**Decidual Prolactin-Related Protein: Heterologous Expression and Characterization**

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**ABSTRACT**

As a first step in understanding the role of decidual PRL-related protein (dPRP) during pregnancy, we have generated recombinant dPRP protein. In this report, we present data on the generation, purification, and characterization of recombinant dPRP protein. The dPRP complementary DNA was subcloned into the pMSXND vector, and the vector was transfected into Chinese hamster ovary (CHO) cells by electroporation. After appropriate selection, amplification, and induction procedures, recombinant dPRP was purified from conditioned medium of the CHO-dPRP cells using ultrafiltration, size-exclusion chromatography, and reverse phase HPLC. Recombinant dPRP was found to possess electrophoretic mobility, immunoreactivity, and N-terminal amino acid sequence identical to those of dPRP isolated from decidual tissue. Polyclonal antibodies were generated to the recombinant dPRP and used for Western blot analysis. dPRP is capable of binding heparin, and a significant fraction of synthesized dPRP resides within the decidual extracellular matrix. Recombinant dPRP failed to bind to PRL receptors and showed no stimulatory activity in the PRL-dependent rat Nb2 lymphoma cell proliferation assay. Additional studies have shown that heterologous expression of dPRP in CHO cells significantly increased the ability of CHO cells to form tumors in athymic mice. In conclusion, recombinant dPRP possesses characteristics similar to those of dPRP of decidual origin and is a heparin-binding protein that may facilitate the establishment of pregnancy.

Decidual Tissue represents a specialized uterine compartment arising in association with implantation of the rodent embryo. During its development, decidual cells completely surround the postimplantation embryo and are situated in direct contact with trophoblast cells at the maternal-embryo interface. It has long been speculated that the decidua plays a critical role in the establishment of pregnancy, but knowledge concerning the exact mechanism(s) by which it does so is limited. Postulated functions of decidual cells include 1) providing a nutritive role in maintenance of the embryo before development of the fetal circulatory system, 2) limiting invasion of trophoblast cells into the uterus, 3) preventing immunological rejection of the fetal allograft, and 4) producing hormones that act in paracrine or endocrine modes to influence the functions of embryonic, extraembryonic, or maternal tissues (1–3). The latter function is probably fundamental to each of the responsibilities of decidual cells.

The present study focuses on a protein produced by decidual cells, termed decidual PRL-related protein (dPRP). dPRP was first identified in rat decidual tissue based on its homology with members of the placental PRL family (4). The dPRP protein and complementary DNA (cDNA) have been isolated and characterized (4). dPRP is a 29-kDa glycoprotein with approximately 70% amino acid sequence homology to a member of the placental PRL family, PRL-like protein C (PLP-C) (4, 5). Another member of the PRL family, PRL-like protein B, is antigenically related to dPRP and has been shown to be expressed in rat decidual cells (6). In addition, evidence exists for the presence of a rat decidual protein related to PRL possessing actions on the ovary and uterus (7, 8). Whether dPRP or PLP-B is responsible for those ovarian and uterine PRL-like effects remains to be determined.

To begin to determine the biological actions of dPRP during the establishment of pregnancy, we used the dPRP cDNA to express the dPRP protein. In this report, we describe the isolation and characterization of recombinant rat dPRP, the generation of antibodies to dPRP, and the utilization of these tools to investigate the biology of dPRP.

**Materials and Methods**

Reagents

FBS and donor horse serum were purchased from JRH Bioscience (Lenexa, KS). Reagents for PAGE were purchased from Bio-Rad Laboratories (Hercules, CA). Adjuvant for immunizations was obtained from Organon Teknika Corp. (West Chester, PA). Methotrexate was purchased from Calbiochem (San Diego, CA). All restriction enzymes, polymers, and DNA ligase were purchased from New England Biolabs (Beverly, MA). Lactoperoxidase was acquired from Boehringer Mannheim Biochemicals (Indianapolis, IN). Equine CG was purchased from Calbiochem (La Jolla, CA). Nitrocellulose was obtained from Schleicher and Schuell (Keene, NH). Ovine PRL was obtained from Nobil Laboratories (Sioux Center, IA). Na[125]I was purchased from DuPont-New England Nuclear (Boston, MA). Heparin-Sepharose was obtained from Pharmacia Fine Chemicals (Piscataway, NJ). Biotinylated Delichos biflorus was acquired from Vector Laboratories (Burlingame, CA). Antibodies against von Willebrand factor were obtained from Dako Corp. (Carpinteria, CA). hCG was obtained from Schein Pharmaceutical (Port...
DECIDUAL PRL-RELATED PROTEIN

