A uterine decidual cell cytokine ensures pregnancy-dependent adaptations to a physiological stressor

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In the mouse, decidual cells differentiate from uterine stromal cells in response to steroid hormones and signals arising from the embryo. Decidual cells are crucially involved in creating the intrauterine environment conducive to embryonic development. Among their many functions is the production of cytokines related to prolactin (PRL), including decidual prolactin-related protein (DPRP). DPRP is a heparin-binding cytokine, which is abundantly expressed in uterine decidua. In this investigation, we have isolated the mouse Dprp gene, characterized its structure and evaluated its biological role. Dprp-null mice were made by replacing exons 2 to 6 of the Dprp gene with an in-frame enhanced green fluorescent protein (EGFP) gene and a neomycin (neo) resistance cassette. Heterozygous intercross breeding of the mutant mice yielded the expected mendelian ratio. Pregnant heterozygote females expressed EGFP within decidual tissue in locations identical to endogenous Dprp mRNA and protein expression. Homozygous Dprp-null mutant male and female mice were viable, exhibited normal postnatal growth rates, were fertile and produced normal litter sizes. A prominent phenotype was observed when pregnant Dprp-null mice were exposed to a physiological stressor. DPRP deficiency interfered with pregnancy-dependent adaptations to hypoxia resulting in pregnancy failure. Termination of pregnancy was associated with aberrations in mesometrial decidual cells, mesometrial vascular integrity, and disruptions in chorioallantoic placenta morphogenesis. The observations suggest that DPRP participates in pregnancy-dependent adaptations to a physiological stressor.

KEY WORDS: Dprp (Dtprp), Decidua, Pregnancy, Uterus, Null mutation, Adaptsions to hypoxia, Mouse

INTRODUCTION

The establishment of pregnancy requires maternal adjustments. Hemochorial placentation, which occurs in both primates and rodents, results in the establishment of a close connection between maternal and fetal tissues (Enders and Welsh, 1993; Carson et al., 2000). This close connection facilitates the exchange of nutrients and wastes. Decidual and trophoblast cells are likely to provide the signaling system that coordinates the activities of the maternal compartment. Decidual cells are modified uterine endometrial stromal cells. The differentiation of decidual cells is one of the earliest uterine adaptations to pregnancy (DeFeo, 1967; Parr and Parr, 1989; Aplin, 2000). Decidu al cell differentiation is exquisitely sensitive to the regulatory actions of progesterone, interleukin-11, and activators of cyclic AMP/protein kinase A (Tang et al., 1994; Lydon et al., 1995; Bilinski et al., 1997; Dimitriadis et al., 2005; Brosens and Gellersen, 1998). During gestation, decidual cells are located at the interface separating invading trophoblast cells from the maternal environment. A number of important functions have been attributed to decidua (Bell, 1983; Aplin, 2000; Brosens and Gellersen, 2006): (1) a protective role in controlling trophoblast cell invasion; (2) a nutritive role for the developing embryo; (3) a role in preventing immunological rejection of genetically disparate embryonic/fetal tissues; and (4) an endocrine/paracrine role in controlling maternal adaptations required for the establishment and maintenance of pregnancy. Pregnancy is dependent upon decidual cell acquisition of each of these specialized functions. Disruptions in decidual cell development are not compatible with pregnancy (Lydon et al., 1995; Bilinski et al., 1998; Robb et al., 1998; Mantena et al., 2006). Progress in understanding specialized decidual cell functions has been limited.

