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Organization and Chromosomal Localization of the Human Platelet-Derived Endothelial Cell Growth Factor Gene

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Human platelet-derived endothelial cell growth factor (hPD-ECGF) is a novel angiogenic factor which stimulates endothelial cell growth in vitro and promotes angiogenesis in vivo. We report here the cloning and sequencing of the gene for hPD-ECGF and its flanking regions. This gene is composed of 10 exons dispersed over a 4.3-kb region. Its promoter lacks a TATA box and a CCAAT box, structures characteristic of eukaryotic promoters. Instead, six copies of potential Sp1-binding sites (GGGCGG or CCGCCC) were clustered just upstream of the transcription start sites. Southern blot analysis using genomic DNAs from several vertebrates suggested that the gene for PD-ECGF is conserved phylogenetically among vertebrates. The gene for hPD-ECGF was localized to chromosome 22 by analysis of a panel of human-rodent somatic cell hybrid lines.

Growth of vascular endothelial cells is important in physiological and pathological conditions, e.g., in angiogenesis, which is a very important process during embryonal development, wound healing, and growth of malignant tumors (24). In addition, the integrity of the arterial endothelial cell layer is thought to be crucial in the prevention of atherosclerosis (36). Furthermore, endothelial cells were recently shown to affect the tonus of blood vessels (14, 51).

Human platelet-derived endothelial cell growth factor (hPD-ECGF) is a recently discovered endothelial cell growth factor with angiogenic activity in vivo which was initially purified from human platelets (31). hPD-ECGF seems to be the only endothelial cell mitogen in platelets (30) and is found also in placental tissue and cultured fibroblasts (44, 45). Analysis of hPD-ECGF and its cDNA revealed that the protein is synthesized as a 482-amino-acid precursor which undergoes limited proteolytic processing in the N terminus (19). Interestingly, the hPD-ECGF precursor was found not to have any signal sequence, in analogy with two other endothelial cell mitogens, acidic and basic fibroblast growth factors.

To investigate the mechanism of regulation of hPD-ECGF expression, structural analysis of the gene for hPD-ECGF is important. In this report, we describe the isolation and characterization of the gene for hPD-ECGF. The nucleotide sequence, exon-intron boundaries, and transcription start sites were determined. We also demonstrate promoter activity of the 5′-flanking region of the gene and discuss the characteristic structures of this region. Furthermore, we report the chromosomal localization of the gene for hPD-ECGF.

MATERIALS AND METHODS

Cloning of the gene for hPD-ECGF. Genomic DNA was isolated from human term placenta, subjected to partial *Sau*3AI digestion, ligated into *Bam*HI-digested λEMBL3 arms, and plated on bacterial strain LE392. A total of 500,000 recombinant plaques were screened, and two positive clones that hybridized to PD-ECGF cDNA clone pPL8 (19) were identified.

Sequencing of the gene for hPD-ECGF. A 6.0-kb *Kpn*I-*Sph*I fragment (see Fig. 1) was subcloned into pBluescript (Stratagene) or M13mp18. Sequential deletants were made by using exonuclease III and mung bean nuclease. Single-stranded DNA templates were isolated and sequenced by use of the M13 universal primer. Sequencing was performed with modified T7 polymerase (Sequenase; USB) in dideoxy sequencing reaction mixtures containing [α-^{35}S]dATP (Amersham). Samples were analyzed on a buffer gradient 6% polyacrylamide gel containing 7 M urea (3). Sequences difficult to read were determined by sequencing with deaza-dGTP or dITP with or without single-strand binding protein (United States Biochemicals). Generally, two or three sequencing runs were analyzed for each segment of each DNA strand and both DNA strands were sequenced.

