Prolactin-Like Protein-F Subfamily of Placental Hormones/Cytokines: Responsiveness to Maternal Hypoxia

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The prolactin (PRL) family of hormones/cytokines is involved in the maintenance of pregnancy and adaptations to physiological stressors. In this report, we identify and characterize a new member of the rat PRL family, examine the impact of maternal hypoxia on placental PRL family gene expression, and investigate maternal adaptive responses to hypoxia. Perusal of the PRL gene family locus in the rat genome resulted in the identification of a putative new member of the rat PRL family. The new member is closely related to the previously reported PRL-like protein-F (PLP-F) and has been named PLP-Fβ and the originally characterized PLP-F, now termed PLP-Fα. The two proteins exhibit structural similarities but possess distinct cell- and temporal-specific expression profiles. In vivo hypoxia stimulates placental PLP-Fα and PLP-Fβ mRNA expression in the rat and mouse, respectively. Rhoc-1 trophoblast cells can differentiate into trophoblast giant cells, express PLP-Fα, and exhibit enhanced PLP-Fα mRNA levels when cultured under low oxygen tension (2%). Exposure to hypobaric hypoxia during later part of pregnancy did not significantly impact the expression of PLP-Fβ mRNA. Finally, exposure to hypobaric hypoxia during midpregnancy led to increased maternal red blood cells, hemoglobin concentrations, hematocrit, and increased concentrations of maternal splenic mRNAs for key proteins involved in hemoglobin synthesis, erythroid Krüppel-like factor, erythroid 5-aminolevulinate synthase-2, and β-major globin. In summary, adaptive responses to maternal hypoxia include activation of placental PLP-Fα/E gene expression, which may then participate in maternal hematological adjustments required for maintaining maternal and fetal oxygen delivery. (Endocrinology 148: 559–565, 2007)

PLACENTATION IS ASSOCIATED with modifications of the maternal environment. This is accomplished, in part, through the elaboration of hormones and cytokines. In the rat and mouse, endocrine cells of the placenta, along with lactotrophs of the anterior pituitary, and decidual cells of the uterus produce a family of hormones/cytokines, related to prolactin (PRL) (1, 2). They are termed placental lactogens (PLs), PRL-like proteins (PLPs), PRL-related proteins, prolipherin, and prolipherin-related protein. In the mouse, 23 genes related to PRL have been identified and characterized, whereas 24 PRL-related genes are known in the rat (3–5). Most of the PRL-related genes in the two species are orthologous. The PRL family genes are located on chromosome 13 in the mouse and chromosome 17 in the rat (3, 5). Each member of the family has temporal and tissue-specific expression patterns associated with pregnancy (2). PRL family members target the reproductive tract, liver, hematopoietic and immune cells, vasculature, and brain and participate in adaptations to physiological stressors (2, 6).

Maternal hypoxia is an effective tool to investigate the involvement of the PRL family in adaptations to physiological stressors (7). At least one member of the PRL family, PLP-A, has been shown to contribute to pregnancy-dependent adaptations to maternal hypoxia (8). Other members of the PRL family are involved in regulating key biological processes that are typically associated with adaptations to hypoxia. The latter PRL family members include the PLP-F subfamily, which has been shown to regulate hematopoiesis, including red blood cell development (9–15). Three PLP-F subfamily members have been reported; two from the mouse, PLP-E and PLP-F (16, 17), and one from the rat, PLP-F (18). PLP-E is expressed in trophoblast giant cells (16) and is an effective stimulator of erythropoiesis (10, 15) and could be part of a pregnancy-dependent adaptive mechanism ensuring adequate red blood cell oxygen delivery. Mouse PLP-F is expressed predominantly by spongiotrophoblast cells (16) and has been shown to similarly stimulate hematopoiesis (13). Although rat PLP-F is slightly more similar in structure to mouse PLP-F, its expression pattern more closely resembles mouse PLP-E (18).

In this report, we identify and characterize a new member of the rat PLP-F subfamily and demonstrate that the expression of some PLP-F subfamily members is regulated by maternal hypoxia.