Decidual cell signaling is mediated, at least in part, through the production of cytokines related to prolactin (PRL) (Tang et al., 1994; Orwig et al., 1997; Telgmann and Gellersen, 1998; Jabbour and Critchley, 2001). PRL is a member of a larger collection of structurally-related hormones/ cytokines (the PRL superfamily) with an array of different biological targets and actions (Wiemers et al., 2003; Soares, 2004; Alam et al., 2006). In the rat and mouse, four members of the PRL superfamily are expressed in uterine decidua: decidual prolactin-related protein (DPRP; DTPRP – Mouse Genome Informatics) (Roby et al., 1993; Lin et al., 1997; Orwig et al., 1997b), prolactin-like protein B (PLP-B; PRLPB – Mouse Genome Informatics) (Duckworth et al., 1988; Croze et al., 1990; Cohick et al., 1997; Müller et al., 1998), PLP-J (PRLPJ – Mouse Genome Informatics) (Hiraoka et al., 1999; Ishibashi and Imai, 1999; Toft and Linzer, 1999; Dai et al., 2000) and prolactin itself (Prigent-Tessier et al., 1999; Kimura et al., 2001). Each of these decidual PRL family cytokines can be viewed as a downstream mediator of intrauterine progesterone action.

DPRP is secreted as a glycoprotein by uterine decidual cells and resides in the decidual extracellular matrix where it binds with high affinity to heparin-containing molecules (Rasmussen et al., 1996; Rasmussen et al., 1997; Orwig et al., 1997b; Wang et al., 2000). Little is known about the physiological actions of DPRP. In this report, we explore the biology of uterine decidual cells through investigation of the Dprp-null mouse.
Fig. 1. Mouse Dprp gene, construction of a Dprp-null mutant targeting vector, genotyping analysis, and Dprp mRNA and protein expression. (A) Exons 2-6 of the mouse Dprp gene were replaced with an in-frame EGFP gene followed by an MC1neo cassette. (B) PCR analysis of wild-type (+/+), heterozygous (+/-) and null (-/-) alleles. (C) RT-PCR analysis of Dprp transcripts in gestation day 7.5 decidua from wild-type (+/+) and Dprp-null (-/-) mice. (D) Western blot analysis of Dprp protein in gestation day 7.5 decidua from wild-type (+/+) and Dprp-null (-/-) mice.

MATERIALS AND METHODS

Gene targeting
A genomic DNA library generated from a 129/SvEv strain mouse liver and packaged in the Lambda FIX II vector was a generous gift of Lexicon Genetics (Houston, TX). Approximately 1×10^6 pfu were screened with a mouse Dprp cDNA (Orwig et al., 1997b). Positive plaques were amplified and used to inoculate LE392 Escherichia coli. A series of forward and reverse oligonucleotide primer sets based on the mouse Dprp cDNA were designed and used to sequence exons and exon-intron boundaries. DNA sequencing was performed with an Applied Biosystems Model 310 sequencer and Applied Biosystems Dye Terminator Cycle Sequencing Kits (Foster City, CA). The Dprp targeting vector was constructed by replacing exons 2-6 of the mouse Dprp gene with the enhanced green fluorescent protein (EGFP) gene and MC1neo cassette flanked by loxP sites (Godwin et al., 1998). A 6.6 kb DNA fragment, containing 3.8 kb of 5' flanking DNA and 2.8 kb of exon 1 and intron A of the mouse Dprp genomic construct, was subcloned upstream of EGFP. A 6.0 kb DNA fragment of the Dprp genomic construct containing 3' flanking DNA located immediately downstream of exon 6 was subcloned downstream of the MC1neo cassette and upstream of a herpes simplex virus thymidine kinase gene. The accuracy of vector construction was verified by restriction enzyme and DNA sequence analyses. A schematic representation of the mouse Dprp gene and the targeting vector are shown in Fig. 1. The targeting vector was introduced into R1 embryonic stem cells (Nagy et al., 1993) (a generous gift from Dr Janet Rossant, Samuel Lunenfeld Research Institute, Toronto, Canada) by electroporation. Cells were selected by exposure to G418 and gancyclovir. Southern blot analysis was used to identify clones that appropriately underwent homologous recombination with the targeting vector. Genomic DNA was isolated, digested with SalI, and fractionated in 0.8% agarose gels. Southern blots were performed with a probe derived from intron A. Wild-type alleles were characterized by a 21 kb hybridization signal; homozygous mutant alleles were characterized by a 5.5 kb hybridization signal. Chimeras were generated by injection into C57BL/6 blastocysts and transferred into pseudopregnant (C57BL/6×CBA) F1 females. PCR was routinely used to identify offspring with wild-type and Dprp mutant alleles. A forward primer corresponding to a nucleotide sequence in intron A of the Dprp gene (5'-GAGCTTAAACTTCAAATGTAAGT-3') was used with reverse primers corresponding to nucleotide sequences in intron B of the Dprp gene (5'-GGTTTGCTAATTAGCGTATGAGT-3') and within the EGFP gene (5'-GTATGCTGATTGATCTAGA-3'). PCR was conducted for 30 cycles under the following conditions: preheat, 94°C for 4 minutes; denature, 94°C for 1 minute; anneal, 60°C for 1 minute; and extension, 72°C for 1.5 minutes. PCR products (wild-type allele, 676 bp; mutant allele, 1148 bp) were separated on 1% agarose gels and stained with Ethidium Bromide. Mice with the Dprp mutation were backcrossed for six generations to C57BL/6 or 129SvJ genetic backgrounds.

