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ARTICLE

Differential Expression of Angiogenic and Vasodilatory Factors by Invasive Trophoblast Giant Cells Depending on Depth of Invasion

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The uterine bed undergoes remarkable changes during pregnancy, including proliferation and decidualization of the uterine stroma and remodeling and angiogenesis of the maternal vasculature. Fetal-derived trophoblast giant cells invade into the uterus where they gain access to the maternal blood circulation to ensure sufficient nutrient supply of the embryo. In serial sections through early- to mid-gestation conceptuses, we have determined the exact distance of trophoblast invasion and the expression of angiogenic, vasodilatory, and anticoagulative factors that are likely to influence remodeling and redirection of the maternal circulatory system. Trophoblast derivatives were detected at a distance as far as ~300 μm from the placental border, where they are allocated exclusively along the mid-line of the decidua. The farthest invading cells characteristically expressed proliferin and proliferin-related protein, hormones that affect endothelial cell migration and vascularization. Occasionally, these cells replaced the normal vascular endothelium and acquired a "pseudo-endothelial" shape. Complete vascular disintegration was observed 50–80 μm outside of the placental border where maternal blood was entirely lined by a trophoblast giant cell-derived network of blood sinuses. This transition in blood space lining correlated with trophoblast expression of various vasodilatory and anticoagulative factors that are likely to promote blood flow toward the placenta. Analysis of teratocarcinoma-like tumors demonstrated that trophoblast giant cell-induced promotion and redirection of blood flow is not restricted to the uterine environment. These results show that trophoblast giant cells have the intrinsic capacity to attract and increase blood flow and to gradually displace the vascular endothelium resulting in the formation of canals entirely lined by trophoblast cells. *Developmental Dynamics 227:185–191, 2003.* © 2003 Wiley-Liss, Inc.

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INTRODUCTION

One of the most intriguing features of mammalian embryonic development is the re-organization of the uterine tissue by the conceptus at sites of implantation. Concurrent with decidualization of the uterine stroma, a remodeling of the maternal vasculature occurs during the first days after blastocyst attachment to promote blood flow toward the embryo. The increase in blood flow is achieved by blood vessel growth through angiogenesis as well as by vasodilation (Cross et al., 2002b). The loss of vascular smooth muscle in decidual arteries (Adamson et al., 2002) results in lack of maternal vasoconstrictive control over blood flow rates, thereby also promoting an increase in blood flow.

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Although a decidualizing reaction can be induced by nonspecific stimuli (De Feo, 1963), appropriate blood vessel outgrowth and remodeling requires the presence of a conceptus (Ma et al., 1997). In rodents, the fetal-derived cells that directly interact with maternal tissue are trophoblast giant cells (TGCs; Cross et al., 1994). TGCs mediate blastocyst attachment in early embryogenesis and exhibit invasive characteristics into the uterine stroma after implantation. In the late-stage mouse placenta, TGCs are the site of production of a variety of hormones and cytokines (Muntener and Hsu, 1977; Cross et al., 1994). TGC differentiation from trophoblast stem cells is tightly controlled by a variety of transcription factors that regulate the extent of giant cell formation (Cross et al., 2002a). Characteristically, TGCs are highly polyploid, which results in their unusually large size (Zybina and Zybina, 1996). As judged by their invasiveness and gene expression patterns, TGCs are analogous to extravillous cytotrophoblast of the human placenta (Hemberger and Cross, 2001). This analogy is further supported by the polyploid nature of human extravillous cytotrophoblast (Berezowsky et al., 1995), although its extent of ploidy is less pronounced than that of murine TGCs.

TGCs are associated with maternal blood at all developmental stages. Immediately after implantation, primary TGCs of the parietal yolk sac form an anastomosing network of blood sinuses at the periphery of the embryo (Bevilacqua and Abrahamsohn, 1988). Invasive TGCs are believed to gain access to decidual blood vessels, and in the second half of gestation, TGCs line the entry sites of maternal blood lacunae into the mature placenta by replacing the vascular endothelium (hemochorial placentation) and also invade by means of an endo-/perivascular route upstream into the spiral arteries (Adamson et al., 2002). In addition to their spatial arrangement, TGCs synthesize a variety of angiogenic and vasoactive substances (Cross et al., 2002b), which further strengthens the suggestion that TGCs are capable of redirecting and increasing maternal blood flow toward the embryo.

