Expression of haptoglobin-related protein and its potential role as a tumor antigen
(neoplasia/breast cancer/acute-phase proteins)

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ABSTRACT These studies describe the detection of a haptoglobin species, its characterization as the HPR gene product, and its association with both pregnancy and neoplasia. Previous work showed that the early recurrence of human breast cancer correlated with immunohistochemical staining with a commercial antiserum ostensibly directed against pregnancy-associated plasma protein A (PAPP-A). Use of this antiserum to guide purification of the putative antigen led to the present identification and purification of a strongly immunoreactive protein species distinct from PAPP-A that was present in the plasma of pregnant women at term. Unlike PAPP-A, a homotetramer of 200-kDa polypeptides, the immunoreactive protein consists of a light (α) chain (16.5 kDa) and a heavy (β) chain (40 kDa); protein microsequencing of the β chain showed it to be a member of the haptoglobin family. The α chain of this haptoglobin species differs from ordinary haptoglobin 1 and 2 α chains both structurally and immunologically and represents the product of the HPR gene, haptoglobin-related protein (Hpr), since (i) the apparent molecular mass is the same as that predicted for Hpr α chain, (ii) the peptide map differs from that of haptoglobin 1 in a manner predicted by the HPR nucleotide sequence, (iii) monospecific antibodies that react with epitopes shared by the unique α chain and a synthetic peptide derived from the HPR nucleotide sequence do not detect these epitopes in either haptoglobin 1 or 2, and (iv) sequences of α-chain peptides were consistent with this identification, excluding haptoglobin 1 but not haptoglobin 2. The immunohistochemical reactivity of antibodies raised to the synthetic Hpr peptide is similar to that of anti-PAPP-A. Moreover, staining of neoplastic breast tissue is abolished by preincubation with purified Hpr.

The present studies describe the expression of haptoglobin-related protein (Hpr), the product of the HPR gene, and its association with both pregnancy and neoplasia. Conventional haptoglobins (Hps) have been well studied; the three known alleles each code for a precursor containing both the α and β chains (1–3). Maeda (4) described the HPR locus as a stretch of DNA located 2.2 kilobases downstream from the conventional HP locus. This sequence appears to be an intact gene coding for a theoretical protein whose α and β chains are distinct from, but highly homologous to, conventional Hps, particularly haptoglobin 1 (Hp-1). Nevertheless, several investigators failed to detect Hpr expression (4–6). Interestingly, the locus contains a retrovirus-like element and is duplicated in some individuals (4, 5).

Experiments directly leading to the present studies found that a commercial antiserum to pregnancy-associated plasma protein A (PAPP-A) reacted immunohistochemically with human breast carcinomas and that these breast carcinomas staining positively for the antigen showed an increased probability for tumor invasion and early metastasis. Clinically, these properties manifested as a dramatically worsened prognosis (7–9). In Western blots (immunoblots) of fractions of sera from pregnant women, this antiserum detected a set of proteins whose properties were inconsistent with those of PAPP-A, a homotetramer composed of 200-kDa polypeptides. The studies below describe the purification of the unexpectedly immunoreactive protein species from the plasma of pregnant women, its identification as the HPR gene product, and its relationship to the clinically important antigen expressed in human breast carcinoma.

MATERIALS AND METHODS

Patient Material. Term maternal plasma was obtained from discarded EDTA-anticoagulated whole blood samples from the delivery suite of The Johns Hopkins Hospital and stored at −70°C until use. Paraffin-embedded human breast carcinoma specimens were obtained from the Department of Pathology.

Electrophoresis and Western Blotting. NaDodSO4/polyacrylamide gel electrophoresis used the Laemmli system (10). For Western blotting, Laemmli gels were transferred to 0.45-μm nitrocellulose sheets (Schleicher & Schuell) in 96 mM glycine/12.5 mM Tris/0.1% NaDodSO4/20% (vol/vol) methanol at 40 V, 250–300 mA, at 4°C for 6 hr, evaluated for transfer efficiency by Ponceau S staining, blocked with 3% (wt/vol) bovine serum albumin, then incubated sequentially with antibody for 2 hr and with 125I-labeled protein A for 1 hr with intervening Tris/saline washes (11).

