitation. Purified RNAs were then polyadenylated using poly(A)polymerase from E.coli (BRL) in 5 mM Tris pH 8.0, 100 µM ATP, 10 mM MgCl, 250 mM NaCl, 1 mM MnCl, 1 unit/µl RNasin and 130 units of poly(A) polymerase at 37°C for 30 min. The reaction mixture was extracted twice with phenol/chloroform (1:1) and ethanol precipitated. Finally, the transcripts were resuspended at 500 ng/µl in sterile H_2O for oocyte injection.

Microinjection of PDGF B-receptor mRNA into Xenopus oocytes, labeling and immunoprecipitation of B-receptor protein Healthy Xenopus laevis females were anaesthesized with 2% ethyl m-aminobenzoate (MS-222, Sandoz) and pieces of ovary were removed surgically. Individual oocytes were isolated and follicle cells removed by incubation in 2 mg/ml collagenase (type IV, Sigma) in

modified Barth's solution (MBS) containing antibiotics (pen-strepto, 10 mg/ml) for 12 h at room temperature. After collagenase treatment and extensive washing, stage 5 oocytes [20] were injected with 50 nl of a solution containing 20 ng PDGF B-receptor mRNA.

Following injection, the oocytes were incubated in MBS supplemented with 0.5 mCi/ml [^{35}S]methionine (Amersham) for 24 h at room temperature. Then, the oocytes were homogenized in 20 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1 mM phenyl-methyl-sulfonyl fluoride (PMSF), 0.2% Triton X-100 and the radiolabeled receptor proteins immunoprecipitated with the monoclonal anti-human PDGF B-receptor antibody PR 7212 [21]. Immunoprecipitated material was separated on 12% SDS-polyacrylamide gels [22] and visualized by autoradiography.

Binding of [^{125}I]PDGF to microinjected Xenopus oocytes Binding experiments were performed essentially as described previously for binding to cells [23]. Briefly, 40 h after the microinjection, groups of 10 oocytes were placed in 500 µl of binding medium (Ham's F-12 medium plus 25 mM Hepes (pH 7.4) and 0.25% BSA) containing 2 ng/ml of either [^{125}I]PDGF-BB or [^{125}I]PDGF-AA and incubated for 1 h at room temperature on an oscillating table. The oocytes were then washed several times with 1 ml of ice-cold binding rinse (PBS, pH 7.4, containing 1 mM $CaCl_2$ and 0.1% BSA), transferred into 1.5 ml polypropylene tubes, and the radioactivity was measured. Specific binding was determined as the total radioactivity minus the non-specific binding obtained in the presence of an excess of the corresponding unlabelled ligand (1 µg/ml).

Materials PDGF-BB and PDGF-AA were synthesized in a S.cerevisae expression system [24] and purified to homogeneity (Eggimann et al., in preparation). Protein concentration was determined by quantitative amino acid analysis. Purified PDGF-BB was radioiodinated using the Bolton-Hunter reagent (Du Pont New England Nuclear). PDGF-AA was labelled with Iodo Gen (Pierce, Inc. Rockford) according to the manufacturers instruction. Routine chemicals were from Fluka, and of the highest purity available. The anti-human PDGF B-receptor monoclonal antibody PR 7212 was kindly provided by Dr. C. Hart, University of Washington, Seattle, USA. The PDGF B-receptor cDNA was obtained from Dr. M. Murray, ZymoGenetics Inc., Seattle, USA.

RESULTS AND DISCUSSION

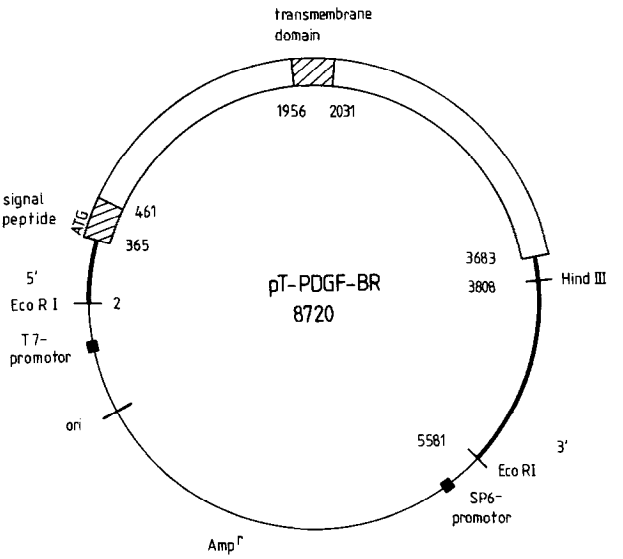
Recent studies show that there are two populations of PDGF receptors, the B- and the A/B-receptor which can be distinguished by their ligand binding specificity [12,13,25].