Primer extention analysis. A synthetic oligonucleotide, 5′-TCGCCCGCTTGCTCCGGATCCCAGCCCAGGTAC CC-3′, complementary to nucleotides 59 to 93 (see Fig. 2) was labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. Fifteen micrograms of human placenta poly(A)$^+$ RNA was mixed with 300 pg of the primer in a volume of 5 μl {50% formamide, 0.4 M NaCl, 40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] [pH 6.5], 1 mM EDTA}, incubated at 68°C for 5 min, and annealed at 50 or 55°C for 12 h. After removal of formamide by ethanol precipitation, the pellet was dissolved in 25 μl of a solution containing 50 mM Tris-HCl (pH 8.3); 40 mM KCl; 6 mM $MgCl_2$; 1 mM dithiothreitol; 55 μM each dATP, dGTP, dCTP, and dTTP; 120 U of RNasin; and 400 U of murine leukemia virus reverse transcriptase (BRL) and incubated at 37°C for 1 h. After digestion of the remaining RNA by RNase A, the extended product was extracted with phenol-chloroform and precipitated with ethanol. To localize precisely the transcription start site with respect to the genomic sequence, samples of primer extension reactions were analyzed on a buffer gradient 6% polyacrylamide DNA sequencing gel containing 7 M urea in parallel with sequencing reactions primed with the same end-labeled primer.

CAT assay. We constructed pScat from pSV2cat (16) by

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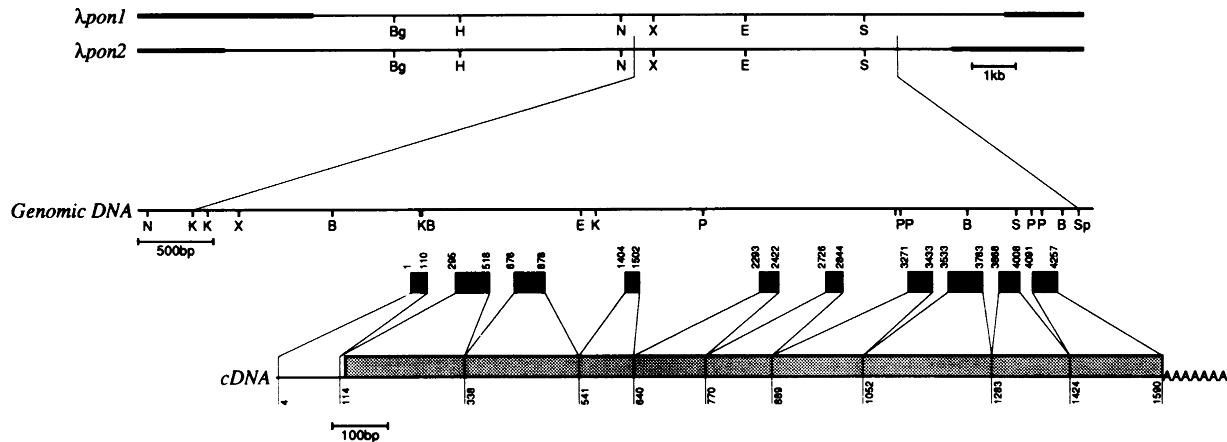

FIG. 1. Organization of the gene for hPD-ECGF. Restriction maps of two positive clones, λpon1 and λpon2, are shown. Thick lines indicate the bacteriophage arms, and thin lines indicate cloned inserts. A detailed restriction map of the 6-kb genomic fragment which contains the gene for hPD-ECGF is shown below. The *Kpn*I-*Sph*I fragment was sequenced. The positions of exons are shown in black boxes with the nucleotide numbers of the borders. See Fig. 2 for numbering of nucleotides. The positions of exon-intron junctions in hPD-ECGF cDNA (pPL8) are also indicated. The numbering of nucleotides is based on a previous report (19). The shaded box represents the open reading frame. Abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, NotI; P, *Pst*I; S, *Sal*I; Sp, *Sph*I; X, *Xho*I.

replacing the replication origin with that of pUC plasmids (52) to increase the copy number of the plasmid. pS0cat was made by removing the simian virus 40 promoter region from pScat. pSPDcat was made by inserting the 1.4-kb *Kpn*I-*Kpn*I fragment (see Fig. 4) in place of the simian virus 40 promoter of pScat. Ten micrograms of each plasmid was transfected by the calcium phosphate precipitation method into hTH7, a human thyroid cancer cell line which expresses hPD-ECGF (44). After 48 h, the cells were harvested and lysed and the chloramphenicol acetyltransferase (CAT) activity was checked by using D-threo-[dichloroacetyl-1-^{14}C] chloramphenicol (Amersham) as previously described (16). The reaction solutions were separated by thin-layer chromatography (Merck Art. 5715) and exposed on Kodak X-Omat AR film for 14 days.

