Murine HIP/L29 Is a Heparin-binding Protein with a Restricted Pattern of Expression in Adult Tissues*

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Heparin/heparan sulfate (Hp/HS)-binding proteins are implicated in a variety of cell biological processes including cell adhesion, modulation of blood coagulation, and cytokine/growth factor action. Hp/HS-interacting protein (HIP) has been identified in various adult tissues in humans. HIP supports high affinity, selective binding to Hp/HS, promotes cell adhesion, and modulates blood coagulation activities via Hp/HS-dependent mechanisms. Herein, a murine ortholog of human HIP is described that is 78.8% identical to human HIP and 99.8% identical at the cDNA level and identical at the amino acid level to a previously described murine ribosomal protein, L29. Western blot analyses and immunohistochemical staining with affinity-purified antibodies generated against two distinct peptide sequences of murine HIP/L29 indicate that HIP/L29 is differentially expressed in adult murine tissues and cell types. In the normal murine mammary epithelial cell line, NMuMG, HIP/L29 is enriched in the 100,000 × g particulate fraction. HIP/L29 can be solubilized from the 100,000 × g particulate fraction with 0.8 M NaCl, suggesting that it is a peripheral membrane protein. HIP/L29 directly binds 125I-Hp in gel overlay assays and requires 0.75 M NaCl for elution from Hp-agarose. In addition, recombinant murine HIP expressed in Escherichia coli binds Hp in a saturable and highly selective manner, compared with other glycosaminoglycans including dermatan sulfate, chondroitin sulfate, keratan sulfate, and hyaluronic acid. Collectively, these data indicate that murine HIP/L29, like its human ortholog, is a Hp-binding protein expressed in a restricted manner in adult tissues.

Heparin/heparan sulfate (Hp/HS) proteoglycans and their respective binding proteins play important roles in diverse biological processes including cell adhesion, cytokine/growth factor action, and modulation of blood coagulation (1–4). Furthermore, several lines of evidence indicate that Hp proteoglycans and their binding proteins participate in aspects of embryo implantation (5). Recently, human Hp/HS-interacting protein (HIP) has been identified in uterine endometrium with expression in luminal uterine epithelial cells, i.e. the cells to which embryos initially attach during the implantation process (6). The expression pattern of HIP coupled with its function as a Hp/HS-binding protein makes it a candidate protein involved in Hp/HS-dependent embryo adhesion.

Previous reports from our laboratory have characterized human HIP as a cell surface, Hp/HS-binding protein expressed in a number of normal human tissues and cell lines (6–7). HIP mRNA is 1.3 kilobases in length and encodes a protein with a calculated molecular mass of 17,750 kDa; however, HIP migrates at an apparent molecular mass of 24 kDa by SDS-PAGE, apparently due to its very high isoelectric point. HIP mRNA was found in a variety of epithelial cell types, but was absent or at markedly reduced levels in two fibroblastic cell lines examined. HIP protein expression was examined immunohistochemically in human uterine endometrial sections where it was localized to the uterine and glandular epithelium and also on the vascular endothelium. Purified human HIP as well as a recombinant HIP protein expressed in Escherichia coli were shown to bind Hp selectively and saturably and also support Hp/HS-dependent cell adhesion (8). In addition to reports on HIP protein, a peptide sequence within HIP has been shown to bind specific Hp/HS sequences within the Hp/HS chain (9). Notably, this peptide domain binds the same Hp/HS pentasaccharide that is recognized by the anticoagulant protein, antithrombin III (10). Consequently, HIP is thought to compete with antithrombin III for this pentasaccharide motif and modulate blood coagulation activities in vivo.

This present study describes the cDNA cloning, expression, and function of a murine ortholog to human HIP that is 99.8% identical to a previously published cDNA sequence for mouse ribosomal protein L29 (11). Using two antibodies generated toward distinct peptide sequences of this protein, the expression pattern of murine HIP/L29 was investigated. Additionally, recombinant murine HIP/L29 expressed in E. coli retains the ability to bind Hp selectively and saturably. Like human HIP, murine HIP/L29 is a protein that binds Hp with a high degree of selectivity and is expressed in a cell type-restricted fashion in adult mouse tissues.