TGCs are associated with the appearance of large blood-filled areas in teratocarcinomas that are derived from embryonic stem (ES) cells deficient for the gene encoding poly(ADP-ribose)polymerase (*Parp1*; Nozaki et al., 1999). Therefore, TGCs may have similar effects in both the normal physiological uterine environment and outside the uterine vascular bed. To examine the potential of TGCs to induce a redirection and promotion of blood flow, we have determined the exact distance of TGC invasion and their spatial arrangement in respect to blood vessels in the transition zone of endothelial cell-lined arteries to open trophoblast-lined canals in normal implantation sites. We also investigated the expression of angiogenic, vasodilatory, and anticoagulative peptides by TGCs as potential mediators to cause or support the increase in blood flow.

RESULTS AND DISCUSSION

In previous work, we have described the pattern of TGC invasion in placentas from the latter half of gestation in mice (Adamson et al., 2002). In this study, the distance of TGC invasion from the main giant cell layer of the ectoplacental cone and placenta into the uterine stroma was analyzed in serial transverse sections through implantation sites at embryonic days (E) 8.5–E11.5. TGCs were detected by in situ hybridization with the giant cell marker proliferin (*Plf*; Linzer et al., 1985) and by immunoreactivity to cytokeratin (Brulet et al., 1980; Adamson et al., 2002). At E8.5, TGCs were observed as far as ~300 μm distal to the tip of the ectoplacental cone. This distance was slightly reduced at later stages, likely reflecting the expanding size and flattening shape of the placenta overall, and varied between 150 and 250 μm in E9.5–E11.5 embryos. When tracing individual spiral arteries from the placenta into the decidua, the interaction and replacement of endothelial cells with TGCs could be visualized (Fig. 1A). This spatial arrangement was particularly obvious from E9.5 onward when the diameter of spiral arteries was increased which allowed their tracking over a long distance. TGC invasion was always confined to the center of the implantation site as an extension of the tip of the ectoplacental cone (Fig. 1). No trophoblast-derived cells were detected in lateral regions of the decidua. Because spiral arteries converge to form a few central canals in the middle of the placenta (Adamson et al., 2002), the cone-like invasion of TGCs spatially correlated with the zone of spiral artery convergence.

TGC morphology and spatial association with maternal blood spaces was conspicuously different at various depths of invasion. Close to the border of the placenta, TGCs were of characteristically large size and easily distinguishable from all other cell types (Figs. 1, 2). Endovascular TGCs that replace endothelial cells of maternal spiral arteries had a spindle-like appearance and smaller nuclei by comparison (Fig. 1B,C). Cytokeratin immunoreactivity confirmed the trophoblast origin of these cells, despite their atypical TGC morphology (Fig. 1B). Although the exact degree of ploidy of the invading endovascular TGCs was not determined, their nuclear and cellular sizes were larger than that of surrounding endothelial and decidual cells. By using transfilter invasion assays with mouse trophoblast stem cell lines, we have similarly found that TGCs, rather than any other trophoblast subtype, are at the "leading edge" of invasion (M. Hemberger, J.C. Cross, unpublished results). Deeply invading, *Plf*-positive TGCs did not express placental lactogen I (*Pl1*), however, which is a marker of early stage TGCs (Carney et al., 1993; Fig. 1C). Instead, *Pl1* was only detectable in regions closer to the forming placenta in a subset of *Plf*-positive cells (Fig. 1C). Endovascular replacement was most prominent within the first 100 μm of invasion, where TGCs often represented the complete vessel lining (Fig. 1A). Further upstream in the spiral arteries, individual endovascular TGCs were interspersed between, and replaced, endothelial cells (Fig. 1A–C). Most *Plf*-positive cells at the front of