In order to obtain human PDGF-B receptor mRNA, a 5.5 kb EcoRI fragment containing the complete PDGF B-receptor cDNA [14] was inserted into plasmid pSPT18 (Pharmacia) downstream of the T7 promoter to generate plasmid pT-PDGF-BR depicted in Figure 1. The full-length cDNA consists of 356 bp of 5'-non coding sequences, a 3318 bp coding region and 1896 bp 3' untranslated region. Capped and polyadenylated 3.8 kb transcripts were synthesized *in vitro* from HindIII linearised pT-PDGF-BR and purified as described in Materials and Methods.

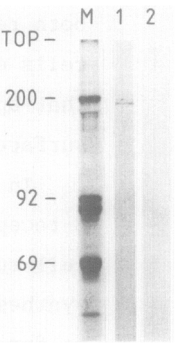
Batches of 100 oocytes were microinjected with 20 ng/oocyte of PDGF-receptor mRNA or, as a control, with water. To monitor the

expression of the human receptor protein groups of microinjected and control oocytes were incubated in the presence of [^{35}S]methionine for 24 h. Following homogenization newly synthesized proteins were immunoprecipitated with the monoclonal antibody PR 7212 which recognizes an extra-cellular epitope of the human PDGF B-receptor [21]. The immunoprecipitated proteins were separated by SDS-gelelectrophoresis and visualized by autoradiography. As shown in Figure 2, two proteins with Mr 200 kd and 190 kd could be immunoprecipitated by PR 7212 from mRNA-injected oocytes, but not from control oocytes.

The size heterogeneity observed is most likely due to differences in glycosylation of the PDGF receptor. Hart et al. [21] described proteins of similar size produced by metabolically labelled human dermal fibroblast cells (SK5), a 180k protein which appears to represent the functionally mature form of the PDGF B-receptor and a smaller form which appears to be a precursor of the mature receptor.



(1)



(2)

Fig.1 Construct for oocyte expression. The vector pT-PDGF-BR consists a 5.5 kb human PDGF B-receptor cDNA subcloned into the in vitro transcription vector pSPT18 (Pharmacia). The full length cDNA consists of 356 bp of 5'-noncoding sequence, a 3318 bp coding region and 1896 bp of 3'-untranslated sequence.

Fig.2 Immunoprecipitation of human PDGF B-receptor from Xenopus laevis oocytes microinjected with receptor RNA. Oocytes were labelled, homogenized and the labeled proteins immunoprecipitated with a monoclonal antibody to the PDGF B-receptor as described in "Materials and Methods". Lanes: M, [^{14}C]methylated proteins (Amersham); 1, immunoprecipitated lysate from PDGF B-receptor RNA-injected oocytes; 2, immunoprecipitated lysate from mock-injected control oocytes.

Table 1

Binding of [^{125}I]PDGF-BB and [^{125}I]PDGF-AA to Xenopus laevis oocytes injected with PDGF B-receptor mRNA

cell type	Binding corrected for non-specific binding	
	[^{125}I]PDGF-BB	[^{125}I]PDGF-AA
	cpm	
Injected oocytes	421	20
Control oocytes	0	2
Swiss 3T3 cells	761	213

Oocytes injected with PDGF B-receptor mRNA, mock-injected control oocytes and Swiss 3T3 cells were incubated with [^{125}I]PDGF-BB and with or without unlabelled competitor PDGF-BB. Binding assays were also performed with [^{125}I]PDGF-AA and unlabelled PDGF-AA as competitor. The values for a typical experiment are shown (the data are expressed as cpm/10 oocytes or cpm/5,5•10^4 cells).

To study the biological activity of the oocyte-produced PDGF receptor, binding assays were performed with highly purified recombinant [^{125}I]labeled PDGF-AA and -BB. The results of a typical experiment are shown in Table 1. Oocytes microinjected with PDGF B-receptor mRNA bound specifically PDGF-BB, but not PDGF-AA. Control oocytes showed only non-specific binding. By contrast, Swiss 3T3 cells bound both homodimers, in agreement with the existence of both receptor types on these cells [25]. Assuming that Swiss 3T3 cells express about 10^5 PDGF B-receptors per cell [25] we estimate that approximately 2.5×10^8 B-receptors were expressed on the cell surface of an individual mRNA-injected oocyte.

In conclusion, our results clearly show that the human PDGF B-receptor can be expressed as a functional protein on the cell-surface of Xenopus laevis oocytes injected with in vitro synthesized mRNA. To the best of our knowledge, this represents the so far largest receptor-ligand complex analyzed with the Xenopus oocyte system. In addition, the ligand binding specificity in this heterologous system supports the concept of the existence of at least two distinct classes of PDGF receptors.

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