Washington, NY). The chemiluminescent detection system was purchased from Amersham Life Science (Arlington Heights, IL). Unless otherwise noted, all other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and tissue preparation

Holtzman rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in an environmentally controlled facility, with lights on from 0600–2000 h, and allowed free access to food and water. Timed pregnancies and pseudopregnancies and tissue dissections were performed as previously described (4,9). The presence of a copulatory plug or sperm in the vaginal smear was designated day 0 of pregnancy. Pseudopregnancy was induced by vagino-cervical stimulation on the evening of proestrus with a mechanical vibrator (10,11). The first day of pseudopregnancy was defined as the first day of leukocytic vaginal smears after stimulation. Decidual responses were induced in pseudopregnant rats by the injection of 50–100 μl sesame oil/uteroine horn on day 4 of pseudopregnancy (12). Decidual and placental cytosol were prepared as previously described (13). Protein concentrations of cytosol preparations were estimated by the method of Bradford (14).

Membrane preparations enriched for PRL receptors were isolated from hepatic and ovarian tissues of prepubertal female rats hormonally treated according to the procedure of Javatilak et al. (15). Briefly, on day 25 postconception, rats were treated with 50 IU equine CG followed by 25 IU hCG approximately 55 h later. One week after CG treatment, animals were killed, and ovaries and uteri were dissected, frozen in liquid nitrogen, and stored at −80°C until used.

BALB/Cathylic mice were obtained from Charles River Laboratories (Wilmington, MA), housed in microisolator cages, and used to evaluate tumor protocols. Protocols for the care and use of animals were approved by the University of Kansas institutional animal care and use committee.

Generation of recombinant dPRP

Construction of the dPRP expression vector. dPRP cDNA was subcloned into an expression vector termed pMSXND, previously constructed by Drs. S.-J. Lee and Daniel Nathans of Johns Hopkins University (16). The pMSXND vector contains a metallothionein promoter, simian virus 40 splicing and polyadenylation signals, a neomycin resistance gene, and a dihydrofolate reductase gene. The protocol we used has been previously described by our laboratory for the generation of recombinant PLP-A (17) and placental lactogen I (PL-I) variant (18).

A 911-bp EcoRI fragment containing the entire coding sequence of dPRP was isolated from a dPRP-Bluescript plasmid after electrophoretic separation in a 5% polyacrylamide gel. Terminal linkers were added to the ends, enabling the dPRP cDNA to be inserted into the XhoI site of the pMSXND vector. Subcloning into the XhoI site situates the dPRP insert downstream of the mouse metallothionein I promoter and upstream of the simian virus 40 splicing and polyadenylation signals. Recombinant plasmid DNA was purified from Escherichia coli JM105 cells by lysozyme and alkaline-SDS lysis of bacterial cells obtained from the American Type Culture Collection (ATCC CCL 61. CHO-K1. Rockville, MD) were used as the host for the dPRP expression vector. CHO cells were routinely maintained in DMEM-MCDB-302 culture medium containing 1 mM proline, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FBS. Transfection (via electroporation, selection with geneticin, G418), and amplification (with methotrexate) were performed as described previously (17,18). The final genetically modified cell line was referred to as CHO-dPRP. Expression of recombinant dPRP protein was monitored by Western blot analysis.

Generation of conditioned medium from CHO cells transfected with the dPRP expression vector. After selection and amplification, the transfected CHO cells were seeded into roller bottles and grown to confluence in DMEM-MCDB-302 medium containing 10% FBS, antibiotics, and 25 mM HEPES. Once confluent, the cells were washed with medium without serum and cultured in the same serum-free medium supplemented with 100 μM cadmium chloride. The conditioned medium was collected at 24-h intervals for 8 days. The first 24-h collection was discarded because of the presence of residual serum. The remainder of the conditioned medium was pooled and stored frozen at −25°C until further analysis.

