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Short communication

Impact of placental growth factor deficiency on early mouse implant site angiogenesis

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ABSTRACT

Effects of placental growth factor (PGF), an angiokine product of fetal trophoblasts and maternal decidual cells, on early decidual angiogenesis are undefined. We used whole-mount immunofluorescence analyses to compare uterus and gestation day 4.5–9.5 mouse implantation sites that differed genetically in fetal or maternal PGF deficiency. Implant site number and embryonic development were similar in $Pgf^{-/-}$ and $Pgf^{+/+}$ females although $Pgf^{-/-}$ lymphatic vessels were anomalous. Correct, fine branching angiogenesis of anti-mesometrial vessels required both conceptus and maternal PGF; correct mesometrial branching angiogenesis depended solely upon conceptus PGF. Thus, PGF is non-redundant for optimizing branching angiogenesis in early decidua.

1. Introduction

In healthy human pregnancies, placental growth factor (PGF) in maternal plasma increases to ~week 30 then declines. Early maternal PGF deficiency is prodromal for approximately half of pregnancies progressing to preeclampsia (PE) [1,2]. Although decidual cells (uterine natural killer (uNK), stromal, and endothelial cells) produce PGF, maternal plasma deficits are usually attributed to poor placental production [3–5]. To identify the roles of maternal versus conceptus PGF in early decidual neoangiogenesis, $Pgf^{-/-}$ mice were studied. $Pgf^{-/-}$ mice are fertile and previous study of their gestation day (GD) 8–12 implant sites reported stalled uNK cell maturation and minor delays in spiral arterial remodeling and placental development [5]. Here, whole-mount immunohistochemistry (WM-IHC) [6–8] was applied to virgin and variously-mated GD4.5–9.5 uteri from normal and $Pgf^{-/-}$ mice to characterize and compare vascular structure during the pre-placental phases of early pregnancy.

2. Methods

129/SvJ ($Pgf^{+/+}$) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA); CD-1 ($Pgf^{+/+}$) from Charles River Laboratories (St-Constant, QC, Canada). Homozygous B6-Tg(UBC-GFP)/30SchaJ ($Gfp^{+/+}$) mice were used as studs to tag conceptus-derived cells [9]. 129-$Pgf^{-/-}$ were barrier husbandry reared at Queen's University [10]. All matings were transplantation antigen compatible (H-2^b). Five CD-1 females were ovariectomized (isoflurane anesthesia) and orthotopically transplanted with $Pgf^{-/-}$ ovaries (CD-1_{OvTx}) [11], rested 3 wk, and mated. Two conceived and were examined at GD8.5. WM-IHC was conducted [6,7] using 3 or more implant sites from 3 or more pregnancies (except CD-1_{OvTx}) per genotype between GD4.5–9.5. Hemisected implantation sites were stained anti-CD31-PE (EC13.3 #553373; BD Pharmingen, Mississauga, ON, Canada), anti-CD45-APC (30-F11 #557235; BD Pharmingen, Mississauga, ON, Canada) and anti-LYVE1-FITC (#FAB2125G; R&D Systems, Minneapolis, MN, USA) examined and photographed as viable tissue. Photomicrographs were analyzed by a single investigator (MTR). Animals were used under institutionally-approved protocols that complied with Canadian guidelines.

Anti-mesometrial lacunarity was measured using ImageJ software (NIH, Berthesda, MD, USA) and analyzed by one-way ANOVA with Tukey's post-hoc test using GraphPad Prism v5 (GraphPad, La Jolla, CA, USA). Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

To identify the source of PGF influencing early decidual neoangiogenesis, $Pgf^{-/-}$ and $Pgf^{+/+}$mating combinations were studied that isolated maternal (M) and conceptus (C) contributions. The matings (female × male) were: **M+C+** ($Pgf^{+/+}$ × $Pgf^{+/+}$, $Pgf^{+/+}$ × $Gfp^{+/+}$), **M+C½** (CD-1 × $Pgf^{-/-}$), **M−C½** ($Pgf^{-/-}$x$Gfp^{+/+}$), **M+C−** (CD-1_{OvTx} × $Pgf^{-/-}$), and **M−C−** ($Pgf^{-/-}$ × $Pgf^{-/-}$) where **+, ½, −** refer to *Pgf* genotypes. In non-pregnant uteri, numerous large subserosal vessels of the external uterine wall were confirmed to be lymphatic vessels by LYVE1 immunoreactivity. These vessels were thin and well defined in $Pgf^{+/+}$, but large and irregularly shaped in $Pgf^{-/-}$, suggesting a role for PGF in uterine lymphatic development

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(Fig. 1G–H). Uterine lymphatic anomalies have been reported in PE women [12]. Internal mucosal vessels of virgin uteri lacked detectable LYVE1, were thin, well defined and indistinguishable between *Pgf*$^{-/-}$ and *Pgf*$^{+/+}$ (data not shown).