Animals and tissue preparation
C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in an environmentally controlled facility, with lights on from 0600-2000 h, and allowed free access to food and water. Timed matings of animals were conducted by placing females with fertile males. The day when a seminal plug was found in the vagina of female mice was designated as day 0.5 of pregnancy. Placentation sites, including uterus, decidua, and placental tissues, were dissected from pregnant animals. Pseudopregnancy was induced by mating with vasectomized males. deciduomal reactions were induced on day 3.5 of pseudopregnancy by injecting 25 µl of sesame oil/uterine horn. Harvested tissues were snap-frozen in liquid nitrogen for RNA and protein analyses. For in situ hybridization and immunohistochemical analyses, tissues were frozen in dry ice-cooled systems. All tissue samples were stored at −80°C until used. Protocols for the above procedures have been described (Deb et al., 2006; Ain et al., 2006). Alkaline phosphatase activities in deciduomal tissue were measured as previously described (Soares, 1987; Arroyo et al., 2005). The University of Kansas Medical Center Animal Care and Use Committee approved all procedures for handling and experimentation with rodents.

Hypobaric hypoxia
Female C57BL/6 pregnant mice were placed in hypobaric chambers beginning on day 5.5 of pregnancy, as previously described (Ho-Chen et al., 2006). Under these conditions, air is circulated at a barometric pressure of

Table 1. Primer sets used for analysis of decidual PRL family transcripts

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tr>
<td>Dprp</td>
<td>5'-TGAATGTCAAAACAGGAGGAA-3'</td>
<td>5'-CAATCTTGCCAGTTATGCGG-3'</td>
</tr>
<tr>
<td>Plp-1</td>
<td>5'-TATGATGGAATACCAATGAA-3'</td>
<td>5'-GGTTTGTGGATTGGCCATGCT-3'</td>
</tr>
<tr>
<td>Plp-2</td>
<td>5'-GCCATTTAAGGTGCATTACT-3'</td>
<td>5'-GTCATATGCTGACTGTTG-3'</td>
</tr>
<tr>
<td>Gapdh</td>
<td>5'-ACACACCTCCATGCCATCC-3'</td>
<td>5'-TCCACACCCCTGTGCTGTA-3'</td>
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~420 Torr, which results in an inspired PO2 of ~78 Torr, equivalent to breathing 11% O2 at sea level. The chambers were opened daily to clean cages and replenish food and water (15-20 minutes).

**Phenotypic analyses of the uteroplacental compartment**

**Western blot analysis**

DPRP protein was detected in tissue extracts by immunoblotting as previously described (Rasmussen et al., 1996; Orwig et al., 1997b). Protein concentrations were determined for each sample using the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA).