Materials and Methods

Animals and tissue preparation

Holtzman rats were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN). CD-1 mice were obtained from Charles River Labora-
tories (Wilmington, MA). Animals were housed in an environmentally controlled facility, with lights on from 0600 to 2000 h and allowed free access to food and water. Timed mating of animals was conducted by placing females with fertile males. The presence of sperm in a vaginal smear of a female rat was designated as d 0.5 of pregnancy. The presence of a seminal plug in the vagina of female mice was designated as d 0.5 of pregnancy. Placentation sites, including uterus, metrial gland, and placental tissues, were dissected from pregnant animals. Placental tissues collected from gestation d 12.5 in the rat were dissected into chorioallantoic and choriovitelline components. On d 18.5 of gestation, the chorioallantoic placenta was dissected into junctional and labyrinth zones. Tissues were snap frozen in liquid nitrogen for RNA analyses. For in situ hybridization, tissues were frozen in dry ice-cooled heptane. All tissue samples were stored at −80 C until used. Protocols for these procedures have been described (19). The University of Kansas Medical Center Animal Care and Use Committee approved all procedures for handling and experimentation with rodents.

In vivo hypoxia

On the designated day of pregnancy, female rats or mice were placed in hypobaric chambers. Two conditions were used. Rats were exposed to conditions, in which air is circulated at a barometric pressure of approximately 380 Torr, which results in an inspired PO2 of approximately 70 Torr, equivalent to breathing 10% O2 at sea level, whereas mice were exposed to conditions, in which air is circulated at a barometric pressure of approximately 420 Torr, which results in an inspired PO2 of approximately 78 Torr, equivalent to breathing 11% O2 at sea level (7). The chambers were opened daily to replenish food and water (15–20 min). Pair-fed and ad libitum-fed control pregnant rats were exposed to ambient conditions (barometric pressure of −760 Torr and inspired PO2 of −149 Torr).

Rcho-1 trophoblast cell culture model

The Rcho-1 trophoblast cell line represents an in vitro model for studying rat trophoblast cells in undifferentiated and differentiated states (20, 21). Rcho-1 trophoblast cells can be maintained in a proliferative state by maintaining the cells or induced to differentiate, primarily along the trophoblast giant cell lineage (20, 21). Rcho-1 trophoblast cells were maintained in a proliferative state by culturing under subconfluent conditions with RPMI 1640 culture medium supplemented with 20% fetal bovine serum (FBS), 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 100 μM of penicillin, and 100 μg/ml of streptomycin (21). Differentiation was induced by growing cells to near confluence in FBS-supplemented culture medium and then replacing the culture medium with NCTC 135 culture medium supplemented with 1% horse serum, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 100 μM of penicillin, and 100 μg/ml streptomycin (21). Trophoblast giant cell differentiation is facilitated by high cell density and the removal of factors capable of stimulating proliferation (removal of FBS) (21). During differentiation, cells were maintained under ambient (21% O2) or low oxygen (2% O2) levels in a BioSpherix OxyCycler incubator/glove box (BioSpherix Ltd., Redfield, NY). These in vitro conditions of low oxygen tension do not necessarily equate with the in vivo maternal hypoxia described above.

Identification and characterization of a new member of the PLP-F subfamily

A new member of the PLP-F subfamily was identified by BLAST analysis (22) using the rat PLP-F nucleotide sequence (18) against the public rat genome assembly (Rat Genome Sequencing Project Consortium, 2004; http://rat.ensembl.org, http://genome.brc.mawu/cgi-bin/hgGateway?org=Rat&db=nm&hgvsid=575405, and http://www.ncbi.nlm.nih.gov/gene/seq/MmBlast.html) and found on a BAC clone localized to chromosome 17 (CH230–190E4; Children’s Hospital-BACPAC Resources, Oakland, CA; Baylor Rat BACtig Assembly: http://www.bacpac.org/rat/). The genomic sequence corresponding to the newly identified PLP-F subfamily member was used to search the expressed sequence tag (EST) database (National Center for Biotechnology Information, Bethesda, MD) (22). Several ESTs with nucleotide sequences matching the genomic sequence were identified and obtained from Invitrogen (Carlsbad, CA). cDNAs were sequenced by the Northwestern Sequencing Facility of Northwestern University (Chicago, IL). The original PLP-F was renamed PLP-Fα and the newly identified PLP-F family member was termed PLP-Fβ. Multiple amino acid sequence alignments were generated with CLUSTAL X (23). Locations of signal peptides were determined with the SignalP software program (version 2.0.b2) (24) and based on homology with other members of the PRL family.