Purification of recombinant dPRP. Approximately 3 liters conditioned medium were processed at one time. The medium was thawed, then centrifuged at 500 × g for 25 min to remove debris. The medium was concentrated using an Amicon ultrafiltration apparatus (mol wt cut-off, 10,000). The concentrated medium was chromatographed on a Sephacryl S-200 column (2.5 × 90 cm) equilibrated with 20 mM sodium phosphate buffer, pH 7.4, containing 300 mM NaCl. Fractions collected from the gel filtration column were monitored by absorbance at 280 nm and by immunoreactivity with antibodies to amino acids 140–159 of PLP-B (4) or later with antibodies to recombinant dPRP. Immunopositive fractions were pooled, concentrated by ultrafiltration, and further purified to homogeneity by reverse phase HPLC using a 15–90% acetonitrile-0.1% trifluoroacetic acid gradient over 35 min at a flow rate of 1 ml/min.

Characterization of recombinant dPRP

Amino-terminal sequence analysis. Amino acid sequences were determined by the Edman degradation method, using a gas phase protein sequencer at the University of California-Los Angeles Protein Microsequencing Facility. Approximately 100 pmol protein were sequenced. Cysteine residues were not protected and thus could not be determined from the analysis.

Generation of antibodies to recombinant dPRP. Purified recombinant dPRP (150 μg/rabbit) was used to immunize two New Zealand White rabbits (Myrtle Rabbitry, Thompson Station, TN). Booster injections (75 μg each) were given at 2-week intervals. The remainder of the immunization protocol and serum collection were performed as previously described (13).

Western blot analysis. Western blot analyses were performed as previously described (13,20). Samples were separated by SDS-PAGE in 12.5% gels under reducing conditions. Proteins from the gels were electroblotted to nitrocellulose. Antibodies generated to a synthetic peptide corresponding to amino acids 140–159 of PLP-B (4), recombinant PLP-A (17), and recombinant dPRP were used as probes. The nitrocellulose filters were then developed by exposure to either an alkaline phosphatase-labeled antirabbit IgG or a peroxidase-labeled antirabbit IgG followed by a chemiluminescent detection system. In some experiments, preimmune serum or antibodies saturated with the respective antigens were used as controls.

Characterization of dPRP heparin interactions

Heparin-Sepharose chromatography. Minicolumns containing either heparin-Sepharose CL-6B or unconjugated Sepharose 6B were prepared and equilibrated with equilibration buffer (10 mM PBS, pH 7.4, containing 0.15 mM NaCl) (21). Samples were diluted to 1 bed vol with equilibration buffer, applied directly to the minicolumns, and placed on a platform rocker for 30 min at 4°C. Unbound proteins were collected by centrifugation and precipitated with cold acetone. Minicolumns were washed with 5 bed vol equilibration buffer, which was also collected and precipitated with cold acetone. Bound proteins were eluted from the resin by boiling in a buffer containing 20% glycerol, 125 mM Tris-HCl, and 1.4 M NaCl (21). The existence of a possible heparin-binding domain located downstream of amino acids 93–100 of dPRP was also evaluated (22). This region was synthesized (Bio-Synthesis, Lewisville, TX), and heparin-Sepharose minicolumns were prepared as before and pretreated with either 800 μl synthetic peptide or equilibration buffer. After washing, 8 μl dPRP were loaded onto the minicolumns, and fractions were separated by SDS-PAGE and immunoblotted with antibodies to dPRP as described above.
Effects of dPRP on angiogenesis

PRL RRA. The procedures used for radiiodination and membrane preparation, and the assay conditions were based on the protocols of Haro and Talamantes (26) and have been described in detail previously (18).

PRL in vitro bioassay. PRL-like bioactivity was assessed by evaluating the proliferation of Nb2 rat lymphoma cells as previously described (18, 27, 28).