**Histological analyses**

Analyses were performed on 10 μm tissue sections prepared with the aid of a cryostat. Sections were stained with Hematoxylin and Eosin, or subjected to biotinylated Grifonia simplicifolia lectin I isolectin B4 (Vector Laboratories, Peterborough, UK) histochemistry, or used for immunocytochemistry. Immunocytochemical analyses were used to determine the distribution of GFP, natural killer (NK) cells, trophoblast cells and endothelial cells (Ain et al., 2003) (T.K., L. A. Rempel, J. A. Arroyo and M.J.S., unpublished). GFP was monitored by fluorescence and immunoreactivity with rabbit anti-GFP polyclonal antibodies (Chemicon International, Temecula, CA). NK cells were detected with a rabbit polyclonal anti-perforin I antibody (Torrey Pines Biologs, Houston, TX). Trophoblast cells were monitored with a rat monoclonal anti-mouse cytokeratin antibody (TROMA-1; Developmental Studies Hybridoma Laboratory, Iowa City, IA). Endothelial cells were localized using a rat monoclonal anti-mouse endoglin antibody (Developmental Studies Hybridoma Laboratory, Iowa City, IA) and a rat monoclonal anti-mouse CD31 antibody (BD Pharmingen, Franklin Lakes, NJ). TUNEL assays were performed with the In Situ Cell Death Detection Kit (Roche Applied Science, Penzberg, Germany) according to the manufacturer’s instructions. All processed tissue sections were examined and images recorded with a Leica MZFLIII stereomicroscope equipped with a CCD camera (Leica Microsystems GmbH, Wetzlar, Germany).

**PRL superfamily mini-array assay**

The PRL superfamily mini-array assay is a hybridization-based tool for simultaneously monitoring expression of each member of the PRL superfamily (Dai et al., 2002). The assay has been effectively used to monitor the phenotypes of decidua and placenta. The PRL superfamily mini-array assay was performed as previously described (Dai et al., 2002).

**Northern blot analysis**

Northern blot analysis was performed as described previously (Faria et al., 1990). Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA (15 μg per lane) was resolved in 1% formaldehyde-agarose gels, transferred to nylon membranes and crosslinked. Blots were probed with [α-32P]-labeled cDNAs for Dprp (Orwig et al., 1997b), Plp-j (Dai et al., 2000), Plp-b (Müller et al., 1998) and metallothionein-I (MtiL) (Liang et al., 1996). Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) cDNA was used to evaluate the integrity and equal loading of RNA samples. At least three different tissue samples from three different animals were analyzed with each probe for each time point.

**RT-PCR analysis**

Dprp, Plp-j and Plp-b mRNA levels were estimated by RT-PCR. Total RNA was isolated from uterine tissues from days 5.5 to 7.5 of gestation. Total RNA (2 μg) and 0.5 μg of oligo d(T) were used for reverse transcription reactions with SuperScript II reverse transcriptase (Invitrogen). PCR was conducted using Platinum Taq DNA High Fidelity polymerase (Invitrogen) and Dprp-, Plp-j-, Plp-b- or Gapdh-specific primers (Table 1). PCR was performed for 30 cycles (denature, 95°C for 45 seconds; anneal, 55°C for 45 seconds; extension, 72°C for 1 minute). The amplified products were resolved by electrophoresis in 1% agarose gels and Ethidium Bromide staining.

**In situ hybridization**

The localization of mRNAs within tissues was performed as described previously (Ain et al., 2003; Weimers et al., 2003). Cryosections (10 μm) of tissues were prepared and stored at –80°C until used. Plasmids containing cDNAs for mouse Dprp and Plp-j (Orwig et al., 1997b; Dai et al., 2000) were used as templates to synthesize sense and antisense digoxigenin-labeled riboprobes according to the manufacturer’s instructions (Roche Molecular Biochemicals, Indianapolis, IN).

**Statistical analysis**

The data were analyzed by data of variance and post hoc comparisons determined by the Newman-Keuls Test.

