Journal of Cellular Biochemistry 47:208–210 (1991)

# Platelet-Derived Endothelial Cell Growth Factor

**Carl-Henrik Heldin, Kensuke Usuki, and Kohei Miyazono**
Ludwig Institute for Cancer Research, S-751 24 Uppsala, Sweden

**Abstract** Platelet-derived endothelial cell growth factor (PD-ECGF) is a 45 kDa single chain polypeptide which stimulates endothelial cell growth and chemotaxis in vitro and angiogenesis in vivo. Analysis of a full length PD-ECGF cDNA revealed an open reading frame coding for 482 amino acids without homology to other known proteins. No signal sequence was observed, and analysis of the biosynthesis and processing of PD-ECGF in a thyroid carcinoma cell line revealed that PD-ECGF is released only very slowly. PD-ECGF becomes covalently associated with nucleotide triphosphates (e.g., ATP) in vivo, as well as in vitro. The physiological significance of this posttranslational modification remains to be elucidated. The tissue distribution and target cell specificity of PD-ECGF suggest roles in angiogenesis (e.g., during wound healing and in the developing placenta), as well as in the maintenance of the integrity of the endothelial cell lining of large vessels.

**Key words:** endothelial cells, growth factor, angiogenesis, platelets, placenta

The formation of new blood vessels is a complicated process which involves the migration, proliferation, and organisation of endothelial cells. Angiogenesis occurs, for example, during the fetal development and in wound healing, as well as in tumor growth [1]. The process is stimulated by certain polypeptide growth factors, some of which stimulate endothelial cell growth, whereas other do not, and thus most likely induce angiogenesis by an indirect mechanism. The most well-characterized of the endothelial cell mitogens are members of the fibroblast growth factor (FGF) family [2,3]. Another endothelial cell mitogen which recently was purified and cloned is vascular endothelial growth factor (VEGF; also called vascular permeability factor, VPF) [4,5].

Platelets were found to contain an angiogenic activity as early as 1977 [6], and later endothelial cell growth promoting activity was demonstrated in platelet lysate [7–10]. An endothelial mitogen was subsequently purified from human platelets [11] and named platelet-derived endothelial cell growth factor (PD-ECGF). This review summarizes the current knowledge of the structural and functional properties of PD-ECGF.

Received July 11, 1991; accepted July 22, 1991.

Address reprint requests to Carl-Henrik Heldin, Ludwig Institute for Cancer Research, Box 595, S-751 24 Uppsala, Sweden.

© 1991 Wiley-Liss, Inc.

## PURIFICATION AND CLONING OF PD-ECGF

PD-ECGF has been purified from human platelets by a five-step procedure [12,13]. The purified product is a single chain protein of 45 kDa with a pI of 4.8. Recently, PD-ECGF was also purified from human term placenta; PD-ECGF from this source was found to have a slightly larger size [14].

Using information of the amino acid sequence of PD-ECGF, oligonucleotide probes were constructed which allowed the cloning of a 1.8 kb full length cDNA from a human placental library [15]. The open reading frame codes for a 482 amino acid protein without sequence homology to other known proteins. Interestingly, the sequence lacks a hydrophobic signal sequence and it is not clear how PD-ECGF is transported out of the producer cell. The lack of signal sequence is a property that PD-ECGF shares, for example, with acidic and basic FGF. There appears to be a limited proteolytic processing of PD-ECGF in the N-terminus; Ala-11 and Thr-6 were found to be the N-terminal amino acids in PD-ECGF purified from platelets [15] and term placenta [14], respectively. It is not known whether or not there are functional differences between the platelet and placenta forms. There are seven cysteine residues in PD-ECGF; since the molecule is monomeric, it thus contains at least one free SH-group. Other interesting features of the PD-ECGF sequence include the

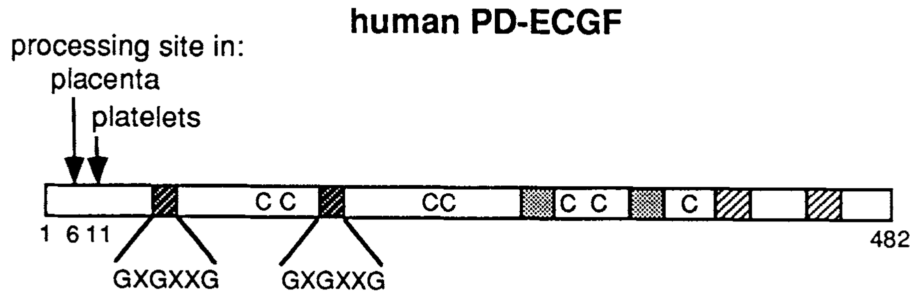


**Fig. 1.** Schematic illustration of the structural properties of PD-ECGF as deduced from its primary sequence. Numbers indicate amino acid numbers in the sequence and "C" indicates the localization of cysteine residues. Two nucleotide binding motifs (GXGXXG) are indicated in the aminoterminal part, and two different areas of internal repeats, each repeated twice, in the carboxyterminal part.

presence of sequence repeats in the C-terminal part and two nucleotide binding motifs in the N-terminal part (GXGXXG; see Fig. 1, and below).

The human PD-ECGF gene is composed of 10 exons, which are dispersed over a 4.3 kb region of chromosome 22 [16]. The PD-ECGF promoter lacks a "TATA box" and a "CCAAT box," but a cluster of six copies of potential Sp1 binding sites was observed just upstream of the transcription start site.