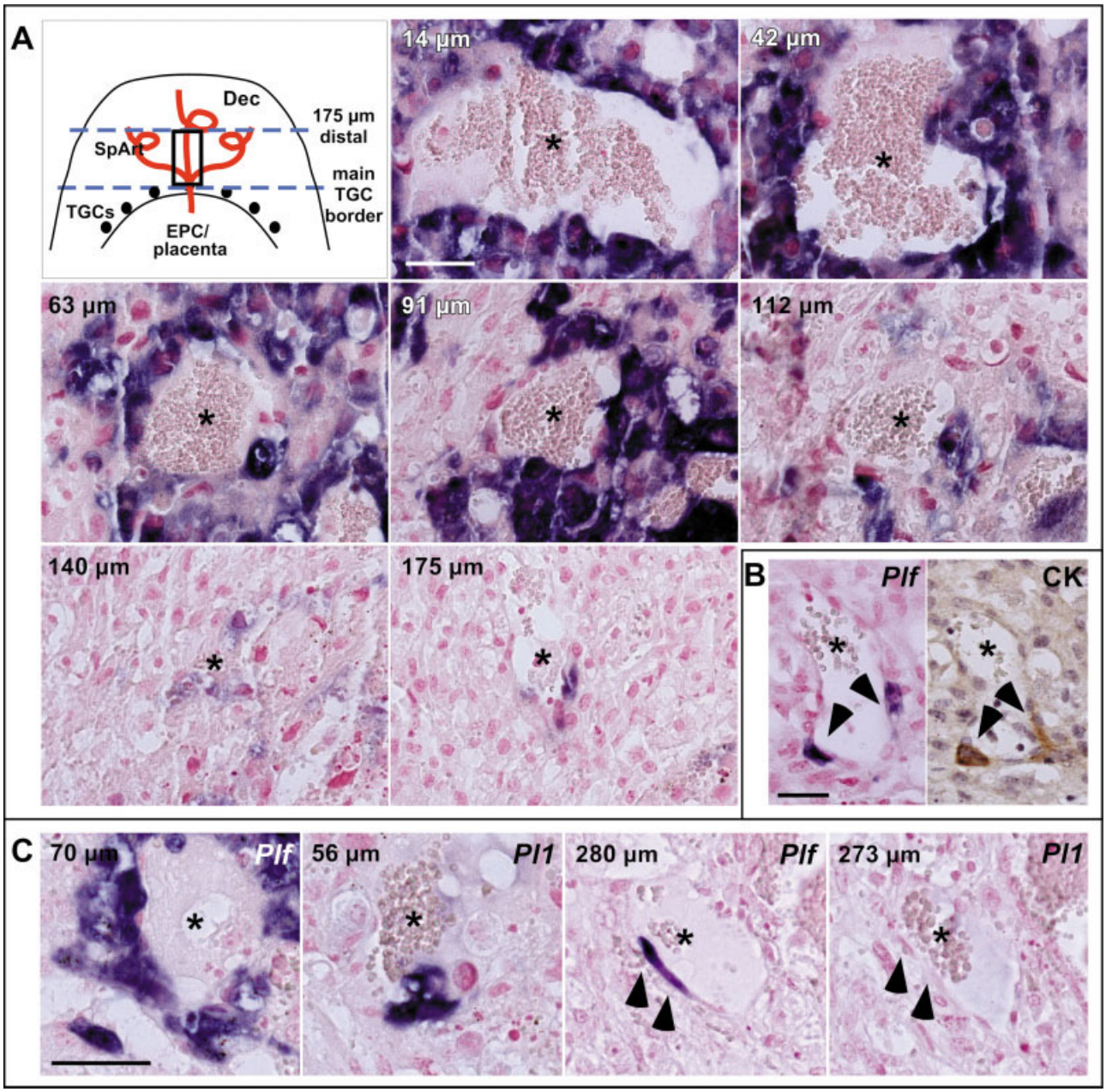

Fig. 1. Trophoblast giant cell invasion into the decidua. **A:** Expression of *Plf* mRNA by endovascular trophoblast giant cells along the length of their invasion path. A single spiral artery (asterisks) of an embryonic day (E) 9.5 implantation site is traced in transverse sections after in situ hybridization with a *Plf* probe. The distance from the main placental border is given for each section. The area depicted in the photographs is indicated by the open box in the diagram. Dec, mesometrial decidua; EPC, ectoplacental cone; SpArt, spiral arteries; TGCs, trophoblast giant cells. **B:** Spiral artery after in situ hybridization with a *Plf* probe and anti-cytokeratin (CK) immunohistochemistry on serial transverse sections of an E10.5 conceptus. Endovascular *Plf*-expressing cells are cytokeratin-positive, which demonstrates their trophoblast origin. **C:** In situ hybridizations with *Plf* and *Pl1* on E8.5 implantation sites at intermediate and distal regions from the main giant cell border. A subset of *Plf*-positive giant cells with typical giant cell morphology is expressing *Pl1* at intermediate distances. More distally located endovascular trophoblast giant cells (arrowheads) do not express *Pl1*. Scale bars = 40 µm in A,C, 20 µm in B.

invasion were located within one or a few cell layers underneath the vascular endothelium. In addition to the association with spiral arteries, TGCs were also consistently observed close to and breaching the epithelial-lined remnants of the blood-filled uterine lumen at early stages (E8.5 and E9.5) of development, representing another source of blood opening into trophoblast-lined sinuses (Welsh and Enders, 1991).