**RESULTS**

**Generation of a Dprp-null mouse**

Screening of a mouse genomic library with the mouse Dprp cDNA resulted in the isolation of a phage clone containing the entire coding sequence for mouse Dprp. The Dprp gene possesses a 6-exon organization, similar to rat Dprp and other members of the PLP-C (PLP-C – Mouse Genome Informatics) subfamily (Dai et al., 1996; Orwig et al., 1997a; Weimers et al., 2003; Alam et al., 2006). Dprp-null mutant mice were generated by gene-targeting strategies culminating in the replacement of a region of

<table>
<thead>
<tr>
<th>Strains</th>
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<th>+/–</th>
<th>–/–</th>
<th>Male:Female</th>
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<td>28.5±3.80 (n=100)</td>
<td>28.49±0.6 (n=61)</td>
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<tr>
<td>C57BL/6</td>
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<td>24.83±2.09 (n=47)</td>
<td>23.57±1.75 (n=26)</td>
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<tr>
<td>129/SvJ</td>
<td>25.6±2.21 (n=20)</td>
<td>24.67±2.63 (n=43)</td>
<td>23.65±1.61 (n=21)</td>
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Table 3. Body weight at 55 days of age

There are no significant differences in body weight (g) among the male or female wild-type (+/+), heterozygous (+/–) or Dprp-null mutant (–/–) mice. Values are expressed as the means±d.

<table>
<thead>
<tr>
<th>Strains</th>
<th>+/-</th>
<th>+/–</th>
<th>–/–</th>
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<tbody>
<tr>
<td>Mixed</td>
<td>79.2±2.35 (n=12)</td>
<td>90.8±2.19 (n=12)</td>
<td>78.3±1.59 (n=12)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>8.71±1.58 (n=21)</td>
<td>8.24±2.53 (n=21)</td>
<td>8.66±1.8 (n=16)</td>
</tr>
<tr>
<td>129/SvJ</td>
<td>6.6±1.69 (n=16)</td>
<td>6.4±1.47 (n=22)</td>
<td>7.27±1.18 (n=18)</td>
</tr>
</tbody>
</table>

Table 4. Reproductive performance of Dprp mutant mice

Values are expressed as the means±d.

<table>
<thead>
<tr>
<th>Strains</th>
<th>+/- × +/-</th>
<th>+/- × +/–</th>
<th>+/– × +/-</th>
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<tbody>
<tr>
<td>Mixed</td>
<td>21.41±3.32 (n=21)</td>
<td>23.57±1.75 (n=26)</td>
<td>24.67±1.74 (n=27)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>18.63±1.74 (n=27)</td>
<td>24.83±2.09 (n=47)</td>
<td>24.67±2.63 (n=43)</td>
</tr>
<tr>
<td>129/SvJ</td>
<td>19.76±2.21 (n=21)</td>
<td>23.65±1.61 (n=21)</td>
<td>24.67±2.63 (n=43)</td>
</tr>
</tbody>
</table>

Table 2. Genotypic distribution of offspring from heterozygous matings

Heterozygous breedings yielded the expected mendellean ratio (1:2:1). Male:female ratio of wild-type (+/+), heterozygous (+/–) and Dprp-null mutant (–/–) mice were also comparable to each other.
ES cell lines (No. 44 and No. 96) with a normal karyotype were injected into blastocysts in order to generate chimeras. The No. 44 cell line gave rise to a >95% male chimera. The No. 96 cell line gave rise to three chimeras, including two males of 40% and 75% chimerism, and a female of 60% chimerism. Male chimeras from both the No. 44 and No. 96 lines were bred to C57BL/6 females and successfully transmitted the Dprp mutant allele to their offspring. Subsequent analyses were derived from mouse line No. 44. Breeding of mice heterozygous for the Dprp-null mutation resulted in offspring genotypes that did not significantly deviate from the expected mendelian ratio (Table 2). The mutation was moved to two inbred strains (C57BL/6 and 129SvJ) following six generations of backcrosses. Homozygous Dprp-null mutant male and female mice were viable on a mixed 129 SvJ and C57BL/6 genetic background and following transfer to C57BL/6 and 129SvJ genetic backgrounds. The offspring exhibited normal postnatal growth rates and were fertile (Tables 3 and 4). Genetic background did not significantly affect the phenotype of mice with the Dprp-null mutation. Genotyping and Dprp expression analyses are shown in Fig. 1. The gene targeting strategy successfully disrupted Dprp mRNA and protein expression.