EXPERIMENTAL PROCEDURES

Materials—Heparin, dermatan sulfate, chondroitin sulfate C, keratan sulfate, hyaluronic acid, sodium chloride, sodium citrate, Tris base, glycine, bovine serum albumin (BSA), and phenylmethylsulfonyl fluoride were purchased from Sigma. [3H]Hp (0.44 mCi/mg) was purchased from NEN Life Science Products. [125I]Protein A (45 mCi/mg) was purchased from ICN Biochemicals Inc. (Irvine, CA). Sodium dodecyl sulfate (SDS), β-mercaptoethanol, and Tween 20 were purchased from Bio-Rad. All chemicals used were reagent grade or better.

cDNA Library Screening—A mouse uterine cDNA library constructed in the λ ACT vector (provided by Dr. Joe Miano, M. D. Anderson Cancer Center) was used for cDNA library screening. Nitrocellulose filters (Schleicher & Schuell) lifted off the plated cDNA library were prehybridized at high stringency (50% (v/v) formamide, 2× SSC, 0.5% (w/v) SDS, and 100 μg/ml denatured, sonicated salmon sperm DNA) or

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The abbreviations used are: Hp, heparin; HS, heparan sulfate; BSA, bovine serum albumin; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis.
low stringency (35% (v/v) formamide, 2× SSC, 0.5% (w/v) SDS, and 100 μg/ml denatured, sonicated salmon sperm DNA) for 74 h at 42 °C. Hybridization was performed using the 3P-labeled human HIP full-length cDNA as a probe (2× 10⁶ cpm/ml) in fresh solutions of identical composition for 16 h at 42 °C. The blots then were washed with 2× SSC and 0.1% (w/v) SDS at 55 °C followed by a fresh solution of identical composition at 42 °C for 2 h. The blots then were exposed to Kodak X AR films at −70 °C overnight. Positive plaques were isolated and further analyzed using the same hybridization and screening conditions.

Computer Analysis—Nucleotide and protein sequence analyses were carried out using GCG and the DNAStar sequence analysis software of the day at the GenBank (release 105.0), EMBL (release 51.0), and SWISS-PROT (release 35.0).

Northern blot analysis—A cDNA fragment corresponding to the entire coding sequence of the murine HIP/L29 was used to probe a Northern blot of purified poly(A)+ RNA (mouse MTN, CLONTECH) from various mouse tissues. After prehybridization at 68 °C for 30 min in ExpressHyb solution (CLONTECH), the cDNA labeled murine HIP/L29 probe was added to fresh ExpressHyb solution (10⁶ cpm/ml) and allowed to interact with the membrane for 1 h. A 30-min rinse in 2× SSC (3 M NaCl, 0.3 M sodium citrate) and 0.1% (w/v) SDS at 65 °C was followed by four washes of 20 min each in 0.1× SSC, 0.1% (w/v) SDS at 50 °C. The membrane was exposed to x-ray film (Kodak XAR 5) at −70 °C overnight. Positive plaques were isolated and further analyzed using the same hybridization and screening conditions.

Membrane preparations—NMuMG cells were grown to 70% confluency in 100-mm tissue culture plates in a humidified incubator (5% CO2 and 95% air) at 37 °C in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal calf serum and antibiotics. Annexin V-negative cells were collected by low-speed centrifugation, washed once with PBS, and homogenized on ice. The homogenate was centrifuged at 100,000 g for 20 min at 4 °C. The supernatant was removed and the pellets were extracted with extraction buffer and then precipitated and prepared for SDS-page as described above.

NaCl Extraction of Membranes—High speed (100,000 × g) particulate preparations were subjected to differential salt extraction with 4 M NaCl followed by 0.8 M NaCl as described above. The proteins were then separated by two-dimensional gel electrophoresis (16) and transferred to nitrocellulose as described above for Western blotting. The unblocked nitrocellulose was incubated with 125I-Bolton-Hunter reagent-derivatized Hp (17) in 0.15 M NaCl, in the presence of 0.1% (w/v) sodium azide, overnight at 4 °C, then was washed three times with PBS before drying for autoradiography. The same nitrocellulose membrane was blocked and then probed with affinity-purified antibodies directed against the peptide sequence, CRFPAKKNKKGLKLM, and binding subsequently visualized with a peroxidase ABC system using a diaminobenzidine substrate kit as described by the manufacturer’s instructions (Vector Laboratories, Burlingame, CA). A parallel gel run under exactly the same conditions was silver-stained as described (18) to visualize the migration positions of all proteins on the gel.