The expression of various TGC-expressed factors with angiogenic, vasodilatory, and anticoagulative functions was compared throughout the invasion zone. These factors included the prolactin-like hormones *Plf* (Lee et al., 1988) and proliferin-related protein (*Plfr/Prp*; Carney et al., 1993), which promote and inhibit endothelial cell migration in vitro and neovascularization in vivo, respectively (Jackson et al., 1994).

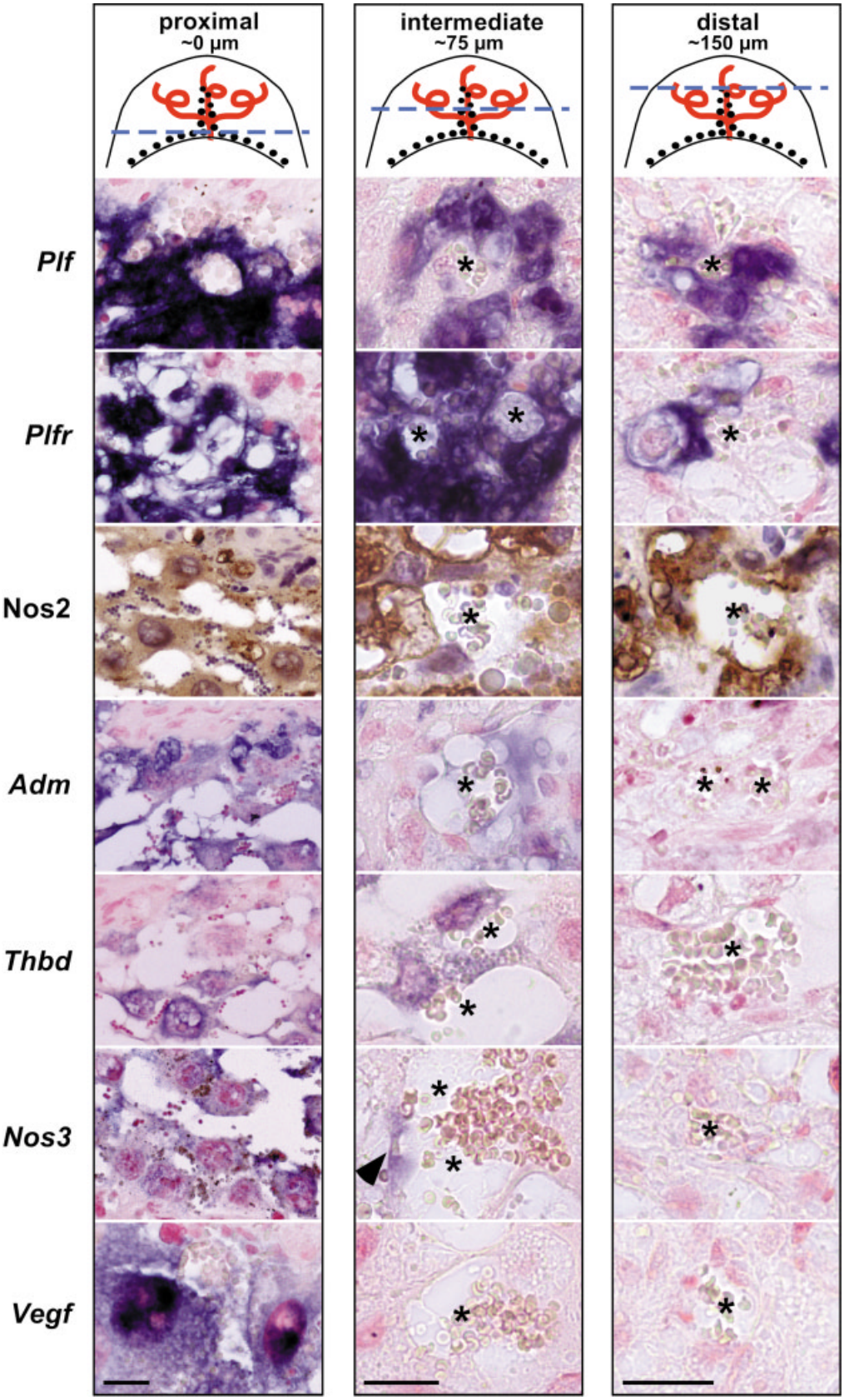

Fig. 2. Comparative expression of genes encoding angiogenic, vasodilatory, and anticoagulative factors in embryonic day 8.5 implantation sites. The indicated probes were hybridized to serial sections from three regions: within the main giant cell layer, and in giant cells associated with spiral arteries (asterisk) on transverse serial sections at either intermediate or distal regions outside of the placental border. Note that the farthest invading giant cells are strongly expressing *Plf*, *Plfr/Prp*, and *Nos2*. Scale bars = panel of proximal region, 40 μm; panels of intermediate and distal regions, 20 μm.