Protein Purification. Proteins reactive with IgGs in the whole IgG fraction of a rabbit anti-PAPP-A antiserum (Dako, Santa Barbara, CA) were purified by successive chromatographic steps monitored by OD280, analysis of fractions by Coomassie blue-stained Laemmli gels, and Western blotting. Initially, 80 ml of plasma was loaded onto a 2.5 × 30 cm Cibacon Blue F3G-A Sepharose (Pharmacia) column equilibrated in 50 mM sodium phosphate (pH 6.8) at 4°C. The flow-through containing detectable immunoreactive material was dialyzed against 20 mM sodium phosphate (pH 6.8 at 4°C) with 1 mM Na2SO4 and applied to a 2.5 × 10 cm column of DEAE-cellulose (Pierce) equilibrated in the same buffer. The immunoreactive material was eluted with 0.2 M NaCl in the starting buffer, dialyzed against 20 mM Tris-HCl (pH 8.5 at 4°C) containing 0.15 M NaCl, 1 mM 2-mercaptoethanol, and 1 mM NaN3, then loaded onto a 2.5 × 60 cm column of Fast Flow Q-Sepharose (Pharmacia) equilibrated in the same buffer. The column was eluted with a 500-ml linear gradient of 150–500 mM NaCl at a flow rate of 100 ml/hr at 4°C. The immunoreactive proteins eluted between 0.25 and 0.28 M NaCl. Fractions containing immunoreactive protein were run on a SDS/PAGE gel and visualized by Coombs and Goldblum stains.

Abbreviations: Hp, haptoglobin; Hpr, haptoglobin-related protein; PAPP-A, pregnancy-associated plasma protein A; KHL, keyhole limpet hemocyanin; CEA, carcinoembryonic antigen.

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pooled, dialyzed against 200 mM Tris acetate (pH 7.5 at 4°C) with 1 mM 2-mercaptoethanol/1 mM NaN₃, and applied to a 5 × 90 cm Sepharose CL-4B (Pharmacia) column. To separate the individual immunoreactive chains under denaturing conditions, fractions containing immunoreactive protein were pooled and dialyzed against 20 mM Tris-HCl, pH 8.5/6 M urea/10 mM 2-mercaptoethanol, followed by HPLC fractionation. Waters system with a 5 × 50 mm Mono Q HR5/5 (Pharmacia) column monitoring OD₂₈₀. The sample was injected in starting buffer, a 10-ml wash followed, and a linear gradient of 30 ml over 15 min to final conditions of 0.5 M added NaCl was applied. The immunoreactive 40-kDa protein was eluted in a volume of 0.85 M NaCl.

**Amino Acid Sequencing.** Protein chains purified by anion-exchange HPLC were homogenous on Laemmli gels. For sequencing, samples were dialyzed against three changes of 0.2 M NH₄HCO₃ and thrice lyophilized from HPLC-grade water. Gas-phase sequencing was carried out on an Applied Biosystems model 470A sequencer by P. Shenbagamurthi (Protein Chemistry Facility, The Johns Hopkins University School of Medicine). Samples of intact protein chains generally consisted of 100 μg of protein by Lowry assay (12) yielding 0.5–2 nmol as quantitated by the sequencer.

**Peptide Mapping.** Peptide mapping was performed using the Elder technique (13, 14).

**Preparation of Anti-Hpr Antibody.** A synthetic peptide corresponding to the 34 N-terminal residues of the predicted HPR gene product (4) was synthesized by the Protein Chemistry Facility on an Applied Biosystems model 43A peptide synthesizer (15). Because histidine coupling is inefficient, lysine was conservatively substituted for the histidine in position 28. For immunization, the peptide was conjugated to keyhole limpet hemocyanin (KLH) (Boehringer Mannheim) essentially as described (16). The KLH-maleimidobenzoyl-n-hydroxysuccinimide ester (MBS) conjugate was separated from unreacted MBS by gel filtration on a Pharmacia PD-10 column. Hpr peptide (1.2 mg) was added directly to the KLH-MBS and the mixture was incubated a further 30 min at room temperature. Coupling efficiency was estimated using Pharmacia Superose 12 FPLC by peak integration and comparison to standards of peptide run alone. The KLH-peptide was separated from unreacted peptide by gel filtration in isotonic phosphate-buffered saline (PBS) on a PD-10 column. This method yielded an approximate substitution ratio of 4.6 peptides per KLH molecule.