## BIOSYNTHESIS AND PROCESSING OF PD-ECGF

Screening of a number of different cell lines revealed the presence of PD-ECGF protein and mRNA in certain carcinoma cell lines, as well as in human foreskin fibroblasts and vascular smooth muscle cells [14,17]. A pulse chase analysis of PD-ECGF biosynthesis in a human thyroid carcinoma cell line revealed that PD-ECGF remained as a 45 kDa component inside the cell for a long time period; only after 24 h of chase was there a small amount seen in the conditioned medium [17]. This is consistent with the lack of signal sequence in the PD-ECGF molecule. No evidence was found for glycosylation of PD-ECGF, suggesting that the single possible acceptor site for N-linked glycosylation in the primary sequence is not used.

Incubation of A431 cells, which synthesize PD-ECGF, with $^{32}$P-orthophosphate led to the incorporation of $^{32}$P-radioactivity into PD-ECGF at serine residues [18]. Further analysis revealed that PD-ECGF become covalently linked with nucleotide-triphosphates, possibly ATP, in intact cells. Covalent linkage between PD-ECGF and nucleotides could also be demonstrated in vitro in the absence of proteins other than PD-ECGF; this reaction was promoted by dithiothreitol, $Mn^{2+}$ or $Mg^{2+}$-ions, denaturing conditions, and heating [18]. The possibility that the two nucleotide-binding motifs in PD-ECGF (Fig. 1) are involved in the reaction, as well as the functional significance of the nucleotidylation of PD-ECGF, remain to be elucidated.

## BIOACTIVITY OF PD-ECGF

PD-ECGF stimulates the growth of vascular endothelial cells in vitro, including cells derived from large vessels and capillaries. Half maximal stimulation occurred at 16 ng/ml (0.35 nM); at higher concentrations, 200 ng/ml or more, the growth promoting activity decreased [17]. PD-ECGF also stimulates other cells, including certain epithelial cell types and choriocarcinoma cells [14; Miyazono et al., unpublished data]. PD-ECGF also stimulates directed migration, chemotaxis, of endothelial cells, as determined by the Boyden chamber method [15].

In vivo, PD-ECGF stimulates angiogenesis. When assayed by the chorioallantoic membrane (CAM) assay, PD-ECGF was found to cause a marked invasion of capillaries [15]. Furthermore, transfection of cDNA for PD-ECGF into *ras*-transformed NIH3T3 cells, followed by injection of the cells into nude mice, led to the formation of tumors with a marked invasion of capillary vessels, whereas tumors formed by control cells were considerably less vascularized [15].

## POSSIBLE IN VIVO FUNCTION OF PD-ECGF

The in vivo function of PD-ECGF is not known. The fact that it is present in the platelets suggests that it may be released at sites of injuries and thus may play a role in wound healing. It is also present in vascular smooth muscle cells and it is thus possible that PD-

ECGF may help to maintain the integrity of the endothelial cell layer of large vessels. The finding that PD-ECGF is present in large amounts in the stromal parts of the placenta, together with the findings that it stimulates the growth of endothelial cells as well as choriocarcinoma cells, which are derived from trophoblasts, furthermore suggests that PD-ECGF may play a role in the formation of vessels, as well as trophoblast structures, in the developing placenta.

Future studies on PD-ECGF will aim at elucidating its in vivo role in normal and diseased tissues, as well as understanding its mechanism of action, including the identification of cell surface receptors and the mechanism of release of PD-ECGF from the producer cells.

## REFERENCES

1. Folkman J, Klagsbrun M: Science 235:442–447, 1987.
2. Rifkin DB, Moscatelli D: J Cell Biol 109:1–6, 1989.
3. Burgess WH, Maciag T: Annu Rev Biochem 58:575–606, 1989.
4. Leung DW, Cachianes G, Kuang W-J, Goeddel DV, Ferrara N: Science 246:1306–1309, 1989.
5. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT: Science 246:1309–1312, 1989.
6. Maca RD, Fry GL, Hoak JC, Loh PT: Thromb Res 19:473–483, 1977.
7. Clemmons DR, Isley WL, Brown MT: Proc Natl Acad Sci USA 80:1641–1645, 1983.
8. King GL, Buchwald S: J Clin Invest 73:392–396, 1984.
9. Miyazono K, Okabe T, Urabe A, Yamanaka M, Takaku F: Biochem Biophys Res Commun 126:83–88, 1985.
10. Miyazono K, Okabe T, Ishibashi S, Urabe A, Takaku F: Exp Cell Res 159:487–494, 1985.
11. Miyazono K, Okabe T, Urabe A, Takaku F, Heldin C-H: J Biol Chem 262:4098–4103, 1987.
12. Miyazono K, Heldin C-H: Biochemistry 28:1704–1710, 1989.
13. Heldin C-H, Hellman U, Ishikawa F, Miyazono K: Methods Enzymol 198:383–390, 1991.
14. Usuki K, Norberg L, Larsson E, Miyazono K, Hellman U, Wernstedt C, Rubin K, Heldin C-H: Cell Regulation 1:577–584, 1990.
15. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W, Heldin C-H: Nature 338:557–562, 1989.
16. Hagiwara K, Stenman G, Honda H, Sahlin P, Andersson A, Miyazono K, Heldin C-H, Ishikawa F, Takaku F: Mol Cell Biol 11:2125–2132, 1991.
17. Usuki K, Heldin N-E, Miyazono K, Ishikawa F, Takaku F, Westermark B, Heldin C-H: Proc Natl Acad Sci USA 86:7427–7431, 1989.
18. Usuki K, Miyazono K, Heldin C-H: J Biol Chem (in press).