Heparin-Agarose Affinity Chromatography—High speed (100,000 × g) membrane preparations were extracted overnight at 4 °C with 0.4 M NaCl in 5 mM Tris (pH 8.0), and then centrifuged at 100,000 × g for 1.5 h. The 0.4 M NaCl-insoluble pellet was subsequently extracted with 0.8 M NaCl in 5 mM Tris (pH 8.0) for 4 h and centrifuged 1.5 h at 100,000 × g. Murine HIP/L29 protein was eluted in the 0.8 M NaCl extract that was diluted subsequently to achieve a final concentration of 0.15 M NaCl, applied to a 0.5-mL pellet of pre-rinsed heparin-agarose (Sigma) and incubated overnight batchwise with constant rotary agitation at 4 °C. Stepwise salt elution from heparin-agarose was performed with NaCl extending from 0.15 to 2 M in 5 mM Tris (pH 8.0). All fractions were trichloracetic acid-precipitated and prepared for SDS-page and Western blotting as described above.

Expression and Isolation of Recombinant HIP—PCR primers were made with BamHI adaptors to the murine HIP/L29 sequence nucleotides 21–504. The forward and reverse primers were 5′-GGGGATCC-CCATGGCAAGTCCAAG and 5′-GGGATCCCTTATGGCGGCGTTCAGTTGTCAC. The amplified product containing the entire open reading frame of murine HIP/L29 was subcloned in-frame into the BamHI site of the Oligo-HIS vector, pV2a, as described previously (19). The product of this fusion added 17 N-terminal amino acids containing the oligohistidine motif. E. coli transformed with the plasmid construct were grown to A600 = 0.62 prior to induction with 0.2 mM isopropyl-β-D-thiogalactopyranoside. The cells then were grown under induction for 5 h and harvested by centrifugation at 2000 × g for 15 min. The cells then were extracted with binding buffer (20 mM Tris-Cl, pH 8.0, 2 mM NaCl, 0.1% (w/v) Triton X-100, and 20% (v/v) glycerol) under sonication. The ruptured cell lysate then was centrifuged at 10,000 × g for 10 min to remove any insoluble material. The cleared lysate then was poured onto a TALON™ (CLONTECH) cobalt metal affinity column that was pre-equilibrated with binding buffer. The proteins were allowed to bind batchwise for 10 min and washed three times for 10 min each, batchwise with binding buffer. The slurry then was loaded onto a column and washed with five column volumes of wash buffer (20 mM Tris-Cl, pH 8.0, 500 mM NaCl, 20% (v/v) glycerol, and 10 mM imidazole). The proteins were finally eluted with elution buffer (20 mM Tris-Cl, pH 8.0, 50 mM NaCl, 200 mM (v/v) glycerol, 100 mM imidazole) in 10 drop fractions into 500 μl of 0.25 M EDTA, pH 7.4. The individual fractions were analyzed by SDS-PAGE and stained with Coomassie Blue to identify fractions containing murine HIP/L29. Murine HIP/L29-containing fractions were pooled and used in subsequent assays.

[HIP]HIP Binding to Recombinant HIP/L29—[3H]HIP was used in solid-phase murine HIP/L29-binding assays performed in 96-well
FIG. 1. cDNA sequence and translated amino acid sequence of murine HIP/L29 with comparison to orthologous sequences. 

A. Overlapping clones of murine HIP/L29. The numbers indicate the length of each overlapping clone with respect to the largest clone, 16–15.