Two Pasteurella-free New Zealand White rabbits (Hazelton Research Products, Reston, VA) were each inoculated on day 0 with 200 μg of antigen in 1 ml of an emulsion comprised of equal parts of PBS and complete Freund's adjuvant; the inocula were divided among three or four subcutaneous and intramuscular sites. On day 14, the animals were given booster inoculations with similar amounts of antigen in incomplete Freund's adjuvant. The rabbits were bled on day 32; serum from the positive rabbit was collected over the ensuing month.

To purify the antibody, a 10 cm × 5 mm SelectiSpher-10 activated-tresyl HPLC affinity column (Pierce) was derivatized with the Hpr synthetic peptide. The column was washed with 30 ml of PBS at 2 ml/min, then 27 mg of peptide dissolved in 4.5 ml of PBS was applied at a rate of 1 ml/min. Unreacted tresyl groups were capped with 30 ml of 0.2 M Tris-HCl (pH 8.0) through the column. For antibody purification, the column was equilibrated in 300 mM NaCl/20 mM sodium phosphate, pH 7.5. Serum (10–20 ml) was loaded at 0.5 ml/min. After loading, the column was washed with buffer until the OD₂₈₀ of the effluent returned to zero. Antibody was eluted with 6 M urea in the same buffer, and the antibody-containing fractions were immediately dialyzed into Tris-buffered saline with 1 mM NaCl.

**Affinity Chromatography.** Hp-1 and the Hpr synthetic peptide were immobilized on agarose beads derivatized with 1,1’-carbonyldimidazole [Reacti-Gel (6X), Pierce], which results in a stable N-alkylcarbamate linkage. Gel (5 ml) was washed with 0.1 M borate/0.15 M NaCl, pH 8.5, at room temperature to remove the acetonitrile. Hp-1 (4 mg) (Sigma) diluted to 1 mg/ml in borate buffer or 50 mg of Hpr synthetic peptide diluted to 12.5 mg/ml in borate buffer was added to 5 ml of gel and incubated with agitation at 4°C for 30 hr. After incubation, the supernatant was decanted and an equal volume of 0.2 M Tris was added to quench any remaining reactivity. The gels were extensively washed with Tris/saline buffer. The Hpr peptide column was then denatured with 100 ml of 0.1% NaDodSO₄ in Tris/saline buffer.

Monospecific anti-Hpr antibodies were isolated from the crude anti-PAPP-A serum by sequential affinity chromatographic steps. First, 100 μl of crude anti-PAPP-A antibody in 10 ml of Tris/saline buffer was incubated with the Hp-1-gel overnight at 4°C with agitation. The gel was next washed extensively with Tris/saline and the flow-through was collected over a 5-ml hydroxyapatite column. Antibodies that bound to the Hp-1 column were eluted with 20 ml of acetate buffer (0.05 M sodium acetate/0.15 M NaCl, pH 3.5, at room temperature). The antibodies that flowed through the column were eluted from the hydroxyapatite column with 20 ml of 0.4 M sodium phosphate. Both sets of antibodies were dialyzed against 2 liters of Tris/saline buffer.

The antibodies that did not bind to the Hp-1 column were then incubated with the denatured Hpr peptide gel overnight at 4°C with agitation. Antibodies that bound to the Hpr peptide were eluted with 20 ml of acetate buffer and dialyzed against Tris/saline.

**Immunohistochemistry.** Immunohistochemistry was performed using the unlabelled antibody immunoperoxidase technique for formalin-fixed paraffin-embedded tissues (7).

**RESULTS**

**Purification of Immunoreactive Proteins from Pregnancy Serum.** The initial goal was to characterize the substance in human breast carcinoma that reacted with the crude IgG fraction of a commercially produced antisera to PAPP-A. The antisera both guided the purification of the homologous antigen from pregnancy serum and helped characterize the immunoreactive material in breast cancer. PAPP-A is a homotrimer of 200-kDa polypeptides (17); surprisingly, anti-PAPP-A failed to detect such a protein in Western blots of pregnancy serum or various subfractions. Instead, three immunoreactive chains were consistently seen with apparent molecular masses of 40 kDa, 20 kDa, and 16.5 kDa in Laemmli gels, the 16.5-kDa species reacting most strongly and the 20 kDa reacting most weakly. Reactivity with several unidentified protein species and the undetectable immunoreactivity with Hpr-PAPP-A in Western blots suggested that one or more of the unidentified bands might be related to the immunoreactive material in neoplastic breast tissue.