Southern blot hybridization of DNAs from different species. High-molecular-weight genomic DNAs from human placenta, mouse liver, quail liver, frog (*Xenopus laevis*) liver, and crucian carp liver were prepared. Ten micrograms of each was digested with *Hin*dIII or *Xba*I, separated on a 0.7% agarose gel, and transferred to a Hybond-N nylon filter membrane (Amersham). The blots were hybridized with a ^{32}P-labeled DNA probe synthesized by random primer extension (12) in 5× SSPE (1× SSPE is 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA [pH 7.4])–0.1% Ficoll–0.1% polyvinylpyrrolidone–0.1% bovine serum albumin–10% dextran sulfate–0.5% sodium dodecyl sulfate–100 μg of *Saccharomyces cerevisiae* tRNA per ml–50 or 30% formamide at 42°C and washed in 0.2× SSC (1× SSC is 150 mM NaCl, 15 mM Na-citrate [pH 7.0]) at 65 and 53°C, respectively. The 1.5-kb *Eco*RI-*Pst*I fragment of pPL8 (19), which contains full-length hPD-ECGF cDNA except for its 3′ end with the poly(A) tail, was used as a probe.

Somatic cell hybrids. DNAs from human-rodent somatic cell hybrid mapping panel 1, obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, N.J., were used for chromosomal assignment of the gene for hPD-ECGF. Human-mouse somatic cell hybrids GM09925 through GM09940 were derived from fusions of fetal human male fibroblasts (IMR-91) with thymidine kinase-deficient mouse cell line B-82 (32, 42). Hybrids GM10324 and GM10567 were generated by fusing human fibroblast cell lines GM00144 and GM02860 with hypoxanthine phosphoribosyltransferase- and adenine phosphoribosyltransferase-deficient mouse cell line A9 (10), while hybrid GM10611 resulted from microcell fusion of retroviral vector SP-1-infected human lymphoblast cell line GM07890 with Chinese hamster ovary line UV-135 (48). In addition, hybrid GM10498, derived from fusion of human fibroblast cell line GM00271 with mouse LMTk cells (47), was also included in the mapping panel. The human chromosome content of the hybrid lines was determined by cytogenetic analysis, as well as by Southern blot and in situ hybridization analyses. High-molecular-weight DNAs isolated from mouse, Chinese hamster, and human parental cell lines and the 19 hybrid cell lines were digested with *Bam*HI, fractionated in 0.8% agarose gels, and transferred to nylon filters as described by Southern (40). A ^{32}P-labeled hPD-ECGF cDNA probe was prepared from the 1.6-kb insert of pPL5 by the oligonucleotide labeling reaction (12) and hybridized to the filters. The filters were washed for 60 min each at 65°C in 6× SSC–0.5% sodium dodecyl sulfate (SDS) and in 2× SSC–0.5% SDS.

Nucleotide sequence accession number. The GenBank accession number of the nucleotide sequence reported here is M58602.

RESULTS

Isolation and sequencing of the gene for hPD-ECGF. A single copy of the gene for hPD-ECGF is present in the human haploid genome (19). A human genomic library was constructed in λEMBL3 by using human placenta genomic DNA partially digested with *Sau*3AI. Screening of 500,000 clones with an hPD-ECGF cDNA clone (pPL8; reference 19) yielded two different hybridizing clones, λpon1 and λpon2. These clones were subjected to restriction endonuclease mapping and shown to be colinear (Fig. 1). Comparison of the restriction maps of the genomic clones and the cDNA clone showed that the regions corresponding to the 1.8-kb hPD-ECGF cDNA sequence was included within a 6.0-kb *Kpn*I-*Sph*I fragment (Fig. 1). This fragment was subcloned

into pBluescript and M13mp18. Sequential deletion mutants were prepared, and the nucleotide sequence was determined. The gene for hPD-ECGF is composed of 10 exons (Fig. 2). Exon 1 is a noncoding exon and contains an upstream stop codon inframe with the open reading frame. The translation start codon is in exon 2, and the translation stop codon, which is part of the poly(A) additional signal, is in exon 10. Except for two copies of *Alu* repetitive sequences in intron 4, the nucleotide sequence shown here has no apparent similarity to other genes in GenBank data base release 56.

Sequences around the exon-intron junctions all conform to the GT-AG rule (7). A pyrimidine cluster is at the 5' side of each acceptor site.