Vascular endothelial growth factor (*Vegf*) is a key angiogenic molecule that is produced by TGCs (Voss et al., 2000). TGCs also produce the potent vasodilators adrenomedullin (*Adm*; Yotsumoto et al., 1998) as well as nitric oxide (Gagioti et al., 2000), a product of inducible (iNOS, *Nos2*) and endothelial (eNOS, *Nos3*) nitric oxide synthase. Thrombomodulin (*Thbd*) is important to prevent clotting of maternal blood floating within trophoblast-lined lacunae of the parietal yolk sac and the placenta as it is expressed by TGCs and syncytiotrophoblast (Weiler-Guettler et al., 1996).

Trophoblast-derived cells at the distal most reach of invasion (i.e., 150–300 μm beyond the placental trophoblast border) were always identified by coexpression of *Plf* and *Plfr/Prp* (Fig. 2). iNOS/*Nos2* expression was present in TGCs at sites of farthest invasion (Fig. 2). iNOS/Nos2 immunoreactivity was also widely distributed throughout the decidua, although it was more concentrated toward the midline of the implantation site. *Adm*, *Thbd*, and eNOS/*Nos3* were only expressed in TGCs in relative proximity (within 50–80 μm) to the placenta (Fig. 2). Expression of these hypotensive and anticoagulative factors, therefore, coincided spatially with the occurrence of entirely TGC-derived blood sinuses. *Vegf* mRNA could only be detected in typically large TGCs of the conceptus not protruding into the invasion zone (Fig. 2).

ES cell-derived tumors that are deficient for the nuclear enzyme Parp1 contain large numbers of giant cells (Nozaki et al., 1999) that belong to the trophoblast cell lineage based on their typical TGC morphology and expression of trophoblast-restricted genes such as *Pl1* and *Pl2* (Hemberger et al., 2003). Sites of TGC accumulation within these tumors are characterized by the occurrence of large blood lakes in direct contact with TGCs. As in the parietal yolk sac and trophoblast invasion zone of embryos, TGCs formed an extended network of blood sinuses in *Parp1*-deficient tumors and these areas lacked a normal, endothelial-lined vasculature (Fig. 3A). When tracing individual TGC clusters in serial sections of these tumors, scattered TGCs were found at a distance of up to 350–500 μm from the main hemorrhagic area (Fig. 3B). These distant TGCs were often relatively small in size, expressed *Plf*, but no other TGC markers, and were mostly located in proximity to blood vessels and occasionally displaced their endothelial lining (Fig. 3C,D). Similar to the implantation sites, only TGCs in the region of complete vascular disintegration expressed *Adm*, eNOS/*Nos3*, *Thbd*, and *Vegf*, mainly in large, differentiated TGCs (Fig. 3E–H). In contrast to the conceptuses, *Plfr/Prp* expression was weak in the tumors and not present in *Plf*-positive cells at farthest distances from the hemorrhagic areas (Fig. 3E,F). Given that *Plfr/Prp* is thought to counteract *Plf* function in vascular outgrowth (Jackson et al., 1994), lack of *Plfr/Prp* expression might indicate an unbalanced TGC action in the tumors resulting in massive accumulations of blood-filled areas. iNOS/*Nos2* reactivity was not detected in TGCs of the tumors (data not shown).