Grossly, GD-matched implant site sizes were similar for all mating combinations. WM-IHC revealed no differences in angiogenesis before GD6.5. At GD6.5, **M-C-** implant sites had wider, less distinct anti-mesometrial blood vessels than **M**+**C**+ and **M**+**C**− indicating maternal PGF optimizes early anti-mesometrial angiogenesis. In all matings, CD31 reactivity was acquired by non-vascular embryonic crypt stromal cells and leukocytes, suggesting cell activation [6,9,13]. No mesometrial differences were observed (data not shown).

By GD8.5, differences were more distinct. Anti-mesometrially, all groups were similar except **M**−**C**− which had deficient branching (Fig. 2), indicating that conceptus PGF joins maternal PGF in importance for anti-mesometrial angiogenesis as pregnancy progresses. Image analysis revealed that **M**−**C**− matings had ~3 × greater lacunarity than each of the other matings ($p < 0.001$). Anti-mesometrial vessel branching was likely not influenced by leukocyte-derived PGF, since leukocytes were infrequent anti-mesometrially. Mesometrially, in decidua highly enriched for leukocytes, abnormally straight, unbranched vessels were seen in **M**+**C**− and **M**−**C**− matings (Fig. 1), indicating conceptus-derived PGF regulation of mesometrial decidual angiogenesis. In all matings, many leukocytes present in the mesometrial decidua basalis acquired CD31 expression and formed conjugates, suggesting PGF deficiency does not impair major leukocyte functions. Ultrastructural studies (data not shown) confirmed our previous finding of altered uNK cell cytokinesis in **M**−**C**− sites [5]. Since anti-mesometrial decidua regresses in later pregnancy, our data suggest that maternal plasma PGF fluctuations over mid-to-late pregnancy characterize conceptus rather than maternal biology.

At GD9.5, vessels continued to grow and develop in **M**−**C**− decidua, but remained distinctly delayed in maturation of pruning mesometrially versus all other matings (data not shown). PGF deficiency had no detected impact on trophoblast invasion into decidua basalis (GD6.5) or into its vessels (GD9.5). Thus PGF expression by trophoblast or decidual cells is not required to initiate or sustain trophoblast invasion.

Maternal plasma PGF concentration patterns are similar in mouse and human pregnancies [5,14], suggesting analogous function. A dominant action of PGF is as a decoy ligand for VEGFR-1, allowing VEGFA to bind and signal through VEGFR-2, promoting angiogenesis [15–17]. PGF deletion would thus lead to available VEGFR-1 for VEGFA binding which would decrease angiogenic signaling through VEGFR-2 and result in a phenotype characteristic of VEGF deficiency. In humans, VEGFA and VEGFR-2 are highly expressed by early placentas, while PGF and VEGFR-1 are more highly expressed from mid-gestation until term [18,19]. Low plasma PGF in women progressing to PE, may reflect a functional rather than absolute VEGF deficiency. PGF is considered to be redundant during development since *Pgf*$^{-/-}$ mice are viable without overt pathology [10]. Our data refute this tenet by showing that PGF, particularly from conceptuses, is essential for normal early decidual angiogenesis.