Effects of dPRP on angiogenesis

Endothelial cell proliferation assay. Bovine aortic endothelial cells (29) or mouse heart small vessel endothelial cells (30) were grown in DMEM plus 10% FBS and 10% calf serum or DMEM-MCDB-302 medium plus 10% FBS, respectively. To assess proliferation, cells were plated at a density of 1 x 10^5 cells/well in 24-well plates containing 1 ml medium. Cells were allowed to attach for 24 h, then were incubated with varying concentrations of dPRP and serum in medium (100 pg to 10 μg dPRP per well without heparin; 0-10% FBS or 10% calf or horse serum). Cells were incubated for 5 days, with a change in medium after 1 week. At the end of the 5-day culture period, cultures were washed with 10 ml PBS, pH 7.3, and stained with crystal violet (300 μl/well; 5% formalin, 50% ethanol, 150 mM NaCl, and 0.5% crystal violet) for 5 min. Plates were extensively washed with water and eluted with 1 ml ethylene glycol. Cell density was estimated by absorbance of the eluate at 600 nm (31, 32).

In vitro transplantation of genetically engineered CHO cells. CHO cells transfected with expression vectors for dPRP (present study), PLP-A (17), or control CHO cells were grown in 75-cm² flasks in DMEM-MCDB-302 medium plus 10% FBS. Parental CHO cells (nontransfected) were grown as a control. Cells (1 x 10^6) were SC injected into the upper dorsal areas of female athymic mice (33). To stimulate the expression of dPRP through the metallothionein promoter, ZnSO₄ was added to the drinking water (6 mM final concentration). Tumor growth and volume were monitored either frozen in liquid nitrogen for analysis of transgene expression or with antipeptide antibodies to amino acids 140-159 of PLP-B. Lanes A and B were incubated with antipeptide antibodies to amino acids 140-159 of PLP-B. Lanes C and D were incubated with anti-PLP-B antibodies. Lanes E were incubated with anti-PLP-B antibodies. Lanes F were incubated with anti-dPRP antibodies. Lanes G were incubated with anti-PLP-B antibodies. Lanes H were incubated with anti-dPRP antibodies.

Results

Heterologous dPRP expression and purification

CHO cells transfected with the dPRP expression vector produced a 29-kDa protein with immunochemical, electrophoretic, and biochemical characteristics similar to those of native dPRP (Figs. 1-3). dPRP production was initially examined with antipeptide antibodies directed to amino acids 140-159 of PLP-B. These antibodies are known to recognize dPRP (4) and specifically cross-react with a 29-kDa protein.
we hypothesized that dPRP may have a more restricted activity in the rat Nb subcellular fraction (Fig. 6) and showed negligible stimulatory activity in the rat Nb lymphoma cell proliferation assay (data not shown). Studies with other heparin-binding proteins have demonstrated a consensus heparin-binding domain. Two motifs, -B-B-X-B- and -B-B-B-X-B-, where B represents a basic residue and X represents a nonbasic residue, have been shown to be heparin-binding domains (22). A region of dPRP spanning amino acids 84-98 resembles a heparin-binding domain. The putative heparin-binding domain was synthesized and tested for its ability to bind heparin. In a variety of experiments, we failed to show any significant affinity of the putative dPRP heparin-binding domain for heparin. To further characterize dPRP's ability to bind to heparin in vivo, CHO-dPRP cells were incubated with the glycosaminoglycan-degrading enzyme heparinase. As shown in Fig. 5, heparinase-treated cultures released dPRP from the cellular monolayer. In contrast, there was little release of dPRP from vehicle-treated cultures. Collectively, these results suggest that dPRP may bind to a heparan sulfate proteoglycan. To further establish an in vivo relationship between dPRP and decidual ECM, we extracted ECM from decidual tissue and tested for the presence of laminin and dPRP. Western blot analysis with antibodies to rat laminin revealed the presence of laminin in the decidual ECM preparation (data not shown). Our results also show the presence of dPRP in the ECM of decidual tissue (Fig. 5).

Characterization of PRL-like activities
dPRP did not displace ovine PRL from ovarian or hepatic PRL receptors (Fig. 6) and showed negligible stimulating activity in the rat Nb lymphoma cell proliferation assay (Table 1). These results indicate that dPRP does not use the PRL signaling pathway.