Our study on serial sections of conceptuses and trophoblast giant cell-containing tumors suggests a fine-tuned regulation of gene expression, depending on the depth of trophoblast invasion into adjacent tissue. Production of vasodilatory and anticoagulative factors coincides with trophoblast adopting vasculature function by forming a network of anastomosing blood sinuses thereby promoting blood flow toward the TGC-containing area. This function is exerted similarly in conceptuses and TGC-containing tumors. Blastocyst explants placed underneath the kidney capsule result in teratocarcinomas that are also associated with blood-filled regions at sites of TGC accumulation (Avery and Hunt, 1972). These results clearly show that the blood flow promoting activities are intrinsic to TGCs and are not dependent on the physiological environment of the uterine bed. *Plf* expression at sites of farthest invasion is likely to play a role in the attraction of angiogenic blood vessel growth because of its function in promoting endothelial cell migration in vitro and its angiogenic activity in vivo (Jackson et al., 1994). Indeed, decidual neovascularization is perturbed in embryos with reduced *Plf* expression levels (Ma et al., 1997). Spatially coordinated trophoblast invasion, therefore, regulates angiogenic remodeling of the maternal vasculature of the conceptus to achieve maximal blood volume concentration at the entry site into the placenta.

EXPERIMENTAL PROCEDURES

Tissue Samples and Histology

For normal conceptuses, laboratory strain CD1 mice were mated and pregnant females were dissected at embryonic days E8.5, E9.5, E10.5, and E11.5 (counting the day of the vaginal plug as E0.5). Whole uterine horns were fixed in 4% paraformaldehyde for 2–4 hr, then cut after every second or third implantation site and allowed to continue fixing overnight at 4°C. After routine processing for paraffin histology, tissue samples were embedded for transverse sectioning and serial sections were cut at 7 μm through whole implantation sites. ES cell-derived tumors were obtained by injecting *Parp1*-deficient ES cells subcutaneously into the flanks of BALB/c *nu/nu* mice (CLEA Japan, Tokyo) as described previously (Nozaki et al., 1999). Developing teratocarcinoma-like tumors were dissected 3 weeks after injection, fixed in 10% formaldehyde, and processed for paraffin histology. Sections were cut at 7 μm.

RNA In Situ Hybridization

Linearized plasmids for *Adm* (2-kb *Sac*I fragment of mouse *Adm* cDNA; gift of Dr. Smithies' laboratory), eNOS/*Nos3* (880-bp *Eco*RI fragment of 3′-region of mouse *Nos3* cDNA; gift of Dr. Marsden's laboratory), *Pl1*, *Pl2*, *Plf*, *Plfr/Prp* (Carney et al., 1993), *Thbd* (Isermann et al., 2001), and *Vegf* (Breier et al., 1992) were used to generate digoxigenin-labeled riboprobes according to the manufacturer's instructions (Roche). In situ hybridizations were carried out at 53°C overnight using standard procedures. Signals were detected by