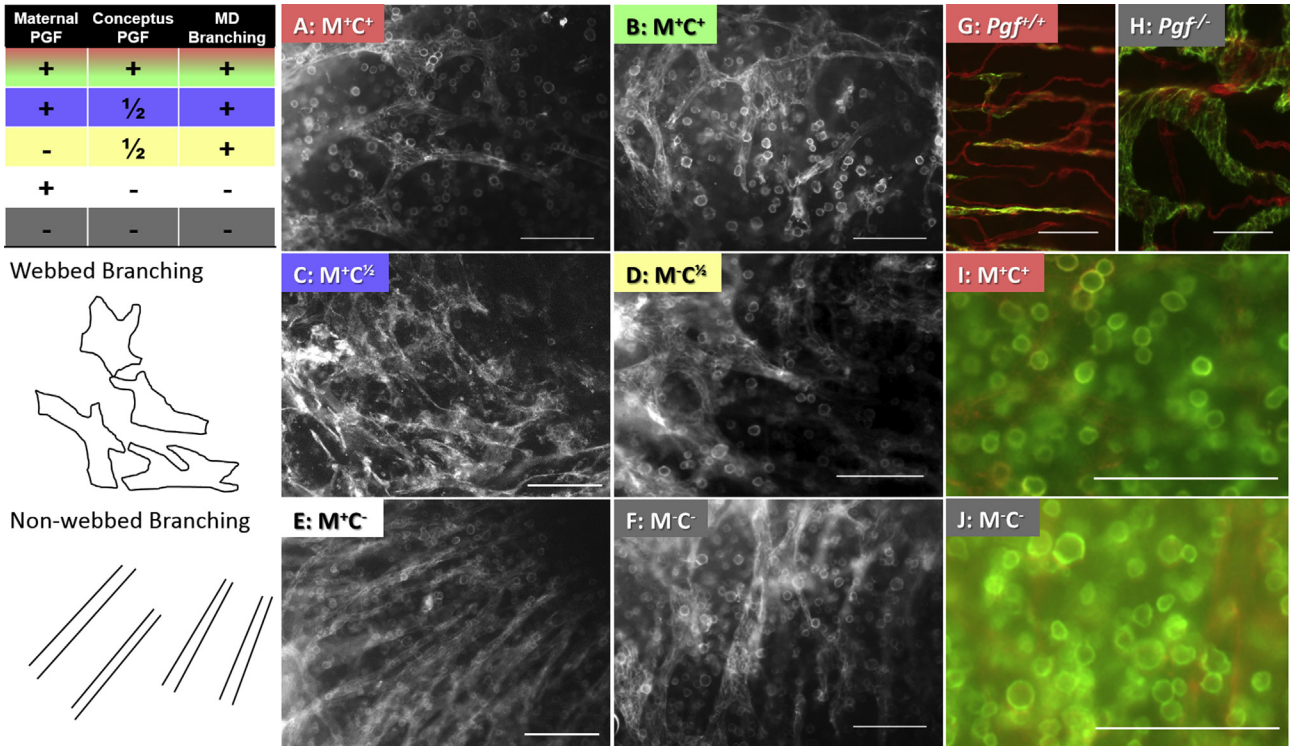

Fig. 1. Mesometrial decidual angiogenesis is deficient only when conceptus-derived PGF is absent. Whole mount immunofluorescence photomicrographs of CD31 stained GD8.5 mesometrial decidua of *Pgf*$^{+/+}$ × *Pgf*$^{+/+}$ (A), *Pgf*$^{+/+}$ × *Gfp*$^{+/+}$ (B), CD-1 × *Pgf*$^{-/-}$ (C), *Pgf*$^{-/-}$ × *Gfp*$^{+/+}$ (D), CD-1$_{OvTx}$ × *Pgf*$^{-/-}$ (E) and *Pgf*$^{-/-}$ × *Pgf*$^{-/-}$ (F) mated mice. Distinct vascular plexuses representing active angiogenesis were seen in the *Pgf*$^{+/+}$ × *Pgf*$^{+/+}$, *Pgf*$^{+/+}$ × *Gfp*, CD-1 × *Pgf*$^{-/-}$, *Pgf*$^{-/-}$ × *Gfp* mated mice (A-D; illustrated in left middle panel). These were very infrequent in the CD-1$_{OvTx}$ × *Pgf*$^{-/-}$ and *Pgf*$^{-/-}$ × *Pgf*$^{-/-}$ mated mice where the vessels remained uniform in width and lacked branching (E, F; illustrated in left bottom panel). The top left panel illustrates the differences in maternal vs. conceptus derived PGF expression and resulting effect on mesometrial decidual (MD) vessel branching. Lymphatic vessels on the external serosal surface of the uterus were identified using CD31 (red) and LYVE1 (green) in *Pgf*$^{+/+}$ (G) and *Pgf*$^{-/-}$ (H) virgin mice. Virgin serosal lymphatic vessels were very straight and regularly spaced in *Pgf*$^{+/+}$ mice (G) but displayed anomalous morphology in *Pgf*$^{-/-}$ mice (H). GD8.5 decidual CD45+ leukocytes (green) were relatively larger in *Pgf*$^{-/-}$ × *Pgf*$^{-/-}$ (J) mated mice, compared to each of the other matings (*Pgf*$^{+/+}$ × *Pgf*$^{+/+}$ shown in panel I). Similar proportions of leukocytes stained CD31+ (red) in each mating combination. Images are representative of 3 implant sites from each of 2–3 litters per mating combination. The bottom left panel illustrates a representative sagittally hemisected whole mounted GD8.5 implant site showing conceptus (green) and CD31+ endothelium (red). Scale bars represent 100 μm all panels.