Fig. 2. Electrophoretic separation of protein samples from various steps in the purification of dPRP from CHO cell-conditioned medium. Samples were separated in 12.5% polyacrylamide gels and stained with Coomassie blue (lanes B-D) or transferred to nitrocellulose and immunoblotted with antibodies to recombinant dPRP (lanes E-G). Lanes B and E, Concentrated conditioned medium from CHO cells transfected with the pRDPRP expression vector; lanes C and F, pooled peak fractions containing dPRP collected from the Sephacryl S-200 gel chromatography column; lanes D and G, peak collected from reverse phase HPLC. Lane A, standards (×10^(-9)).

Fig. 3. Characterization of antibodies generated to recombinant dPRP. Lanes A and D, Medium conditioned by decidual cell cultures; lanes B and E, deciduoma cytosolic preparations; lanes C and F, conditioned medium from CHO-dPRP cells. Samples were separated by SDS-PAGE in 12.5% gels, electrophoretically transferred to nitrocellulose, and examined by Western blot analysis using an antiserum to recombinant dPRP at a final dilution of 1:20,000. Antibodies used in lanes D, E, and F were saturated with recombinant dPRP. M, standards (×10^(-9)) are shown.

Abrogated its ability to bind heparin, providing evidence for the specificity of the dPRP-heparin interaction (data not shown). Studies with other heparin-binding proteins have demonstrated a consensus heparin-binding domain. Two motifs, -B-B-X-B- and -B-B-B-X-B-, where B represents a basic residue and X represents a nonbasic residue, have been shown to be heparin-binding domains (22). A region of dPRP spanning amino acids 84-98 resembles a heparin-binding domain. The putative heparin-binding domain was synthesized and tested for its ability to bind heparin. In a variety of experiments, we failed to show any significant affinity of the putative dPRP heparin-binding domain for heparin. To further characterize dPRP's ability to bind to heparin in vivo, CHO-dPRP cells were incubated with the glycosaminoglycan-degrading enzyme heparinase. As shown in Fig. 5, heparinase-treated cultures released dPRP from the cellular monolayer. In contrast, there was little release of dPRP from vehicle-treated cultures. Collectively, these results suggest that dPRP may bind to a heparan sulfate proteoglycan. To further establish an in vivo relationship between dPRP and decidual ECM, we extracted ECM from decidual tissue and tested for the presence of laminin and dPRP. Western blot analysis with antibodies to rat laminin revealed the presence of laminin in the decidual ECM preparation (data not shown). Our results also show the presence of dPRP in the ECM of decidual tissue (Fig. 5).

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Fig. 4. dPRP was not detectable in serum from pseudopregnant decidualized rats, as measured by Western blot analysis (data not shown). In contrast, other members of the PRL family are abundantly present in maternal serum (34). Thus, we hypothesized that dPRP may have a more restricted distribution and possibly associate with the decidual ECM. Heparan sulfate proteoglycan is known to bind an array of cytokines and growth factors and was evaluated as a possible reservoir for dPRP (35). Heparin-Sepharose affinity chromatography has been used to characterize and purify a number of heparin-binding molecules, such as basic fibroblast growth factor (23), lipoprotein lipase (21), and heparin-binding epidermal growth factor (36). In the present experiments, dPRP was shown to bind strongly to heparin, requiring treatment with SDS for dissociation. Both native and recombinant dPRP bound to heparin in vitro (Fig. 4). dPRP did not bind to unconjugated Sepharose, and heat denaturation of dPRP

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Effects of dPRP on angiogenesis