The nucleotide which corresponds to nucleotide 1 of cDNA clone pPL8 (19) was absent in the genomic sequence. It is possible that this nucleotide was added artificially during construction of the cDNA library. In addition, two nucleotide differences were found between the sequence assembled from the 10 exons determined in this study and the reported sequence of the cDNA. (i) ACT at position 3568 to 3570 (exon 8) is ACC in the cDNA. However, this change does not affect the predicted amino acid sequence because both ACT and ACC encode threonine. (ii) TCG at position 4201 to 4203 (exon 10) is TTG in the cDNA. TCG encodes serine, while TTG encodes leucine. The sequence of hPD-ECGF purified from human platelets (19) conforms to the genomic sequence at this position. Since the genomic and cDNA libraries were derived from different placentas, these differences may represent polymorphism.

We found neither a TATA box nor a CCAAT box in the region sequenced. Instead, the 5'-flanking region was found to contain many G and C nucleotides and six copies of potential Spl-binding motifs (GGGCGG or CCGCCC) (9) were found clustered just upstream of exon 1 (Fig. 2). In addition to the Spl-binding motifs, we detected several regions homologous to the promoter sequences of other genes which also have high G+C contents and lack both TATA and CCAAT boxes, such as the genes for the insulin receptor (2), transforming growth factor α (20), the epidermal growth factor receptor (18), hypoxanthine phosphoribosyltransferase (23), and N-ras (17) (Fig. 2).

Determination of transcription start sites by primer extention. To determine the transcription start site in the gene for hPD-ECGF, we used the RNase protection assay and the primer extention method. Despite several attempts using various conditions, we could not assign the transcription start site by the RNase protection assay, probably because of the high G+C content in this region. However, primer extension using a primer complementary to nucleotides 59 to 93 gave five bands (Fig. 3). The lower two bands were only 1 nucleotide apart. This may be due to the cap effect caused by prematurely terminated reverse transcription at the methylated residue next to the cap site (49). Thus, this doublet may represent only one transcription start site. We designated the C corresponding to band D nucleotide 1.

Although we could not verify the 5' end of the hPD-ECGF mRNA by RNase protection assay, it is likely that the four bands indicated in Fig. 3 represent the actual transcription start sites. The following evidence supports this notion. (i) The 5' end of cDNA clone pPL5 corresponds to band D, and another clone, pPL8, is only 2 nucleotides longer. Usually, cDNA made by the conventional method is shorter in its 5' end than mRNA. However, since the cDNA library from which pPL5 and pPL8 were derived was made by tailing with G after first-strand synthesis and priming by oligo(dC) for second-strand synthesis (19), full-length cDNA clones can be anticipated. (ii) Six potential Spl-binding sites are clustered around and just upstream of this region. In genes that lack both TATA and CCAAT boxes, transcription usually starts just downstream of, or within the cluster of, potential Spl-binding sites and some of these genes have divergent transcription start sites (23, 34).

Identification of promoter activity in the 5'-flanking sequence of the gene for hPD-ECGF. We examined the presence of promoter activity in the 5'-flanking region of the gene for hPD-ECGF by constructing plasmids with the gene for CAT as the reporter gene. A plasmid with a 1.4-kb sequence from the 5'-flanking region and a part of exon 1 (nucleotides −1360 to +64) of the gene for hPD-ECGF as an insert (pSPDcat) was transfected into hTH7, a human thyroid cancer cell line which expresses hPD-ECGF (44), and CAT activity in the cell lysate was examined. Significant CAT activity was detected in the cell lysate (Fig. 4). For comparison, a construct with the simian virus 40 early-gene promoter (pScat) was used as a positive control and a plasmid with no insert (pS0cat) was used as a negative control. These results indicate that the 1.4-kb 5'-flanking region acts as a promoter for the gene for hPD-ECGF.

Southern blot hybridization of DNAs from different species. To see whether the gene for PD-ECGF was conserved among vertebrates, human, mouse, bird, frog, and fish genomic DNAs were hybridized with hPD-ECGF cDNA (pPL8) under stringent and relaxed conditions.

Under stringent hybridization conditions, only human and mouse DNAs showed specific bands, indicating that a sequence highly related to the hPD-ECGF gene exists in mouse DNA. Under relaxed conditions, several faint bands appeared in all lanes. PD-ECGF thus seems to be conserved among vertebrates (data not shown).