Fig. 1 summarizes the purification of the unidentified immunoreactive bands. The figure shows a Coomassie blue-stained 10–15% polyacrylamide Laemmli gel corresponding to Western blot. Sequential chromatographic steps involved removal of albumin and additional protein species on Cibacron Blue F3GA Sepharose (lanes a), step elution from a DEAE-cellulose column (lanes b), gradient elution from a Fast-Flow Q Sepharose anion-exchange column (lanes c), and gel permeation chromatography on Sepharose CL-4B (lanes d). Interestingly, gel filtration incompletely resolved the immunoreactive proteins into three consecutively eluting species consisting of the 40-kDa species co-eluting with the weakly reactive 20-kDa species, the 40-kDa band co-eluting with a weakly immunoreactive 16.5-kDa species, and lastly,
as a separate, albeit overlapping peak, the 40-kDa species co-eluting with a strongly immunoreactive 16.5-kDa band.

The relationship between the purified protein and the immunoreactive material in breast cancer was explored using a human breast carcinoma known to stain positively both with anti-PAPP-A and with anti-carcinoembryonic antigen (CEA). Preincubation of anti-PAPP-A with a mixture of the purified protein species ablated immunostaining, but preincubation of anti-CEA under similar conditions had no effect, thus demonstrating an immunologic relationship between the material present in breast cancer and that found in pregnancy serum (data not shown).

Identification as a Hp by Protein Microsequencing. The 40-kDa chain was isolated by anion-exchange HPLC under denaturing conditions. N-terminal sequencing yielded 20 residues. Using the protein sequence database of the National Biomedical Research Foundation (November 1987) and the DFASTP alignment program, three protein sequences showed 100% homology over the entire 20-amino acid stretch with the B chain of human Hp-1, the B chain of human haptoglobin 2 (Hp-2), and the predicted B chain of the Hpr precursor (6, 18–21). Hp molecules are composed of light (α) and heavy (β) chains synthesized as a single chain and then cleaved to form the final product (22). Hps migrate aberrantly slowly on Laemmli gels with apparent molecular masses of 39.5 kDa for the glycosylated β chains and of 16.5 kDa for the carbohydrate-free α chain of Hp-1 and 20 kDa for the α chain of Hp-2 (1). The molecular masses found by gel electrophoresis for both the heavy and light chains of the purified protein are similar to those reported for Hp-1.

Hp Purified from Pregnancy Plasma Possesses Distinctive Structural Features. Although sequence analysis placed the pregnancy plasma protein unequivocally in the HP gene family, more specific assignments could not be made, since the N-terminal sequence for the β chains of all species is identical. Fig. 2 shows the results of an immunologic analysis performed with purified Hp-1 and -2 standards, the Hp purified from pregnancy serum, rabbit anti-Hp, and anti-PAPP-A. Fig. 2A shows a 10–15% polyacrylamide Coomassie blue-stained Laemmli gel of Hp-1, Hp-2, and the Hp purified from pregnancy serum. The corresponding Western blot with anti-Hp mimics the Coomassie blue-stained gel in its intensity and distribution, recognizing all three Hp chains; the relative immunoreactivity of the 16.5-kDa chain of the purified protein is commensurate with the relative intensity of its Coomassie blue staining. Anti-PAPP-A also reacts with Hp heavy chains and Hp-2 light chain; however, its relative immunoreactivity with the 16.5-kDa chain from pregnancy serum shown in lane c is disproportionately intense in comparison to its Coomassie blue staining, particularly when compared to the disproportionately weak relative immunoreactivity of the Hp-1 α chain shown in lane a. Thus, the α chain isolated from pregnancy plasma is immunologically distinct, containing epitopes seen by the anti-PAPP-A that are not present in the α chains of either Hp-1 or Hp-2.

In addition to immunologic analysis, peptide mapping (13, 14) was performed to identify potential structural differences between heavy and light chains from the Hps and purified pregnancy protein. Peptide mapping by this technique detects only tyrosine-containing peptides. Using the published amino acid sequences, the family of tryptic peptides from the α chains of Hp-1 and Hp-2 should each be similar since Hp-2 resulted from a partial reduplication of Hp-1 (1) (Fig. 3 A and C). The principal differences in amino acid sequence of Hpr, Hp-1, and Hp-2 lie in the α chains. Within the α chains, these differences cluster principally at the N termini. The deduced Hp α-chain sequence predicts the same tryptic peptides as those obtained from Hp-1 plus one additional tyrosine-containing peptide. Fig. 3B shows the peptide map of the purified α chain from the pregnancy-associated protein with an arrow highlighting the one additional peptide obtained experimentally. These results thus raise the possibility that the Hp purified from pregnancy plasma might represent the HPR gene product. Fig. 3D is a mixing experiment where Hp-1 and the purified pregnancy-associated protein α chain were mixed before mapping. Again, the additional peptide is present but with less relative intensity, while the shared peptides all co-migrate.