Members of the PRL family have been shown to have effects on angiogenesis (37, 38). This information in addition to the localization of dPRP to an area of the pregnant uterus with limited vacularization (39) led us to hypothesize that dPRP may be involved in the control of uterine blood vessel development. To examine the effects of dPRP on endothelial cells in vitro, we tested its ability to affect endothelial cell proliferation. Our experiments showed that dPRP had limited effects on endothelial cell proliferation (data not shown). A number of factors can stimulate angiogenesis in vivo without stimulatory effects on endothelial cells in vitro (40). To determine whether dPRP modulates angiogenesis in vivo, we tested its ability to affect tumor development in athymic mice, similar to the procedure described by Ueki et al. (33). Control CHO cells or CHO cells expressing dPRP or PLP-A generated solid tumors after sc transplantation in athymic mice. Histological examination of tumor tissue revealed no significant differences in the architecture or morphology of dPRP-producing tumors compared to that of control tumors (Fig. 7). Lectin histochemical detection of endothelial cells detailed prominent neovascularization in both types of tumors (Fig. 7). Immunoreactive dPRP was identified in the...
CHO-dPRP tumors by Western blot analysis (Fig. 8). Although dPRP expression did not affect tumor structure, a striking effect was noted in the incidence of the tumors (Table 2). There was a significantly greater percentage of mice injected with CHO cells expressing dPRP-generated tumors than of mice injected with control CHO cells or CHO cells expressing PLP-A (Table 2). The difference in tumor incidence was not correlated with in vitro growth rates of the cell populations. Control CHO cells grew significantly faster in vitro (1.1 ± 0.4 absorbance units) than either CHO-dPRP cells (0.6 ± 0.1 absorbance units) or CHO-PLP-A cells (0.7 ± 0.03 absorbance units).

**Discussion**

In the present report, we expressed dPRP in CHO cells, characterized recombinant dPRP protein, generated poly-

![Graph](image)

**Table 1.** Effects of dPRP and ovine PRL on the proliferation of rat Nb2 lymphoma cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell no. (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0 ± 0.02</td>
</tr>
<tr>
<td>dPRP (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>1.1 ± 0.08</td>
</tr>
<tr>
<td>1.0</td>
<td>1.1 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>1.0 ± 0.08</td>
</tr>
<tr>
<td>100</td>
<td>0.9 ± 0.07</td>
</tr>
<tr>
<td>1000</td>
<td>1.0 ± 0.02</td>
</tr>
<tr>
<td>Ovine PRL (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>2.9 ± 0.08</td>
</tr>
<tr>
<td>1.0</td>
<td>7.0 ± 0.34</td>
</tr>
<tr>
<td>10</td>
<td>8.8 ± 0.61</td>
</tr>
<tr>
<td>100</td>
<td>8.8 ± 0.11</td>
</tr>
<tr>
<td>1000</td>
<td>7.4 ± 0.42</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. *Significantly different than control cultures, P < 0.01.
FIG. 7. Morphology and vessel formation in CHO cell tumors. Tissue sections from control CHO cell tumors (A and C) and CHO-dPRP-expressing tumors (B and D) were stained with hematoxylin and eosin (A and B; magnification, ×100) or Dolichos biflorus agglutinin (C and D; magnification, ×40). Both types of tumors show spindle-shaped cells with prominent nuclei and are well vascularized.

TABLE 2. Tumor incidence in mice injected with parental CHO cells and transfected CHO cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. of mice injected</th>
<th>% Tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>13</td>
<td>39</td>
</tr>
<tr>
<td>CHO-dPRP</td>
<td>15</td>
<td>87</td>
</tr>
<tr>
<td>CHO-PLP-A</td>
<td>5</td>
<td>40</td>
</tr>
</tbody>
</table>

FIG. 8. Western blot analysis of dPRP expression in CHO-dPRP tumors. CHO-dPRP tumor cytosolic preparations (designated 1 and 2 and obtained from tumors from different mice) were electrophoretically separated on a 12.5% polyacrylamide gel, transferred to nitrocellulose, and examined for the presence of dPRP by Western blot analysis using antiserum to dPRP. Recombinant dPRP was used as a control. M, standards (×10^-5) are shown.

The actions of members of the extrapituitary PRL family can be distinguished based on their utilization of the PRL receptor signaling pathway. PL-I, PL-II, and PL-I variant bind to PRL receptors and appear to exhibit biological actions similar to those of pituitary PRL (classical members) (18, 42, and 45-47) (Dai, G., and M. J. Soares, unpublished results). dPRP does not use the PRL receptor and does not effectively stimulate the proliferation of lactogen-dependent Nb lymphoma cells (present study). Gibori and colleagues have demonstrated that the decidual luteotrophin acts on the cor-