Chromosomal assignment of the gene for hPD-ECGF. Southern blot analysis of DNAs from 19 human-rodent somatic cell hybrids retaining 1 to 19 human chromosomes were used for assignment of hPD-ECGF. The human chromosome contents of the hybrid clones are shown in Table 1. A major 3.8-kb hPD-ECGF fragment was resolved from the 8.6- and 1.4-kb mouse fragments and the 3.2- and 1.2-kb Chinese hamster fragments in *Bam*HI digests of parental and hybrid DNAs. The presence of the 3.8-kb hPD-ECGF fragment in the hybrid clones correlated only with the presence of human chromosome 22 (Table 1). Of the 19 hybrids analyzed, 6 were positive for the hPD-ECGF fragment while 13 were negative. No discordancies were found for chromosome 22, whereas all other chromosomes could be excluded by at least four discordant hybrids. These data thus show that the gene for hPD-ECGF maps to chromosome 22.

DISCUSSION

We show in this communication that the gene for hPD-ECGF consists of 10 exons that span 4.3 kb and that the 5'-flanking region contains promoter activity, as demonstrated by a CAT assay. The 5' end of hPD-ECGF mRNA was determined by primer extension.

The promoter region of the gene for hPD-ECGF has several prominent features, i.e., high G+C content (75%), six potential Spl-binding motifs, and no TATA or CCAAT box. The G+C-rich islands in the 5'-flanking region of the gene for hPD-ECGF contains many CpG dinucleotide sequences, which are rare in vertebrate genes (Fig. 5). In other genes which are associated with G+C-rich islands, there is evidence that many of the cytosine residues of CpG se-