B. cDNA sequence of the three overlapping clones with translated sequence. Deduced amino acid sequence is below the cDNA sequence. Nucleotides are numbered from the beginning of the cDNA sequence, and amino acids are numbered from the starting methionine. The starting ATG is indicated.
microassay plates. Approximately 600 ng of HIP, 600 ng of denatured BSA, or an equal volume of purified proteins from E. coli transformed with an expression vector containing murine HIP/L29 in the reverse direction were added to each well and dried at 37 °C overnight. Coating efficiency was determined by increasing the amount of HIP added to the well during the coating procedure until no further increase in [3H]HIP binding could be achieved. Under these conditions, essentially all of the recombinant human HIP up to 1.2 μg/well bound to the surface. The next day, each well was rinsed with PBS three times, and blocked with 100 μL of 0.1% (w/v) denatured BSA in a 37 °C incubator for at least 1 h. Each well then was rinsed with PBS three times, and then [3H]HIP (typically 1.0 × 10^6 dpm) was added in a final volume of 50 μL in PBS containing 0.1% (w/v) BSA, and incubated in a 37 °C incubator for 2 h. For glycosaminoglycan competition assays, unlabeled Hp (sodium salt, grade I-A from porcine intestinal mucosa), chondroitin sulfate C (from shark cartilage), dermatan sulfate (sodium salt, from porcine skin), heparan sulfate (sodium salt, from bovine kidney), keratan sulfate (sodium salt, from bovine cornea), and hyaluronic acid (from human umbilical chord) were added to the binding assays at a final concentration of 10 μg/ml. At the end of each experiment, unbound [3H]HIP was removed by rinsing three times with PBS. Bound [3H]HIP was extracted with 100 μL of extraction buffer (4 μL guanidine HCl, 25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, and 0.02% (w/v) sodium azide) 3–4 h at 37 °C. All of the extract was counted in a Beckman scintillation counter.

RESULTS

Isolation and Characterization of a cDNA Encoding Murine HIP/L29—To identify a murine homologue to human HIP, a mouse uterine cDNA library was screened using a full-length human HIP cDNA (7). Using both low and high stringency screening techniques, three different clones, 16–15, 18–14, and 18–4b, were identified (Fig. 1A). These clones contained inserts with sizes of 970, 515, and 181 base pairs, respectively. Clones 18–14 and 18–4b had identical, overlapping sequences to the largest clone 16–15. Clone 16–15 contained the entire open reading frame of murine HIP/L29 (GenBank accession number L08651) (11) that encodes a protein with a predicted molecular mass of 17,588 Da and an isoelectric point of 11.92 (Fig. 1B). The only base pair substitution was a G → A substitution at position 504, reflecting a conserved change to the stop codon. The coding sequence for murine HIP/L29 cDNA was used to perform a search of the Swissprot protein data base. This search yielded five homologues to mouse HIP/L29 (Fig. 1C). The sequences identified were putative HIP/L29 orthologues of rat (20–22) (Swissprot accession number p25886), human (7, 23), Saccharomyces cerevisiae (24) (Swissprot accession number p05747), Drosophila melanogaster (25) (Swissprot accession number q92366), and Drosophila (26, 27) (Swissprot accession number q24154), respectively, in the order of homology to murine HIP/L29. Several amino acids are conserved throughout the first 50 amino acids of the five sequences and additional amino acids are functionally conserved in this region. The Saccharomyces and Drosophila sequences all demonstrate large C-terminal truncations relative to mouse, rat, and human HIP/L29. Mouse shares 96.6% similarity to rat over 151 amino acids, 84.4% to human over 160 amino acids, 71.7% to S. cerevisiae over 53 amino acids, 63.2% to S. pombe over 57 amino acids, and 58.5% to Drosophila over 70 amino acids. The starting methionine of rat, human, S. cerevisiae, and the first three amino acids of S. pombe were absent from the proteins examined. The peptide sequences underlined (Fig. 1B) were synthesized and antibodies generated toward these sequences.

Northern Blot Analysis of Adult Mouse Tissues—To determine the expression pattern of murine HIP in murine tissue, total, poly(A)^+ RNA from heart, brain, spleen, lung, liver, skeletal muscle, and testis was probed with 32P-labeled murine HIP/L29 cDNA probe. The migration position of HIP is indicated by an arrow and the same blot probed with 32P-labeled cDNA for β-actin. Lane 1, heart; lane 2, brain; lane 3, spleen; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, testis.