Analysis of mRNA expression

PRL family miniarray assay. The PRL family miniarray is a hybridization-based tool for monitoring expression of each PRL family member (25). It has been used to monitor phenotypes of anterior pituitary, uterine, and placental tissues. The PRL family miniarray was performed as previously described (25). Total RNA was extracted using TRIzol reagent (Invitrogen, 26), [32PIDCTP-labeled cDNA probes were produced by reverse transcription using 5 μg total RNA. Hybridization was conducted overnight with labeled probes at 42 C. Membranes were washed, wrapped in plastic wrap, and exposed to Bio-Max film (Kodak, Rochester, NY) for 1–4 h and later exposed for 3–5 h on PhosphoImager cassettes (Amersham Pharmacia Biotech, Piscataway, NJ).

Northern blot analysis. Northern blots were performed as previously described (27, 28). Total RNA was extracted using TRIzol, separated on 1% formaldehyde-agarose gels, and transferred to nylon membranes. Blots were probed with [32P]dCTP-labeled cDNAs for rat PLF-Fα (18), the newly identified rat PLP-Fβ, mouse PLP-F (17), erythroid Krüppel-like factor (EKLF; GenBank accession no. AA926284), β-major globin (GenBank accession no. X15009), or erythroid 5-aminolevulinate synthase-2 (ALAS-2; GenBank accession no. NM_013197). A 28S rRNA cDNA probe was used to assess RNA integrity and as a loading control.

RT-PCR. PLP-Fα and PLP-Fβ transcripts were assessed by RT-PCR. Total RNA was extracted from Rcho-1 trophoblast cells using TRIzol. Five micrograms of total RNA and 0.5 μg of oligo dT were used for reverse transcription reactions with Superscript II RT (Invitrogen). PCR was performed using Platinum Taq DNA High Fidelity polymerase (Invitrogen) with PLP-Fα (forward: 5'-CTA TTA CAC CCA GGC CT-3'; reverse: 5'-AGA ATC TTC ACT ATT GAT GGA TAA) and PLP-Fβ (forward: 5'-CTG TTA AAT AAT GAC ACC AGA GT-3'; reverse: 5'-TCC AAG ACC TTC AAA TAT TAC TCC-3') specific primers. PCR was conducted for 30 cycles under the following conditions: preheat 94 C, denature 94 C for a min, anneal 60 C for a min, and extension 72 C for a min. PCR products were separated on 1% agarose gels and stained with ethidium bromide.

In situ hybridization. In situ hybridization was performed as previously described (29, 30). Ten-micrometer cryosections of tissues were prepared and stored at −80 C until used. Plasmids containing cDNAs for rat PLP-Fα (18) and PLP-Fβ were used as templates to synthesize sense and antisense digoxigenin-labeled riboprobes according to the manufacturer’s instructions (Roche Molecular Biochemicals, Indianapolis, IN). Tissue sections were air dried and fixed in ice cold 4% paraformaldehyde in PBS. Prehybridizations, hybridizations, and detection of alkaline phosphatase-conjugated antidigoxigenin were performed as previously reported (29, 30).

Red blood cell analysis

Blood samples (1–3 ml) were collected by cardiac puncture of anesthetized rats. Blood was collected into Vacutainer tubes and stored at 4 C until analyzed. Hematological measurements were performed using an Act10 hematological analysis system (Beckman Coulter, Miami, FL) at the Hematology Core Laboratory of the University of Kansas Medical Center (Kansas City, KS). Analyses focused on red blood cell numbers, hemoglobin concentrations, and hematocrit.

Statistical analysis

Data generated were analyzed with ANOVA. The source of variation from significant F ratios will be determined by Newman-Keuls multiple comparison test (31).
Results
Identification of two rat genes related to PLP-F

Screening of the rat genome database with the rat PLP-F cDNA sequence (18) resulted in the identification of the PLP-F gene and a closely related gene. Nucleotide sequence for the PLP-F-related gene was used to screen the National Center for Biotechnology Information EST database. Several ESTs were identified with high levels of nucleotide sequence identity, and one full-length EST was characterized (cDNA clone identification: UI-R-CX0-bxe-b-02–0-ULs1, GenBank accession no. BI276593). We termed the original PLP-F gene, clone identification: UI-R-CX0-bxe-b-02–0-ULs1, GenBank identity, and one full-length EST was characterized (cDNA ESTs were identified with high levels of nucleotide sequence identity, and their predicted amino acid sequences share 66% identity (Fig. 1A). Both PLP-Fα and PLP-Fβ consist of 250 amino acid proteins possessing a predicted 29 amino acid signal peptide and five homologously positioned cysteine residues, located at amino acids 100, 210, 215, 232, and 241. PLP-Fα has an additional cysteine located at amino acid 99. The predicted amino acid sequence of PLP-Fα includes three putative N-linked glycosylation sites (amino acids 35, 102, and 134), whereas PLP-Fβ possesses four putative N-linked glycosylation sites (amino acids 50, 102, 134, and 244), Rat PLP-Fα and PLP-Fβ are closely related to mouse PLP-Fα and PLP-Fβ; however, specific orthologous relationships cannot be defined.