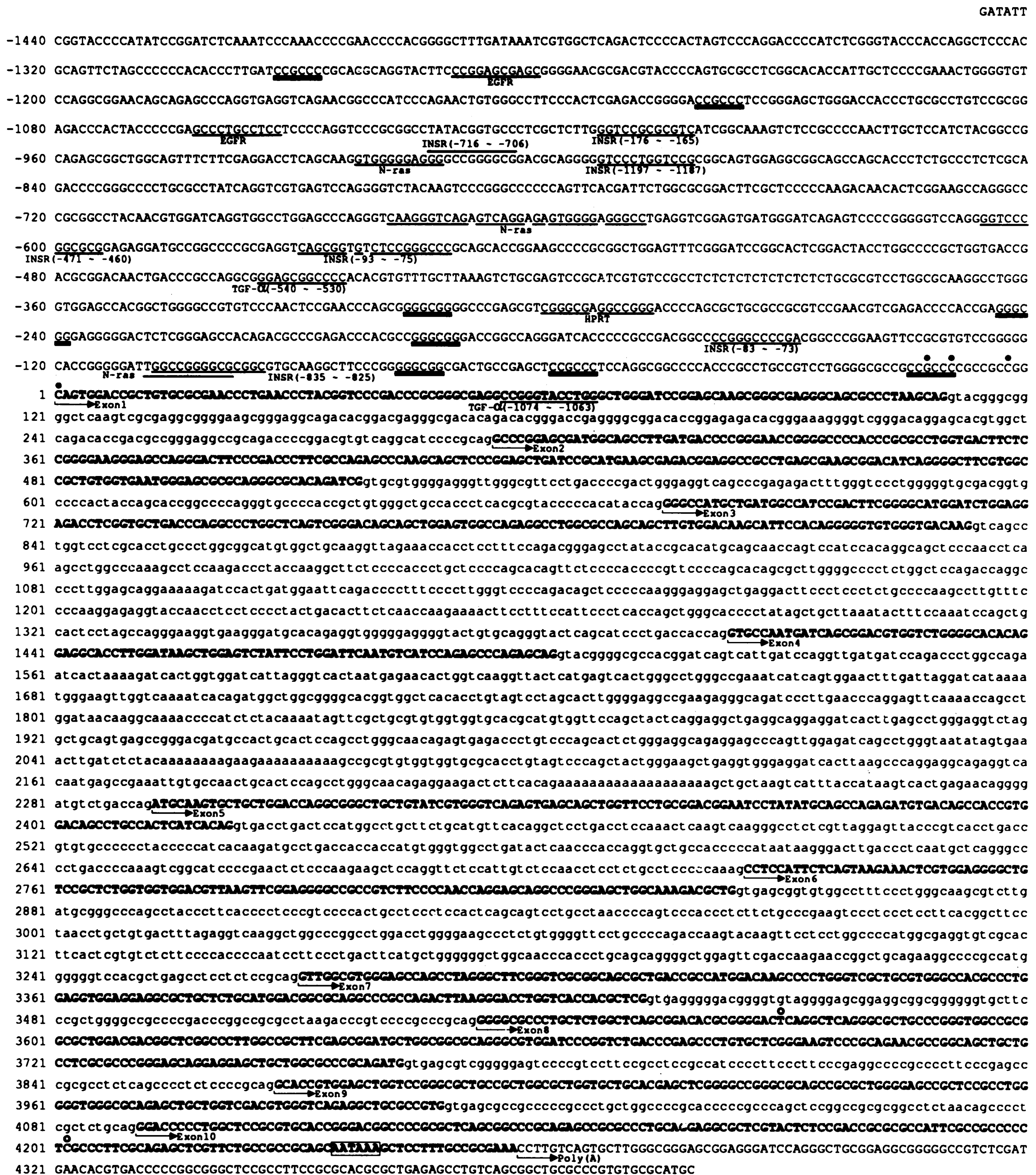

FIG. 2. Nucleotide sequence of the gene for hPD-ECGF and its flanking regions. Nucleotide 1 corresponds to one of the transcription start sites. The sequences of the 5'- and 3'-flanking regions are in lightface uppercase letters. Sequences of exons are in boldface uppercase letters, and introns are in lightface lowercase letters. Four transcription start sites are indicated by closed circles. The two nucleotides different from the cDNA sequence (one in exon 8 and the other in exon 10) are indicated by open circles. The poly(A) additional signal is boxed, and the site of poly(A) addition is indicated. Eight potential Sp1-binding motifs are indicated by thick lines. Sequences homologous to those of the gene for the human epidermal growth factor receptor (EGFR), the human insulin receptor (INSR), human hypoxanthine phosphoribosyltransferase (HPRT), human transforming growth factor α (TGF-α), and human N-ras are indicated by thin lines. Only identical nucleotides are underlined, and the lines are interrupted at mismatched nucleotides. Each number in parentheses is the position of the sequence relative to the transcription start site of the gene. Where several transcription start sites exist, approximate positions are indicated.

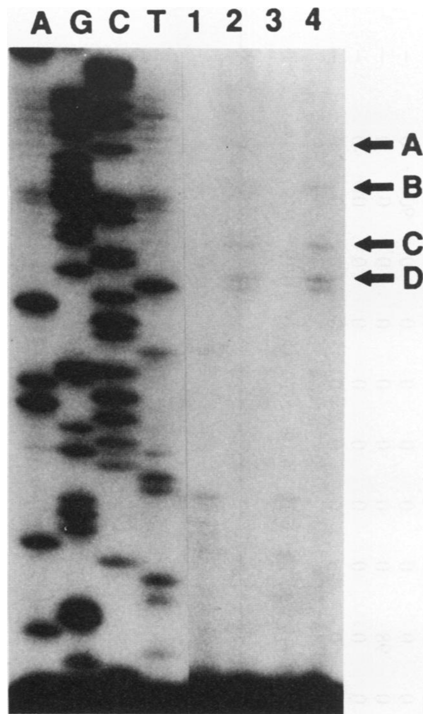

FIG. 3. Mapping of the 5′ ends of hPD-ECGF mRNA by primer extension. The four numbered lanes show primer-extended products obtained by using 15 μg of *S. cerevisiae* tRNA (lanes 1 and 3; negative controls) or 15 μg of human placenta poly(A)$^+$ RNA (lanes 2 and 4). Hybridization was performed at 55°C (lanes 1 and 2) or 50°C (lanes 3 and 4). The C nucleotide corresponding to band D was arbitrarily designated nucleotide 1. The sequence ladder was obtained by using the same end-labeled oligonucleotide as a DNA sequencing primer on an appropriate M13 phage template.