The ubiquitously expressed β-actin probe generated a similar signal in each lane for the 2.0-kilobase α isoform of β-actin. In heart, skeletal muscle, and testis, the 1.8-kilobase γ isoform also was detected as described (28) and is not related to mRNA degradation. The difference in signal intensities between the

*In bold type. Two peptide sequences that were used to create antibodies toward murine HIP/L29 are in bold type, underlined, and named peptide-2, and peptide-3, respectively. The stop codon is indicated by an asterisk (*). The polyadenylation signal, attaaa, is indicated in bold type. C, comparison of murine HIP/L29, denoted by m, to rat L29 (r), human HIP (h), S. cerevisiae YL43 (c), S. pombe YL43 (p), and D. melanogaster 60 S ribosomal subunit L29 (d). The sequences are arranged from top to bottom in the order of percent identity/similarity to murine HIP/L29. Numbers indicate the amino acids of murine HIP/L29 from the starting methionine to the stop codon with four gaps, indicated by dots, made to induce a best fit between rat L29 and human HIP sequences. The stop codon is denoted with an asterisk (*). Amino acids that are identical between the six sequences are in bold type and shaded. The symbol ~ is used to show sequences that are not present and therefore cannot be compared with murine HIP/L29.*
different lanes using β-actin as a control probe is due to the specialization of these tissues. These observations indicated that the large differences observed in HIP/L29 mRNA expression could not be accounted for by differences in gel loading or transfer efficiencies.

**Western Blot Analysis of Murine Tissues**—Murine HIP/L29 protein expression also was examined by Western blot analysis. Testis, lung, serum, kidney, spleen, and liver protein extracts were examined (Fig. 3). Two different antibodies directed to predicted peptide motifs in murine HIP/L29 were generated and used for these studies as described in Fig. 1B. Both antibodies recognized proteins with an apparent molecular mass of 24 kDa. Like its human counterpart, it is assumed that the molecular mass of murine HIP/L29 is higher than the calculated molecular mass of 17.6 kDa because of the large arginine and lysine content (26.9%) of the protein. A higher Mr class of proteins were recognized by the anti-HIP peptide-2 antibody, but not the anti-HIP peptide-3 antibody, in liver and kidney (lanes 4 and 6, Fig. 2A). The identity of the larger Mr proteins is not known. The 24-kDa protein displayed robust expression in testis, spleen, and liver with reduced expression in lung and was not detectable in whole mouse serum samples.

**Immunohistochemical Localization of Murine HIP/L29 in Adult Tissues**—Frozen sections of murine liver, uterus, and testis were probed with an antibody against HIP peptide-3 (Fig. 4). In liver, murine HIP/L29 is localized in discrete areas throughout the tissue (Fig. 4A). Omission of the primary antibody yielded no staining of similar frozen sections of liver (Fig. 4B). In the uterus, murine HIP/L29 expression is largely confined to uterine and glandular epithelium (Fig. 4D). Testis displayed strong, discrete staining of sperm tails and heads (Fig. 4, E and F). Collectively, these data demonstrate that murine HIP/L29 protein is expressed in a highly restricted pattern in several adult mouse tissues.

**Subcellular Distribution of HIP**—To determine if murine HIP/L29, like human HIP, is associated with membranes (5), a subcellular fractionation procedure was used to examine HIP subcellular localization by Western blot analysis (Fig. 5). Like human HIP, murine HIP/L29 is enriched in the 100,000 g particulate fraction, suggesting that a portion of the NMuMg’s murine HIP/L29 population is membrane-associated. Murine HIP/L29, as well as human HIP, lacks a potential transmembrane spanning peptide motif. Therefore, it was considered that murine HIP/L29 was peripherally associated with membranes. To test this hypothesis, the 100,000 g particulate fraction was extracted with NaCl concentrations up to 1.6 M. Murine HIP/L29 was quantitatively eluted from membranes with NaCl concentrations of 0.8 M or above (Fig. 6). These characteristics were consistent with the properties expected of a peripheral membrane protein.

