THREE ISOFORMS OF PLATELET-DERIVED GROWTH FACTORS ALL HAVE THE CAPABILITY TO INDUCE ANGIOGENESIS *IN VIVO*

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Three isoforms of platelet-derived growth factors (PDGFs) composing of AA, AB and BB chains all exhibited angiogenic activity in a dose-dependent manner in an *in vivo* assay system involving the chorioallantoic membrane of chick embryo. The order of potency was BB>AB=AA. They, however, failed to stimulate proliferation of vascular endothelial cells, suggesting that their effects are indirect. These data suggest the possibility that three PDGF isoforms are indirect angiogenic factors.

KEYWORDS angiogenic activity; platelet-derived growth factor; chorioallantoic membrane assay

Tumor-related angiogenesis is a fundamental event in progressive growth and metastasis of tumors.¹⁻⁴⁾ This phenomenon is recognized to be mediated by a diffusible factor(s) derived from tumor cells themselves and/or host-derived cells like activated macrophages.¹⁾ These angiogenic factors include fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), which is also called vascular permeability factor, angiogenin and transforming growth factor- β (TGF- β). The last two peptides appear to be indirect angiogenic factors because they have no ability to promote vascular endothelial cell proliferation *in vitro*.

We previously found that rat mammary carcinomas induced by 7,12-dimethylbenz[a]anthracene (DMBA) had relatively potent angiogenic activity *in vivo*,⁵⁾ and that a DMBA-induced rat mammary tumor cell line secreted two distinct peptide-like angiogenesis factors into the conditioned medium.⁶⁾ Interestingly, these two angiogenic factors were unable to stimulate vascular endothelial cell growth *in vitro*. These findings directed our attention to peptide-like growth factors without endothelial cell mitogenic activity because one of our interests is to identify an angiogenic factor(s) able to express its function *in vivo*.

Platelet-derived growth factor (PDGF) is a family of three isoforms composing of AA, AB and BB chains.⁷⁾ With respect to involvement of PDGF in angiogenesis, there appear apparently controversial observations: PDGF has the ability to induce angiogenesis *in vivo*,⁸⁾ while it has no effect.⁹⁾ In addition, there is no direct comparison of three isoforms of PDGFs in terms of their angiogenic properties at the same time. Considering these findings, in this study we determined whether or not three PDGF isoforms had angiogenic effects using a chick embryo chorioallantoic membrane (CAM) assay. Their effects on proliferation of vascular endothelial cells were also examined *in vitro*.

PDGF-AA and-BB were obtained from Collaborative Co., Bedford, MA and PDGF-AB from Gibco BRL, Gaithersburg, MD. Fetal bovine serum (FBS) was purchased from Biocell, Carson, CA; Dulbecco's modified Eagle's medium (DMEM) from Sigma, St. Louis, MO; antibiotic from Gibco BRL, Gaithersburg, MD; methylcellulose (4,500 cps) from Tokyo Kasei Kogyo Co., Ltd., Tokyo.

Angiogenic activity was assessed as described previously.⁵⁾ Fertilized chick eggs were cultured at 37°C for 10 days in a humidified egg incubator. The 10-day-old CAM received a methylcellulose disk impregnated with increasing doses of PDGFs or a methylcellulose disk alone, and were treated for 4 days under the same conditions, at which time an angiogenic response was assessed as negative or positive: the response was scored as positive when a methylcellulose disk with a test sample produced a spoke wheel pattern of blood vessels around the disk.

Vascular endothelial cell proliferation was determined as described previously.⁵⁾ After vascular endothelial cells from bovine carotid artery (2×10^4 cells/well) were incubated for 72 h in the wells of a 24-multiwell dish (Sumitomo Bakelite Co., Ltd., Tokyo) containing 1 ml of 2% FBS/DMEM in the presence of the indicated concentrations of PDGFs, the cell number was counted in a Coulter counter after trypsinization.

Fig. 1. Dose-Dependency of Angiogenesis Induction by Three PDGF Isoforms

*P<0.05 compared to the control, whose positive angiogenic activity value was 5% (1/20);
 **P<0.01. compared to the control;
 ***P<0.001 compared to the control (Fisher's exact probability test).

Either of three isoforms of PDGFs showed dose-dependent angiogenic activities in doses ranging from 10 to 300 ng/egg (Fig. 1). Thus their angiogenic potency appears comparable to that of FGFs, VEGF or TGF- β , because these known angiogenic factors were reported to induce angiogenesis in the ng dose range in a CAM assay. This might imply that PDGFs play important roles in various angiogenic responses *in vivo*, including tumor angiogenesis. Representative results of these experiments are shown in Fig. 2. They all produced spoke wheel blood vessel patterns, representing a positive angiogenic response. Among them PDGF-BB showed the most potent angiogenic activity, although the mechanism of the angiogenic action of PDGFs is unknown. By contrast, all PDGF isoforms failed to promote proliferation of vascular endothelial cells at concentrations up to 100 ng/ml (data not shown), suggesting that their angiogenic effects *in vivo* are mediated via a direct angiogenic factor(s) derived from attracted host cells, including activated macrophages, in the methylcellulose disks containing PDGFs. This might

be supported by the facts that PDGFs have the abilities to migrate and activate macrophages⁷⁾ and that the activated macrophages secrete various direct angiogenic inducers, including VEGF.¹⁰⁾ These results suggest that three isoforms of PDGFs are all potent angiogenesis inducers. Further study is necessary to elucidate the mechanism of angiogenic action of PDGF isoforms, as well as their effects on other angiogenic components, including plasminogen activator production by vascular endothelial cells and endothelial cell migration.

Fig. 2. Angiogenic Activities on the 4th Day after Implantation of a Disk Impregnated with BSA (A), PDGF-AB (B), -AA (C) or -BB (D) into a Chick Embryo CAM

Note the spoke wheel vascular patterns caused by three isoforms of PDGFs (100 ng/disk). Magnification, x 6.

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REFERENCES

- 1) J. Folkman, Y. Shing, *J. Biol. Chem.*, **267**, 10931 (1992).
- 2) T. Oikawa, *Blood Vessel Endothelium*, **2**, 470 (1992).
- 3) T. Oikawa, *Gendai Iryo*, **26**, 1849 (1994).
- 4) T. Oikawa, *J. Clin. Exp. Med. (Igaku No Ayumi)*, **170**, 544 (1994).
- 5) T. Oikawa, A. Hiragun, Y. Yoshida, H. Ashino-Fuse, T. Tominaga, T. Iwaguchi, *Cancer Lett.*, **43**, 85 (1988).
- 6) T. Oikawa, K. Hirotsu, K. Yamaoka, T. Katayama, T. Iwaguchi, H. Ito, I. Morita, S. Murota, T. Tominaga, A. Hiragun, *Cancer Lett.*, **59**, 57 (1991).
- 7) R. Ross, E.W. Raines, D.F. Bowen-Pope, *Cell*, **46**, 155 (1986).
- 8) W. Risau, H. Drexler, V. Mironov, A. Smits, A. Siegbahn, K. Funai, C.-H. Heldin, *Growth Factors*, **7**, 261 (1992).
- 9) J. Wilting, B. Christ, H.A. Weich, *Anat. Embryol.*, **186**, 251 (1992).

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