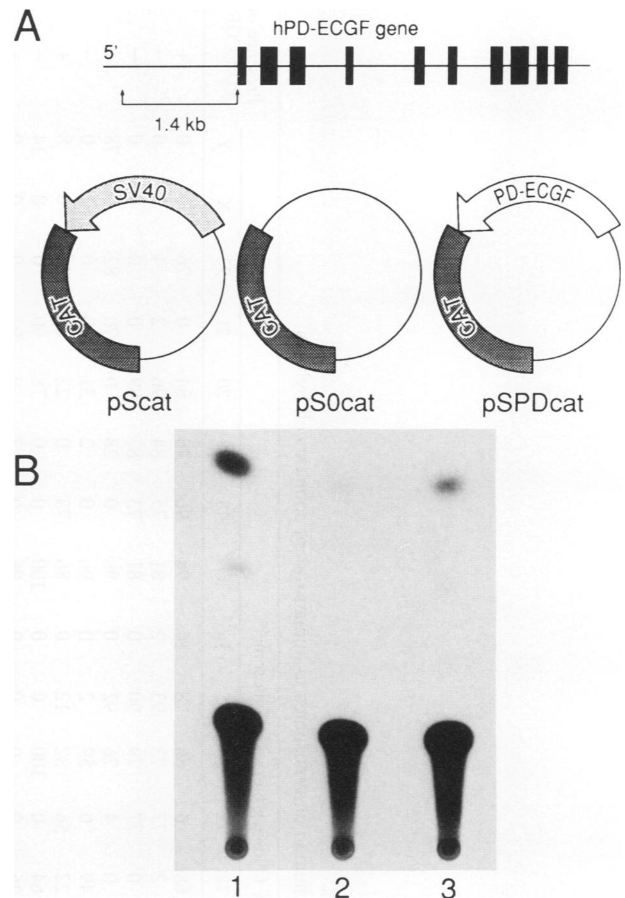

FIG. 4. CAT assay of the hPD-ECGF promoter. (A) The 1.4-kb fragment indicated was inserted 5′ of the coding region of the gene for CAT to give pSPDcat. The structures of this plasmid and the control plasmids are illustrated. (B) Autoradiogram illustrating CAT activity associated with each plasmid after transfection into hTH7 cells. Lanes: 1, pScat, a positive control; 2, pS0cat, a negative control; 3, pSPDcat. SV40, Simian virus 40.

quences in G+C-rich islands are not methylated (5) and that G+C-rich regions influence expression of the associated genes (22, 25, 50).

Most of the genes associated with G+C-rich islands have a TATA box, a transcription initiation stabilizer usually located approximately 30 bp upstream of the transcription start site (8), and a CCAAT box, a consensus sequence recognized by several transcription factors (27). However, among the genes associated with G+C-rich islands, there is a group of genes that lack both a TATA box and a CCAAT box. This group includes the hamster gene for HMG coenzyme A reductase (34) and the human genes for hypoxanthine phosphoribosyltransferase (23), the epidermal growth factor receptor (18), adenosine deaminase (46), the insulin receptor (2), transforming growth factor α (20), and N-ras (17). Many of these genes have a housekeeping character; i.e., expression occurs in many tissues (15, 41). Frequently, these genes have multiple transcription start sites, probably because they lack the TATA box stabilizer.

The promoter regions of most other growth factor genes differ from that of hPD-ECGF. The promoters of the genes of interleukin-1β (11), interleukin-2 (13), interleukin-5 (43), and granulocyte-macrophage colony-stimulating factor (29) are not associated with G+C-rich islands and have TATA-boxes. The promoters of the genes for the platelet-derived growth factor A (6, 35) and B (33) chains, angiogenin (26), and basic fibroblast growth factor (1) are associated with G+C-rich islands and have TATA-boxes. Among the genes we investigated, only the promoter of the gene for transforming growth factor α (20) has structural similarities to the promoter of the gene for hPD-ECGF; it is associated with G+C-rich islands, lacks both TATA and CAATT boxes, and has some sequence similarity (Fig. 2).