**Murine HIP/L29 Binds Hp**—To test the function of murine HIP/L29 as a Hp-binding protein, three procedures were em-
ployed. In the first, HIP/L29-containing fractions solubilized by 0.8 M NaCl extraction were separated by two-dimensional PAGE. Many proteins were detected in this fraction by silver staining (Fig. 7A); however, only a subset of these proteins directly bound 125I-Hp in an overlay assay (Fig. 7B). Out of these 125I-Hp-binding proteins, only one was recognized by anti-HIP/L29 (Fig. 7C). This protein migrates at a highly basic pH with an apparent molecular mass of 24 kDa. Parallel 125I-Hp overlays were done in the presence of either 500 μg/ml unlabeled chondroitin sulfate C or 500 μg/ml unlabeled Hp. Although 125I-Hp binding was markedly diminished in the presence of 500 μg/ml unlabeled Hp, no reduction was observed in the presence of 500 μg/ml chondroitin sulfate (data not shown). These data indicated that direct binding of 125I-Hp to murine HIP/L29 was highly selective for Hp versus other polyanions.

The second procedure involved fractionation of the 0.8 M NaCl solubilized HIP/L29 on Hp-agarose (Fig. 8). The relative affinity of murine HIP was examined by determining the salt concentration required for elution. In this procedure, murine HIP bound Hp-agarose efficiently at physiological salt concentrations and eluted sharply and completely with 0.75M NaCl. These observations demonstrated that murine HIP/L29 bound Hp-agarose avidly at physiological pH.

A third procedure examined the ability of murine HIP/L29 to bind [3H]Hp in a solid phase assay. A recombinant form of murine HIP/L29 containing an oligohistidine sequence fused to

**Fig. 4.** Murine HIP/L29 is expressed in a cell-type specific manner in adult tissues. Frozen, methanol-fixed sections of murine liver, uterus, and testis were incubated with an antibody directed against peptide-3 using the immunohistochemical procedure described under “Experimental Procedures.” Panel A shows a liver section probed with anti-peptide-3 antibody. Panel B is a liver section serial to panel A probed identically as panel A, but omitting the peptide-3 antibody. Panel C is a liver section serial to panel A stained with hematoxylin to show tissue morphology. Panel D is a section of proestrus uterus probed with an anti peptide-3 antibody. Note the intense staining of the lumenal and glandular epithelium with relatively little staining of the surrounding stroma. Panel E is a cross-section through several seminiferous tubules in the testis probed with anti-peptide-3 antibody. Panel F is a 5× higher magnification of a region of panel E. Note the intense staining of developing sperm heads and tails, with little or no staining of surrounding Sertoli or interstitial cells. The magnification for panels A–E is given in panel B.
the N terminus was expressed in *E. coli* and purified by immo-
obilized metal affinity chromatography. Recombinant murine
HIP/L29 was purified routinely to >95% homogeneity by this
procedure and used directly in the subsequent functional as-
says (Fig. 9). [3H]Hp binding to recombinant murine HIP was
concentration-dependent and saturable as indicated in Fig.
10A. Half-maximal binding was achieved at [3H]Hp concentra-
tions of 5–10 nM, suggesting a high affinity interaction. Excess
unlabeled Hp was an effective competitor; however, other gly-
cosaminoglycans including chondroitin sulfate C, dermatan
sulfate, bovine kidney heparan sulfate, keratan sulfate, and
hyaluronic acid displayed little or no ability to compete for
[3H]Hp binding (Fig. 10B). Consequently, it appeared that recombinant murine HIP/
L29, rather than contaminating proteins, was responsible for
Procedures." The extract first was diluted to 0.15 M NaCl and then applied to heparin-agarose. Murine HIP/L29 was eluted sequentially from heparin-agarose with increasing concentrations of NaCl as indicated at the top of the panel. The proteins from each fraction were analyzed by SDS-PAGE and Western blotting with an antibody directed against peptide-2. Extract is the starting material, and Unbound is the material that eluted in the flow through. The migration positions of Mr markers (in kDa) are shown to the left of the panel.

the selective and saturable binding of [3H]Hp. Collectively, these studies demonstrated that murine HIP/L29 had similar Hp binding properties as its human counterpart, despite the fact that there were some amino acid sequence differences in the C-terminal region.