Northern blotting analyses were used to determine gestational expression profiles of the rat PLP-Fα (Fig. 1B). mRNAs for both PLP-Fα were predominantly expressed in the junctional zone of the chorioallantoic placenta. Temporally, PLP-Fα was most abundant during midgestation, whereas PLP-Fβ was dominant during the later stages of pregnancy.

Rho-1 cells represent a rat trophoblast cell line capable of differentiating into trophoblast giant cells (21). RT-PCR analysis was used to determine the expression patterns of the two PLP-Fα in differentiating Rho-1 trophoblast cells (Fig. 1C). Differentiating Rho-1 trophoblast cells (trophoblast giant cells) primarily expressed PLP-Fα transcripts. Dissected midgestation trophoblast tissues (chorioallantoic placenta) and late gestation junctional zone served as positive controls for the PLP-Fα.

The PLP-Fα mRNAs were localized to gestation d 11.5 and gestation d 18.5 trophoblast tissues by in situ hybridization (Fig. 2). PLP-Fα exhibited predominantly a trophoblast giant cell distribution, and PLP-Fβ showed primarily a spongiontrophoblast cell expression pattern. Consistent with the Northern blot analysis, PLP-Fα expression was more prominent at d 11.5 of gestation and PLP-Fβ expression was predominant at gestation d 18.5.

In summary, rat trophoblast cells express two structurally related PLP-Fα subfamily genes (PLP-Fα and PLP-Fβ). The two genes are expressed predominantly by two different cell types (trophoblast giant cells and spongiontrophoblast cells) during different phases of pregnancy (midgestation and late gestation).

In vivo maternal hypoxia impacts PLP-F subfamily gene expression

Members of the PRL family have been shown to participate in adaptations to physiological stressors, including maternal hypoxia (8, 32, 33). Additionally, at least one member of the PLP-F subfamily specifically activates erythropoiesis (10, 15). Consequently, we investigated the effects of maternal hypoxia on PRL family gene expression using the PRL family miniarray assay (25). Pregnant female rats were exposed to the equivalent of 10% oxygen from d 6.5 to 12.5 of gestation (Fig. 3A). PRL gene family expression was assessed in chorioallantoic and choriovitelline placentas on gestation d 12.5. PLP-Fα was the only PRL family member significantly stimulated by maternal hypoxia (Fig. 3B). Choriovitelline pla-

Fig. 1. The rat possesses two genes related to PLP-F: PLP-Fα and PLP-Fβ. A, Amino acid sequence comparison of PLP-Fα (18) and PLP-Fβ (current study). Predicted signal peptide cleavage sites are indicated by an arrow. Homologous cysteine residues are shown in blue-outlined boxes and N-linked glycosylation sites are highlighted in red-shaded boxes. Asterisks below the sequences denote identity, and dots below the sequences denote similarity. B, Expression of PLP-Fα and PLP-Fβ mRNA in rat placenta during the second half of gestation. Placental tissues were isolated, dissected into junctional and labyrinth zones, extracted for total RNA, and analyzed by northern blotting. C, RT-PCR analysis of PLP-Fα and PLP-Fβ in placental tissue and Rho-1 trophoblast cells. Specific primer sets were used to identify and discriminate between PLP-Fα and PLP-Fβ. Lane A, Day 12.5 chorioallantoic placenta (CA) placenta; lane B, d 18.5 junctional zone (JZ) of the chorioallantoic placenta; lane C, Rho-1 trophoblast cells; lane D, positive control PLP-Fα plasmid; lane E, positive control PLP-Fβ plasmid. Glycerol-3-phosphate dehydrogenase (G3PDH) primers were used as a positive control for the RNA samples in lanes A–C.
Central PLP-Fα mRNA responses were more robust and consistent than chorioallantoic placental PLP-Fα responses (data not shown). The effects of maternal hypoxia in stimulating choriovitelline placental PLP-Fα mRNA were verified by Northern blot analysis (Fig. 3C).