Characterization of the uterine compartment in Dprp mutant mice

DPRP is known to be expressed in decidual and deciduomal tissues from both pregnant and pseudopregnant animals (Rasmussen et al., 1996; Rasmussen et al., 1997; Lin et al., 1997; Orwig et al., 1997b). The Dprp-null allele contains an EGFP gene inserted into the Dprp locus. Pregnant heterozygous (+/−) and homozygous null (−/−) females faithfully expressed EGFP within decidual tissue in locations similar to endogenous DPRP expression in pregnant wild-type (+/+ ) females (Fig. 2). However, the tissue distribution of EGFP in the mesometrial compartment of Dprp-null mice was less than the tissue distribution of DPRP protein in the mesometrial compartment of wild-type mice (Fig. 2). This difference continued to be evident on day 11.5 of gestation (Fig. 3).

Pregnancy proceeded in the absence of detectable Dprp mRNA. DPRP deficiency influenced the expression of another member of the decidual PRL superfamily, Plp-j (Fig. 4A). Plp-j mRNA levels were decreased in Dprp-null mutant decidua. DPRP deficiency did not significantly affect the expression of two other decidual products, Plp-b and Mt1 (Fig. 4A), and did not significantly affect expression of other members of the PRL superfamily within the placenta on days 12.5 or 17.5 of gestation (Fig. 4B).
We next examined decidualization in pseudopregnant wild-type and Dprp-null mice (Fig. 5). Deciduoma formation was similar in mice of both genotypes with only subtle differences, including a modest but significant decrease in deciduomal weight, when expressed per body weight (Fig. 5D). DPRP protein in wild-type and GFP in Dprp-null mice localized predominantly to the antimesometrial deciduomal compartment (Fig. 5F-H). Similar to pregnancy, Plp-j mRNA expression was also down-regulated in Dprp-null deciduoma (Fig. 5I).

The organization of the maternal-fetal interface was examined. Distributions of endothelial (endoglin and CD31) and NK cell (perforin 1) markers and TUNEL activity did not differ between wild-type and Dprp-null uteroplacental compartments on gestation days 7.5 and 9.5 (data not shown).

Overall, the DPRP deficiency appeared to have only modest consequences for the establishment and maintenance of pregnancy and the organization of the maternal-fetal interface under standard husbandry conditions.
**Impact of maternal hypoxia on the Dprp-null phenotype**

Successful species develop strategies to optimize their reproductive performance. This optimization is likely to include the evolution of genes that specifically permit reproduction in physiologically challenging conditions. The PRL superfamily has been postulated to participate in the regulation of adaptations to physiological stressors (Dorshkind and Horseman, 2001; Ain et al., 2004; Soares et al., 2006). These insights led us to examine a role for DPRP in the regulation of pregnancy-dependent adaptations to physiological stressors. Hypoxia was selected as a physiological stressor because it is well established that low oxygen tension promotes extensive tissue remodeling at the maternal-fetal interface (Zamudio, 2003; Fryer and Simon, 2006; Myatt, 2006).

In order to determine the time course for the physiological challenge, we first examined the ontogeny of decidual PRL family (Dprp, Plp-j and Plp-b) gene expression. Dprp expression was initiated between days 5.5 and 6.5 of gestation (Fig. 6A). Consequently, we challenged pregnant wild-type and Dprp-null mice from days 5.5 to 11.5 (duration of decidual Dprp expression during normal pregnancy) with the equivalent of 11% oxygen (21% oxygen is ambient at sea level). After hypoxia exposure, animals were returned to ambient conditions and examined on day 17.5 of gestation. Maternal hypoxia did not affect decidual Dprp gene expression in wild-type mice possessing the mutant Dprp gene did not adapt to hypoxia as well as did wild-type mice (Fig. 6C-E). Most fetal-placental units were healthy on day 17.5 of gestation in wild-type animals; whereas most fetal-placental units were dying or resorbing in the Dprp-null mice. We conclude from these observations that DPRP participates in pregnancy-dependent adaptations to hypoxia.