Tissue distribution of a hormone or cytokine has direct bearing on its accessibility to target cells. Some extracellular signals circulate free or bound to transport proteins, whereas others act locally, often tethered to the surface of cells or the ECM (35, 44). Western blot analysis of serum from pseudo-pregnant decidualized rats indicated that unlike other members of the PRL family, dPRP did not circulate at detectable levels. dPRP was found to associate with heparin and was localized at least in part within the decidual ECM. Heparin binding is not an attribute previously reported for the PRL family; however, it is a feature of a number of growth factors and cytokines, such as fibroblast growth factor and heparin-binding epidermal growth factor, that influence cellular behavior principally via a paracrine mode of action (23, 36). Modulatory factors present in the decidual ECM, such as dPRP, would be well situated to influence the behavior of decidual cells and other cell types traversing the decidual ECM, such as trophoblast, endothelial, and various immune cells.
pus luteum via its utilization of the PRL receptor signaling system (7). Thus, our findings do not support a relationship between dPRP and the decidual luteotrophin. This places dPRP in a subgroup of PRL structural relatives with distinctive biological actions (nonclassical members). Also included in the nonclassical subgroup are a few members of the rat placental PRL family, PLP-A, PLP-B, and PLP-C (17, 48) (Cohick, C. B, and M. J. Soares, unpublished results); two members of the mouse placental PRL family, proliferin and proliferin-related protein (16, 38); and a 16-kDa fragment of pituitary PRL (37).

Progress in identifying the biological activities of nonclassical members of the PRL family has been limited. Proliferin, proliferin-related protein, and the 16-kDa fragment of PRL have been shown to specifically participate in the control of blood vessel development (37, 38). Proliferin stimulates angiogenesis, whereas proliferin-related protein and the 16-kDa fragment of PRL inhibit angiogenesis. This information coupled with the intrauterine expression pattern of dPRP in a region relatively devoid of vasculature (39) prompted us to hypothesize that dPRP would be antiangiogenic. dPRP did not markedly influence the development of vascular structures, as evaluated in both in vitro and in vivo assays. Although our prediction was not supported by the experimental evidence, results from the in vivo analyses implicated dPRP in alternative physiological roles within the uterus. We used a tumor development model to evaluate the role of dPRP on angiogenesis. In this model, the putative modulator is stably overexpressed in CHO cells and then transplanted into athymic mice (33). Tumor growth is directly related to the vascularization of the transplant. We did not observe any significant differences in the morphology, vascularity, or growth of control vs. dPRP-expressing tumors. Heterologous expression of dPRP in CHO cells was, however, associated with an increase in the ability of CHO cells to form tumors after transplantation into athymic mice. Thus, dPRP expression was correlated with an altered relationship between the tumor cells and their host, thereby increasing the success rate for establishing a tumor. In the future, it will be necessary to directly examine the effects of dPRP on tumor cell-host relationships.

Parallels between host regulatory events in tumor transplantation and establishment of the genetically foreign embryo within the uterus are evident (49). Natural killer (NK) cells and macrophages are potential effector cells in tumor resistance (50, 51) and have also been implicated in the maternal immune response to pregnancy (52, 53). Inhibition of pregnancy. Previous studies have shown that decidua development in athymic mice transplanted with CHO cells overexpressing dPRP. Similarly, dPRP may be regulating maternal immune cell responses during the establishment of pregnancy. Previous studies have shown that decidua products influence macrophage function (54, 55). In addition, and most interestingly, there is a virtual absence of macrophages and NK lineage cells in regions of the uterus that express dPRP (Refs. 53 and 56–58 and the present study) (Rasmussen, C. A, K. E. Orwig, and M. J. Soares, unpublished results). Thus, dPRP is a candidate cytokine responsible for the redistribution and altered function of resident intrauterine immune cells during the initiation of pregnancy. Information on the effects of dPRP on host macrophage and NK cell populations in tumors growing in athymic mice may aid in the evaluation of this prediction.

In conclusion, we have generated reagents that can help expand our understanding of the biological actions of dPRP, one of the principal secretory products of the decidua. dPRP is a heparin-binding cytokine residing at least in part within the decidual ECM that probably assists in the establishment of pregnancy through paracrine actions within the uteroplacental compartment.

Acknowledgments

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References