As indicated above, the mouse possesses two members of the PLP-F subfamily, PLP-E and PLP-F. Mouse PLP-E and rat PLP-Fβ share commonalities in their temporal and spatial expression patterns. Pregnant female mice were exposed to the equivalent of 11% oxygen from d 5.5 to 11.5 of gestation (Fig. 3D). Northern blot analysis revealed increased expression of PLP-E mRNA in animals exposed to hypoxia compared with pair-fed normoxic controls (Fig. 3E).

Finally, we examined the affect of maternal hypoxia on the regulation of PLP-Fα and PLP-Fβ gene expression during the last week of gestation. Pregnant female rats were exposed to the equivalent of 10% oxygen from d 13.5 to 18.5 of gestation (Fig. 4A). Northern blot analyses of gestation d 18.5 junctional zone tissues indicated that PLP-Fα and PLP-Fβ were not responsive to hypoxia (Fig. 4B).

In summary, midgestation trophoblast rat PLP-Fα and mouse PLP-E expression respond positively to exposure to in vivo maternal hypoxia.

Impact of in vitro oxygen tension on trophoblast cell PLP-Fα gene expression

Rcho-1 trophoblast cells represent an excellent in vitro model for investigating aspects of trophoblast giant cell differentiation and function (21). In the following experiments, Rcho-1 trophoblast cells were induced to differentiate while exposed to ambient or 2% oxygen for 10 d (Fig. 5A). Northern blot analyses revealed that PLP-Fα mRNA levels were significantly elevated in the 2% oxygen-exposed cell cultures (Fig. 5B). Thus, both in vivo and in vitro models indicate that trophoblast cell PLP-Fα expression is sensitive to oxygen tension.

Erythroid responses to maternal hypoxia

To better understand the maternal response to hypobaric hypoxia, blood and spleen tissues were collected on gestation d 12.5 after 7 d of hypobaric hypoxia or in pair-fed pregnant animals exposed to ambient conditions (Fig. 6A) and analyzed for various hematological parameters. Exposure to maternal hypoxia significantly increased red blood cell numbers, hemoglobin concentrations, and hematocrit values (Fig. 6B). Additionally, key genes associated with hemoglobin synthesis (EKLF, ALAS-2, and β-major globin) were significantly elevated in splenic tissues from pregnant females exposed to hypobaric hypoxia (Fig. 6C). Collectively, the data suggest that the pregnant female rat responds to hypoxia, at least in part, through stimulation of the erythroid cell lineage.

Discussion

The PRL family has undergone species-specific expansions (5, 6). In the mouse and rat, the PRL family is exceptionally

Fig. 2. Localization of PLP-Fα and PLP-Fβ mRNAs within rat placental tissues. A, Schematic diagram of a midgestation uteroplacental compartment. The black-outlined box is the region of the placenta that is enlarged in C and E. B, Schematic diagram of late gestation uteroplacental compartment. The black-outlined box is the region of the placenta that is enlarged in D and F. In situ hybridization was used to identify the spatial distributions of PLP-Fs in the rat uteroplacental compartment. Cryosections were prepared from gestation d 11.5 (C and E) and d 18.5 (D and F) and hybridized to digoxigenin-labeled PLP-Fα (C and D) and PLP-Fβ (E and F) antisense and sense (data not shown) probes.
In this report, we investigate the rat PLP-F subfamily. This subfamily consists of a number of subfamilies (3, 5). These subfamilies are based strictly on structural relatedness. They exhibit elements of conservation between the mouse and rat and also species specificity. The PLP-F subfamily is of considerable interest because of recent advances in understanding its biological roles during pregnancy (9–15). This subfamily consists of two members in the mouse (PLP-E and PLP-F) and two members in the rat (PLP-Fα and PLP-Fβ).

In this report, we investigate the rat PLP-F subfamily. PLP-Fα and PLP-Fβ exhibit significant sequence similarities and structurally are both more closely related to mouse PLP-F than to mouse PLP-E. However, based on expression profiles, PLP-Fα shares similarities with mouse PLP-E, whereas PLP-Fβ parallels mouse PLP-F. Furthermore, we demonstrated that exposure of pregnant rats to hypoxic hypoxia is an effective technique for investigating pregnancy-dependent adaptations. Maternal hypoxia activates placental signals, including members of the PLP-F subfamily.