Hp α-chain from Pregnancy Serum Contains Hpr Epitopes. If the immunologically and structurally distinct Hpr from pregnancy serum is the HPR gene product, then its α chain should share unique epitopes with a synthetic peptide derived from the deduced Hpr sequence. A 34-amino acid peptide
was synthesized that was identical to the predicted N terminus of Hpr α chain except for one conservative amino acid substitution. This peptide was immobilized on agarose beads for immunoblotting in conjunction with separate columns of Hp-1 and Hp-2. Thus, antibodies reacting with epitopes shared with the conventional Hps should bind to the Hp columns, and antibodies reacting with the synthetic peptide could be positively selected.

Fig. 4 shows, by stepwise immunoselection, that the antibodies that react to the α chain of the Hp from pregnancy serum also react with the Hpr synthetic peptide. Fig. 4A is a 10–15% polyacrylamide gradient Laemmli gel with Hp-1 in the left lane and purified pregnancy-associated Hp in the right lane; B–E are immunoblots of these same proteins. Fig. 4B was incubated with unabsorbed anti-PAPP-A serum, and, as in Fig. 2C, there is scant reactivity to the Hp-1 α chain, weak reactivity to the contaminating 20-kDa Hp-2 α chain, but intense labeling of the 16.5-kDa pregnancy-associated Hp α chain along with the 40-kDa β chains.

Fig. 4C and D show the results of immunoblotting of the anti-PAPP-A antibody on immobilized Hp-1. The blot in C was incubated with the antibodies reactive to Hp-1 that were eluted from the column, while the blot in D was incubated with antibodies in the flow-through. Antibodies reactive to the α chains of pregnancy-associated Hp and Hp-2 flowed through the Hp-1 column, whereas antibodies against the β chains were retarded. The flow-through containing antibodies specific for the α chain of the pregnancy-associated Hp were then incubated with immobilized synthetic Hpr peptide. The positively selected antibodies binding to the Hpr peptide were eluted and incubated with the blot in E. Only the α chain of the pregnancy-associated Hp reacted with antibodies specific for the Hpr synthetic peptide. This demonstrates that the α chain of pregnancy-associated Hp shares epitopes with the Hpr synthetic peptide.

Hpr Epitopes Identified in the Cytoplasm of Human Breast Carcinoma. An immunoperoxidase staining and absorption experiment was performed with anti-Hpr, anti-PAPP-A, and purified Hpr. If the immunoreactivity of human breast carcinoma with the anti-PAPP-A antibody was due to Hpr, preincubation of anti-PAPP-A with native Hpr should abolish the immunoreactivity. Similarly, neoplastic breast tissue that stains with anti-PAPP-A should stain with anti-Hpr, with the same pattern of competition.

When anti-Hpr antibody was incubated for 2 hr at 4°C with native Hpr, cytoplasmic staining was abolished (Fig. 5). To establish specificity of the immunoblotting, when anti-CEA antibodies were incubated under similar conditions with native Hpr, anti-CEA cytoplasmic staining was not abolished. This established that the substance identified by anti-PAPP-A in human breast carcinoma expresses epitopes derived from the N terminus of the predicted Hpr α chain. This antigen must, therefore, represent Hpr, a modified form of Hpr, or a highly cross-reactive protein.