**Analysis of the maternal-fetal interface in Dprp-null mice exposed to hypoxia**

The defects responsible for pregnancy termination in Dprp-null mice exposed to hypoxia were unique. Initial gross inspection and histological examination indicated that only modest effects were evident by day 9.5 of gestation in Dprp-null mice exposed to hypoxia. However, notable pathologies were identified by day 11.5 of gestation; macroscopic lesions were discernible in the mesometrial region or in the mesometrial-anti-mesometrial junction of Dprp-null uteroplacental compartments, but were not evident in wild-type uteroplacental compartments (Fig. 7). Maternal hypoxia did not significantly affect decidual Dprp gene expression in wild-type mice or decidual EGFP expression in Dprp-null mice (data not shown).

Histological examination of tissue sections through the uteroplacental compartments revealed prominent adaptive as well as a range of potentially maladaptive responses to hypoxia in the wild-type and Dprp-null mice (Figs 8, 9). The adaptive responses to maternal hypoxia observed in the wild-type uterine mesometrial compartment included compression of the mesometrial decidua and increased depth of endovascular trophoblast cell invasion. The potentially maladaptive responses in the Dprp-null mesometrial compartment included: (1) enlarged mesometrial blood spaces (Fig. 8A,D); (2) distorted choioallantoic placental organization, including trophoblast giant cell overgrowth (Fig. 8B,E); (3) exaggerated compression of the mesometrial decidua (Fig. 8C,F);
and (4) decreased endovascular trophoblast invasion (Fig. 9). These aberrations may be related to the altered mesometrial decidua in the Dprp-null mouse noted above (Fig. 3). The net result is a failure in the placenta-specific adaptations to hypoxia required to ensure maintenance of pregnancy.

**DISCUSSION**

Decidua is a specialized uterine stromal cell modification found in species with hemochorial placentation (Aplin, 2000). It functions as a supportive structure that facilitates placentation and embryonic development and it is established that pregnancy does not proceed in its absence. In this report, we provide evidence that a secretory product of the uterine decidua is fundamental to the regulation of pregnancy-dependent adaptations to hypoxia. The decidual cell secretory product is a member of the PRL superfamily of hormones/cytokines.

The composition of the PRL superfamily is diverse and species-specific (Forsyth and Wallis, 2002; Soares, 2004). In the mouse and rat the PRL superfamily has expanded, consisting of approximately two dozen genes, whereas in other species (e.g. human and dog) the superfamily has but a single constituent (Wiemers et al., 2003; Alam et al., 2006). Why mammalian genomes evolved differently with respect to this classic hormone/cytokine is unknown. We have gained insights into the PRL superfamily through an examination of the biology of members of the expanded mouse PRL superfamily and have utilized a standard single gene mutation approach. Based on gene expression patterns, the PRL superfamily is linked to pregnancy (Soares, 2004). Previously, we demonstrated that a trophoblast cell-derived PRL family member, PLP-A (PRLPA – Mouse Genome Informatics), targets uterine NK cells and imposes only modest effects on the biology of pregnancy under standard laboratory housing conditions (Müller et al., 1999; Ain et al., 2004). In the current study, we have shown that another member of the PRL superfamily produced by uterine decidual cells, DPRP, also has subtle influences under ordinary husbandry conditions. However, both PLP-A and DPRP modulate pregnancy-dependent adaptations to hypoxia.