A, Schematic representation of in vivo rat experiments designed to evaluate placental responses to maternal hypoxia used to generate data presented in B and C. Pregnant Holtzman female rats were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 380 Torr, which resulted in an inspired PO2 of approximately 70 Torr, equivalent to breathing 10% O2 at sea level. Hypoxia exposure was initiated on d 13.5 of gestation and animals were killed at d 18.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). B, Examination of the effects of maternal hypoxia on choriovitelline placental PRL gene family expression using the PRL family miniarray. Total RNA was isolated from choriovitelline placental tissues on d 12.5 of gestation, radiolabeled by reverse transcription, and used as hybridization probes for rat PRL family miniarrays. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control. PLF-RP, Proliferin-related protein; dt/PRP, decidual/trophoblast PRL-related protein. C, Northern blot analysis of PLP-Fα mRNA in choriovitelline placental tissues from female rats exposed to hypoxia and ambient conditions. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

B. Pregnant Holtzman female rats were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 420 Torr, which resulted in an inspired PO2 of approximately 78 Torr, equivalent to breathing 10% O2 at sea level. Hypoxia exposure was initiated on d 6.5 of gestation and animals were killed at d 12.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). B, Examination of the effects of maternal hypoxia on choriovitelline placental PRL gene family expression using the PRL family miniarray. Total RNA was isolated from choriovitelline placental tissues on d 12.5 of gestation, radiolabeled by reverse transcription, and used as hybridization probes for rat PRL family miniarrays. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control. PLF-RP, Proliferin-related protein; dt/PRP, decidual/trophoblast PRL-related protein. C, Northern blot analysis of PLP-Fα mRNA in choriovitelline placental tissues from female rats exposed to hypoxia and ambient conditions. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

A, Schematic representation of in vivo mouse experiments used to generate data presented in E. Pregnant female CD-1 mice were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 420 Torr, which results in an inspired PO2 of approximately 78 Torr, equivalent to breathing 11% O2 at sea level. Hypoxia exposure was initiated on d 5.5 of gestation and animals were killed at d 11.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). E, Northern blot analysis of PLP-E mRNA in placental tissues from female mice exposed to hypoxia and ambient conditions. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

A, Schematic representation of in vitro rat experiments used to generate data presented in B. Proliferative Rcho-1 trophoblast cells were cultured for 3 d under proliferative conditions and then under differentiating conditions in 2% or ambient O2 for 10 d. B, Northern blot analysis of PLP-Fα mRNA in differentiating Rcho-1 trophoblast cells exposed to ambient conditions or low oxygen. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

A, Schematic representation of in vitro cell culture experiments used to generate data presented in B. Proliferative Rcho-1 trophoblast cells were cultured for 3 d under proliferative conditions and then under differentiating conditions in 2% or ambient O2 for 10 d. B, Northern blot analysis of PLP-Fα mRNA in differentiating Rcho-1 trophoblast cells exposed to ambient conditions or low oxygen. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.
are stimulated by exposure to maternal hypoxia. Although hypobaric hypoxia and pair-fed normoxia controls.

FIG. 6. Effects of maternal hypoxia on red blood cell parameters and expression of hemoglobin synthesis genes. A, Schematic representation of in vivo rat experiments used to generate data presented in B and C. Pregnant Holtzman female rats were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 380 Torr, which resulted in an inspired PO2 of approximately 70 Torr, equivalent to breathing 10% O2 at sea level. Hypoxia exposure was initiated on d 6.5 of gestation and animals were killed at d 12.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). B, Effects of maternal hypoxia on red blood cell parameters. Hematological variables were measured using an Act10 hematological analysis system. C, Expression of genes encoding key proteins involved in the regulation of hemoglobin synthesis from the maternal rat spleen. Northern blot analyses were performed for EKLF, ALAS-2, and β-major globin mRNAs in splenic tissue from animals exposed to hypobaric hypoxia and pair-fed normoxia controls.

During pregnancy, hematopoiesis is also initiated in extraembryonic and embryonic tissues. The midgestation placenta is a site of hematopoietic stem cell development (44–46), and the visceral yolk sac is an active site of erythropoiesis (41, 42). The peak in placental hematopoietic stem cell production and visceral yolk sac erythropoiesis correspond to the expression profile of PLP-F subfamily members; however, whether they have access to these extraembryonic targets and are capable of modulating their functions remains to be determined.

In summary, the placenta orchestrates adaptive responses to hypoxia, at least in part, via the elaboration of hormones and cytokines. PLP-Fa and PLP-E, in the rat and mouse, respectively, represent hormones/cytokines that are responsive to maternal hypoxia and possess biological actions contributing to pregnancy-dependent regulation of oxygen homeostasis.

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