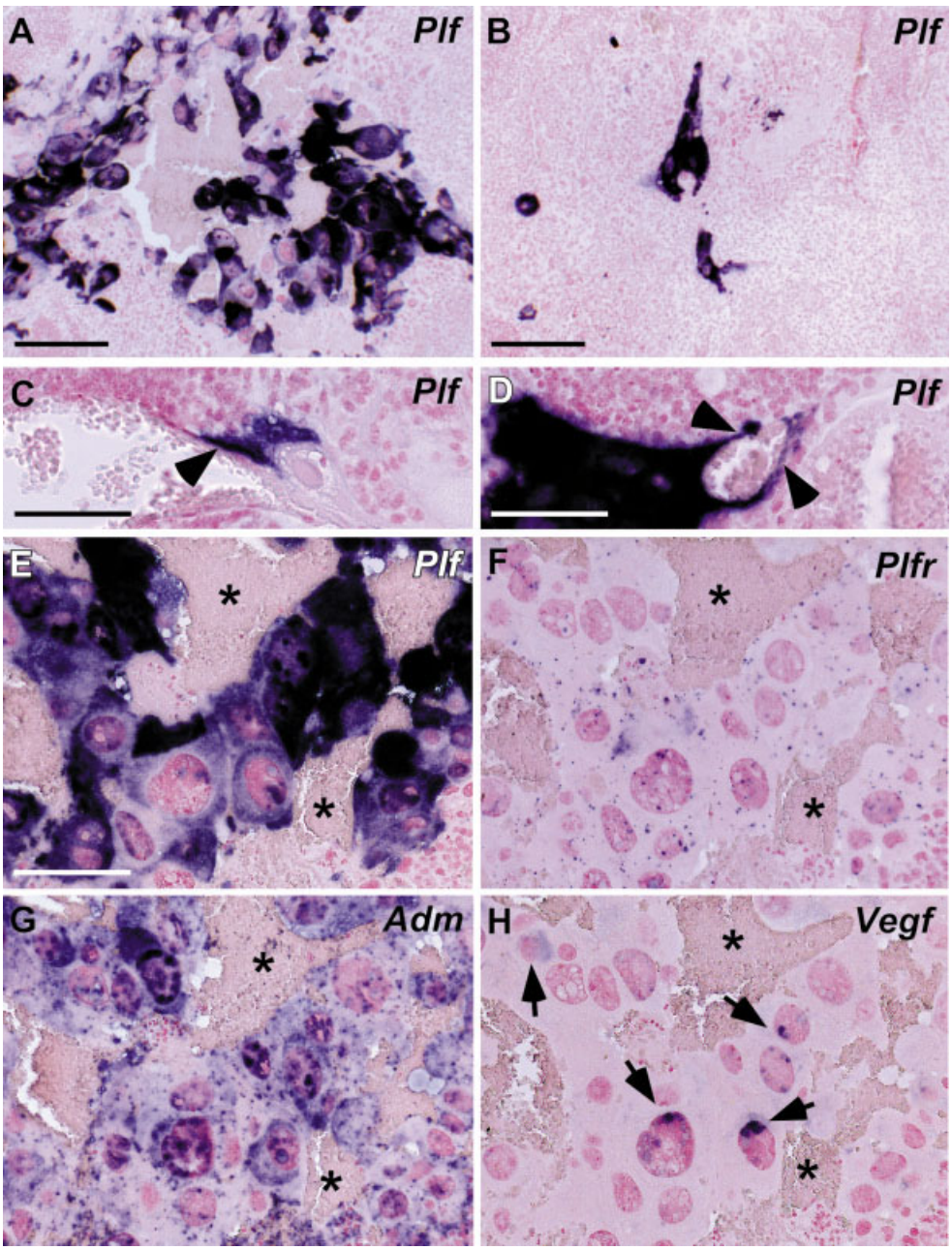

Fig. 3. Comparative expression of genes encoding angiogenic and vasodilatory factors in teratocarcinoma-like tumors containing trophoblast giant cells (TGCs). These tumors were generated from embryonic stem cells that are deficient for the *Parp1* gene. **A:** TGC cluster expressing *Plf* mRNA with open blood spaces lined by trophoblast cells. **B:** Section 420 μm away from the area shown in A, showing only a few *Plf*-positive cells that are comparatively much smaller. **C,D:** *Plf*-positive TGCs displacing the endothelial cell lining of blood vessels (arrowheads). **E–H:** Comparative expression on serial sections of *Plf*, *Plfr/Prp*, *Adm*, and *Vegf* (arrows) in large TGCs within the blood-filled area. Asterisks indicate corresponding regions in the sections. Scale bars = 200 μm in A,B, 50 μm in C,D, 100 μm in E–H.

using an antidigoxigenin alkaline phosphatase-conjugated antibody (Roche), and staining was performed overnight using NBT and BCIP (Roche). Sections were counterstained with nuclear fast red (Dako).

Immunohistochemistry

Antibodies against iNOS/Nos2 (Transduction Laboratories) and von Willebrand factor/factor VIII (Dako) were used at 1:100 and 1:200 dilutions, respectively. Paraffin sections were dehydrated, peroxidase-inactivated by incubation in 3% hydrogen peroxide for 5 min, and treated with 0.1% trypsin (Dako) in 0.01% $CaCl_2$ for 5 min at room temperature. For anticytokeratin staining, sections were heated in 0.01 M sodium citrate pH 6.5 and washed 3× in 50 mM Tris-HCl pH7.5, 0.3 M NaCl, 0.1% Nonidet P-40. The anticytokeratin antibody (Dako) was used at a 1:100 dilution. Tissues were blocked with 10% serum, 1% bovine serum albumin for 20 min before incubating for 3–5 hr with the primary antibody. After three washes in 1× PBS, horseradish peroxidase-conjugated secondary antibodies were used for detection. Staining was carried out with DAB^+ substrate chromogen solution (Dako), and sections were counterstained with hematoxylin.

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REFERENCES

Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, Cross JC. 2002. Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. Dev Biol 250:358–373.

Avery GB, Hunt CV. 1972. Giant cell formation in ectopic mouse trophoblast. Exp Cell Res 74:3–8.

Berezowsky J, Zbieranowski I, Demers J, Murray D. 1995. DNA ploidy of hydatidiform moles and nonmolar conceptuses: a study using flow and tissue section image cytometry. Mod Pathol 8:775–781.

Bevilacqua EM, Abrahamsohn PA. 1988. Ultrastructure of trophoblast giant cell transformation during the invasive stage of implantation of the mouse embryo. J Morphol 198:341–351.