Southern blot analysis of a panel of human-rodent somatic cell hybrid lines was used to assign the gene for hPD-ECGF to chromosome 22. Assignment of the gene for hPD-ECGF to chromosome 22 adds an important DNA marker to this chromosome. A gene for another growth factor from platelets, platelet-derived growth factor B, has previously been mapped to 22q12.3-q13.1 (21). Abnormalities involving chromosome 22 are well documented in human tumors. Loss of one chromosome 22 is a characteristic feature of several types of tumors of the nervous system, including meningiomas, a subgroup of malignant gliomas and schwannomas (4, 39, 41a), and a t(11;22)(q24;q12) is regularly found in Ewing sarcoma (37). Of particular interest in relation to localization of the gene for PD-ECGF are findings of chromosome 22 abnormalities in certain blood vessel tumors. Thus, patients with the sporadic type of Kaposi sarcoma frequently show numerical and structural abnormalities involving chromosome 22 (38) and a t(13;22)(q22;q11) has been reported in

TABLE 1. Correlation of hPD-ECGF sequences with human chromosomes in human-rodent somatic cell hybrids

Hybrid clone	Avg % of cells with the following human chromosome:[a]																								Presence of PD-ECGF
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
GM09925	74	24	0	74	76	60	82	78	0	0	4	68	6	86	78	14	98	96	46	84	0	76	0	0	+
GM09926	69	75	75	65	2	88	85	69	0	68	0	2	77	73	93	2	81	75	84	96	2	4	2	0	−
GM09927	69	83	75	77	0	93	79	73	0	82	0	0	77	79	90	0	81	73	87	89	0	0	0	0	−
GM09928	0	84	58	0	48	32	0	66	0	2	0	0	4	76	92	0	98	0	28	0	70	82	0	78	+
GM09929	0	0	61	59	0	43	2	49	0	0	33	49	0	59	2	0	96	0	2	31	0	0	2	0	−
GM09930A	0	34	62	4	12	0	26	4	0	0	6	22	56	82	12	0	86	78	0	22	82	76	6	8	+
GM09931	0	0	0	0	26	0	78	0	0	46	0	64	0	100	0	0	100	0	0	78	90	0	0	14	−
GM09932	0	0	0	68	86	46	0	80	0	2	28	26	0	0	0	0	96	0	2	0	92	0	0	0	−
GM09933	50	0	84	16	54	76	92	54	0	6	0	50	84	78	92	0	88	70	80	32	94	88	0	32	+
GM09934	0	50	0	0	83	79	4	87	0	0	77	87	0	2	89	0	90	89	0	91	89	2	0	0	−
GM09935A	0	0	52	10	28	12	0	0	0	8	0	22	74	72	0	0	93	59	0	9	91	71	0	0	+
GM09936	0	0	0	18	0	46	70	10	0	16	34	0	2	88	2	0	100	0	44	24	0	18	0	0	−
GM09937	0	0	54	38	0	62	54	70	0	4	0	42	0	70	60	0	96	66	0	0	0	0	0	0	−
GM09938	0	0	2	88	60	88	86	4	0	0	36	92	0	80	4	0	92	0	4	80	76	60	0	2	+
GM09940	0	0	46	0	0	0	84	62	0	0	0	0	0	0	62	0	100	0	0	0	0	0	0	0	−
GM10324	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	90	0	−
GM10567	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0	0	0	0	0	0	0	0	−
GM10611	0	0	0	0	0	0	0	0	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−
GM10498	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96	0	0	0	0	0	0	0	−
Discordancy ratio	6/19	6/19	7/19	9/19	4/19	9/19	8/19	10/19	7/19	9/19	9/19	7/19	5/19	6/19	8/19	7/19	10/19	6/19	6/19	8/19	4/19	0/19	7/19	4/19	

[a] Obtained from cytogenetic analysis of a minimum of 25 cells examined at passage 1 and at harvest.

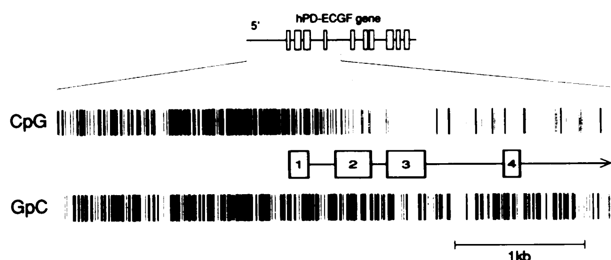

FIG. 5. Positions of CpG and GpC dinucleotide sequences around the transcription start site of the gene for hPD-ECGF. Each vertical line indicates one CpG (upper map) or GpC (lower map) sequence. Open boxes indicate exons.

hemangiopericytoma (28). The localization of the gene for PD-ECGF to chromosome 22 and its possible relationship to any of these tumors, however, remain to be determined.

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