**DISCUSSION**

These studies describe the expression of a protein product derived from the human HPR locus, a gene for a Hp located 2.2 kilobase pairs downstream from the conventional Hp locus (5). The following several pieces of evidence support the hypothesis that the protein isolated from pregnancy plasma is Hpr: (i) affinity-purified antibodies reactive to the α chain of the purified protein also react with a synthetic peptide representing the 34 N-terminal amino acids of the predicted Hpr α chain, but not with the α chains from Hp-1 or -2; (ii) the molecular mass of the α chain of the purified protein is consistent with that predicted for the Hpr α chain,
co-migrating on Laemmlti gels with the Hp-1 α chain; (iii) the peptide map of the purified α chain differs from the maps of the α chains of Hp-1 and Hp-2 in the manner that would be predicted from the Hpr α-chain sequence; and (iv) the Hpr α chain co-purifies with a second polypeptide chain whose size and N-terminal amino acid sequence are consistent with those of Hp β chains. Additional support comes from sequencing. Unfortunately, the N terminus of the purified Hpr α chain is blocked, and attempts to isolate enzymatically cleaved peptides from the N-terminal portion of the molecule were unsuccessful. Two peptides, Gly-Lys-Pro-Lys-Asn-Pro-Ala-Asn-Gln-Val-Gln-Arg and Thr-Leu-Asn-Asp-Lys-Glu-Trp, were isolated whose sequences are common to Hpr and Hp-2 α chains, but are not contained in the sequence of the Hp-1 α chain. In view of the other data, including high molar yields of these peptides and the absence of any detectable contaminating Hp-2, it is most likely that these peptides are derived from the α chain of Hpr.

Hps are acute-phase proteins initially discovered by their ability to bind hemoglobin, enhancing the endogenous peroxidase activity of hemoglobin (8). Traditionally, Hp function has been thought principally to be iron conservation mediated by its ability to bind hemoglobin. Hps would thus prevent renal excretion of free hemoglobin and recycle iron to the liver by a hepatocyte cell surface receptor for the Hp—hemoglobin complex (23–26); additionally, by binding iron, they might also exert an antibacterial effect (27). The present data suggest additional possibilities. The detection of Hp in maternal serum suggests that Hpr may play a role in pregnancy. Unless it represents a fetal Hp that crossed the placenta, the Hpr purified from maternal serum suggests maternal expression during pregnancy to carry out a yet unknown function.

In addition to pregnancy plasma, a species reacting with anti-Hpr peptide antibody was detected immunohistochemically in human breast carcinoma. The anti-Hpr synthetic peptide antibody showed a cytoplasmic staining pattern similar to the commercial anti-PAPP-A antibody used in previous studies (7–9). In both cases, positive staining was abolished when the antibody was preincubated with purified Hpr, whereas neither Hp-1 nor Hp-2 had any effect, thus strongly inferring that Hpr is present in the cytoplasm of breast cancer cells. The immunoreactivity with the anti-Hpr synthetic peptide antibody, like the anti-PAPP-A antibody, distinguished a group of patients with a drain-prognosis (P.K. and G.R.P., unpublished data). The finding of Hp immunoreactivity within cancer cells, the propensity to localize in invasive, rather than in situ tumors, and its consistent association with phenotypically aggressive neoplasia suggests that Hpr or related molecules may serve as a mediator of certain malignant processes. Although the mechanism whereby Hpr might influence the behavior of a malignancy is unclear, previous studies reported unusual Hps, some of which modulate lymphocyte responsiveness (28–30). In all cases, such Hps were detected in abnormal body fluids whose accumulation was directly due to a malignancy; however, none has been precisely characterized. It is thus possible that Hpr might modulate the host response to a malignancy so as to enable it to survive and expand clonally. In addition, Hps from cancerous body fluids have been shown to stimulate collagen synthesis in fibroblasts (31, 32), suggesting that the interface between tumor cells and the extracellular matrix might be another locus of action.

While no expression of the HPR gene has been identified in adult or fetal hepatocyte mRNA (5, 6), Hp synthesis may not be restricted to the liver. In some instances, lymphocyte cultures (33) and brain (34) synthesize Hps, suggesting that an alternative site of synthesis, such as placenta or decidua, might be responsible for Hpr production during pregnancy. In breast cancer, malignant cells might elaborate Hpr themselves, or internalize it by way of a surface receptor. Although the granular cytoplasmic staining for Hpr suggests synthesis and secretion by the breast carcinoma cells, the answer must await biosynthetic studies.

The isolation of Hpr from pregnancy plasma and the localization of immunologically related material, if not Hpr itself, in virulent breast carcinoma broadens the spectrum of pathophysiologic roles of Hps. Detection and quantitation of Hpr may prove to be a useful diagnostic procedure in cancer and, possibly, pregnancy (35). As the comparison between neoplastic states and pregnancy is often drawn, Hpr may subsume similar functions in pregnancy and neoplasia, aiding development in one instance while facilitating tumor invasion and metastasis in the other.

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