Breier G, Albrecht U, Sterrer S, Risau W. 1992. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 114:521–532.

Brulet P, Babinet C, Kemler R, Jacob F. 1980. Monoclonal antibodies against trophectoderm-specific markers during mouse blastocyst formation. Proc Natl Acad Sci U S A 77:4113–4117.

Carney EW, Prideaux V, Lye SJ, Rossant J. 1993. Progressive expression of trophoblast-specific genes during formation of mouse trophoblast giant cells in vitro. Mol Reprod Dev 34:357–368.

Cross JC, Anson-Cartwright L, Scott IC. 2002a. Transcription factors underlying the development and endocrine functions of the placenta. Recent Prog Horm Res 57:221–234.

Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M, Adamson SL. 2002b. Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. Mol Cell Endocrinol 187:207–212.

Cross JC, Werb Z, Fisher SJ. 1994. Implantation and the placenta: key pieces of the development puzzle. Science 266: 1508–1518.

De Feo VJ. 1963. Determination of the sensitive period for the induction of the deciduomata in the rat by different inducing procedures. Endocrinology 73: 488–497.

Gagioti S, Scavone C, Bevilacqua E. 2000. Participation of the mouse implanting trophoblast in nitric oxide production during pregnancy. Biol Reprod 62:260–268.

Hemberger M, Cross JC. 2001. Genes governing placental development. Trends Endocrinol Metab 12:162–168.

Hemberger M, Nozaki T, Winterhager E, Yamamoto H, Nakagama H, Kamada N, Suzuki H, Ohta T, Ohki M, Masutani M, Cross JC. 2003. *Parp1*-deficiency induces differentiation of ES cells into trophoblast derivatives. Dev Biol (in press).

Isermann B, Hendrickson SB, Hutley K, Wing M, Weiler H. 2001. Tissue-restricted expression of thrombomodulin in the placenta rescues thrombomodulin-deficient mice from early lethality and reveals a secondary developmental block. Development 128:827–838.

Jackson D, Volpert OV, Bouck N, Linzer DI. 1994. Stimulation and inhibition of angiogenesis by placental proliferin and proliferin-related protein. Science 266:1581–1584.

Lee SJ, Talamantes F, Wilder E, Linzer DI, Nathans D. 1988. Trophoblastic giant cells of the mouse placenta as the site of proliferin synthesis. Endocrinology 122: 1761–1768.

Linzer DI, Lee SJ, Ogren L, Talamantes F, Nathans D. 1985. Identification of proliferin mRNA and protein in mouse placenta. Proc Natl Acad Sci U S A 82: 4356–4359.

Ma GT, Roth ME, Groskopf JC, Tsai FY, Orkin SH, Grosveld F, Engel JD, Linzer DI. 1997. GATA-2 and GATA-3 regulate trophoblast-specific gene expression in vivo. Development 124:907–914.

Muntener M, Hsu YC. 1977. Development of trophoblast and placenta of the mouse. A reinvestigation with regard to the in vitro culture of mouse trophoblast and placenta. Acta Anat 98:241–252.

Nozaki T, Masutani M, Watanabe M, Ochiya T, Hasegawa F, Nakagama H, Suzuki H, Sugimura T. 1999. Syncytiotrophoblastic giant cells in teratocarcinoma-like tumors derived from Parp-disrupted mouse embryonic stem cells. Proc Natl Acad Sci U S A 96:13345–13350.

Voss AK, Thomas T, Gruss P. 2000. Mice lacking HSP90beta fail to develop a placental labyrinth. Development 127: 1–11.

Weiler-Guettler H, Aird WC, Rayburn H, Husain M, Rosenberg RD. 1996. Developmentally regulated gene expression of thrombomodulin in postimplantation mouse embryos. Development 122:2271–2281.

Welsh AO, Enders AC. 1991. Chorioallantoic placenta formation in the rat: II. Angiogenesis and maternal blood circulation in the mesometrial region of the implantation chamber prior to placenta formation. Am J Anat 192:347–365.

Yotsumoto S, Shimada T, Cui CY, Nakashima H, Fujiwara H, Ko MSH. 1998. Expression of adrenomedullin, a hypotensive peptide, in the trophoblast giant cells at the embryo implantation site in mouse. Dev Biol 203:264–275.

Zybina EV, Zybina TG. 1996. Polytene chromosomes in mammalian cells. Int Rev Cytol 165:53–119.