Wild-type pregnant mice can effectively adapt to hypoxia without fetal loss (Ho-Chen et al., 2006). Adaptations are dependent upon the timing, duration and magnitude of the hypoxic exposure. Among the pregnancy-dependent adaptations are events occurring at the maternal-fetal interface. Most notable are a compression of the mesometrial decidua and alterations in the uterine mesometrial vasculature, including its interactions with trophoblast cells. Null mutations in either the Plp-a gene or the Dprp gene interfere with adaptive responses to hypoxia and result in fetal loss. Under hypoxic conditions, the absence of PLP-A obstructs early stages of trophoblast-vascular interactions, disrupting nutrient delivery and leading to growth restriction (Ain et al., 2004). The hypoxia-exposed Dprp-null placenta is able to satisfactorily progress through this early interaction with the maternal environment but collapses a couple of days later, which is associated with a series of anomalies.
in the uterine mesometrial compartment and placenta. The appearance of vascular lesions, enlarged mesometrial blood spaces, distorted chorialloantoic placentas, and decreased endovascular trophoblast invasion characterize the Dprp-null mutant response to hypoxia. The specific aberration that leads to pregnancy failure is unknown. Some insights into the Dprp-null phenotype may be deduced from inspection of decidual tissue adjoining the developing chorialloantoic placenta.

The orientation of the post-implantation uterus is determined by the entry site of the vasculature. The region associated with vascular entry is referred to as the mesometrial compartment, and the opposite side of the uterus is referred to as the anti-mesometrial compartment. Mesometrial and anti-mesometrial decidua differ structurally and functionally (Kreibiel, 1937; Bell, 1983; Gu and Gibori, 1995). DPRP is expressed in anti-mesometrial decidua and in a smaller population of mesometrial decidual cells situated proximal to the developing chorialloantoic placenta (Orwig et al., 1997b; Rasmussen et al., 1997) (Figs 2, 3). Decidual cell Dprp expression is initiated between days 5.5 and 6.5 of gestation in the mouse. In the present study, abnormalities were not observed in the organization of anti-mesometrial decidua or in its neighboring tissues from Dprp-null mice under normoxic or hypoxic conditions. By contrast, prominent differences were noted in the mesometrial compartments of wild-type and Dprp-null mice. Such observations place more significance on the mesometrial decidual cell source of DPRP. This mesometrial decidual structure may be crucial in coordinating uteroplacental adaptations to hypoxia and may provide a key to understanding the phenotype of the Dprp-null mouse exposed to hypoxia.

DPRP is a cytokine possessing an affinity for heparin-containing structures (Rasmussen et al., 1996; Wang et al., 2000). Evidence suggests that DPRP does not circulate but instead is deposited within the decidua extracellular matrix. Although the DPRP protein is structurally related to PRL, DPRP does not utilize the PRL-receptor signaling pathway (Rasmussen et al., 1996). The mechanism of action of DPRP is unknown but may include an autocrine/paracrine activity required for the differentiation and/or survival of the mesometrial decidua (as suggested by the present study). Alternatively, DPRP may independently modulate mesometrial vascular-trophoblast interactions. Interestingly, PRL is produced by human decidual cells, possesses an affinity for heparin (Khurana et al., 1990), and its targets are likely to be intrauterine (Jabbour and Critchley, 2001). Whether human PRL produced by decidual cells functionally overlaps with DPRP and facilitates adaptations to physiological stressors remains to be determined.

Investigation of the Dprp-null mouse has permitted a dissection of mechanisms controlling decidual cell adaptations to physiological stressors, and has demonstrated the effectiveness of in vivo hypoxia as a tool for elucidating intrinsic regulatory processes controlling placentaion.

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References


Pregnancy-dependent adaptations to hypoxia

Müller, H., Ishimura, R., Orwig, K. E., Liu, B. and Soares, M. J.


Nagy, A., Rossant, J., Nay, R., Abramow-Newerly, W. and Roder, J. C.

Orwig, K. E., Ishimura, R., Müller, H., Liu, B. and Soares, M. J.

Prolactin in early pregnancy.

Homologues for prolactin-like protein-A and B are present in the mouse.