**DISCUSSION**

In this report, a murine orthologue to human HIP, identified by screening a murine uterine cDNA library with human HIP cDNA, is described that is identical in sequence to a previously described mouse protein assigned as large subunit ribosomal protein L29 (11). The current report describes the expression pattern of this protein and its mRNA for the first time in adult tissues. The restricted pattern of expression is not what would be expected for a constitutively expressed, essential ribosomal protein. Although modest variations in cellular/tissue levels of such proteins can occur, these proteins are generally expressed at similar levels in all cell types and tissues (29). In contrast, Northern, Western, and immunohistochemical analyses all indicate marked variations in expression in different tissues and restricted patterns of expression among different cells and cell types within tissues. Although murine HIP/L29 also may play a role as an accessory ribosomal protein, we suggest an extraribosomal role for murine HIP/L29 as a Hp/HS-binding protein with a different subcellular localization.

**FIG. 9.** Recombinant murine HIP/L29 purification from E. coli. Proteins from E. coli transformed with a murine HIP/L29 expression vector with the coding region of murine HIP/L29 in the forward (lane 2) or reverse (lane 3) orientation were purified as described under "Experimental Procedures." The purified proteins were subjected to SDS-PAGE and stained with Coomassie Blue. Lane 1, Mr markers; lane 2, purified murine HIP/L29; lane 3, purified proteins from E. coli transformed with a murine HIP/L29 cDNA inserted in the reverse orientation in the expression vector.

erved function across species, this function has yet to be determined. Full sequence information on YL43 has yet to be obtained, but the Drosophila orthologue complete cDNA has been obtained and exhibits a truncation with respect to mouse, rat, and human. Perhaps the addition of C-terminal amino acids to mammalian orthologues has given this protein an additional function as a Hp/HS-binding protein with a different subcellular localization.

The protein sequence of murine HIP/L29 does not contain a canonical signal peptide sequence or a potential membrane-spanning region. However, >90% of murine HIP/L29 is associated with the 100,000 × g particulate fraction in NMuMG cells. The lack of a transmembrane domain is consistent with experimental evidence that murine HIP/L29 is quantitatively eluted from NMuMG membrane preparations by salt. There are other examples of proteins that are present in the extracellular matrix that have neither a signal peptide nor a transmembrane motif, e.g., FGF-2. Studies done with rat L29 clearly show that it co-purifies with ribosomes (20) and is a nucleic acid-binding protein that is distributed between the cytoplasm and nucleus (22). Both human and murine HIP/L29 co-isolate with the 100,000 × g particulate fraction and are solubilized with high salt. These properties are consistent with those of a peripheral membrane protein. However, redistribution of cellular components may occur during homogenization and so subcellular fractionation may give misleading results regarding murine HIP/L29 localization. Human HIP/L29 is found on cell surfaces (6, 30, 31). Nonetheless, in tissue cross-sections, human and murine HIP/L29 immunoreactivity is found throughout the cell. Thus, it is possible that this protein occurs at multiple cellular sites with potentially different functions. Recent studies of other proteins, previously believed to be only found in cytoplasm, indicate that proteins in addition to HIP/L29 can be found in multiple subcellular locales as well as the cell surface (32–35).

Western blot analysis using two different antibodies against distinct peptide motifs indicate that murine HIP/L29 is not

**FIG. 8.** Murine HIP/L29 binds tightly to heparin-agarose. The 0.8 × NaCl extract of a 100,000 × g particulate fraction was subjected to heparin-agarose chromatography as described under "Experimental Procedures." The extract first was diluted to 0.15 × NaCl and then applied to heparin-agarose. The migration positions of Mr markers (in kDa) are shown to the top of the panel. The proteins from each fractions were analyzed by SDS-PAGE and stained with Coomassie Blue.

Although this homology strongly suggests a domain with con-
HIP is normally expressed by human trophoblasts in close apposition to perlecan-enriched matrices of the fetal-maternal interface (30). Antibodies to HIP markedly inhibit human cytотrophoblast invasion in vitro and HIP expression is reduced in cytotrophoblasts in preeclamptic tissue, i.e. a clinical condition characterized by poor trophoblast invasive properties (30). Thus, HIP may play important roles in terms of trophoblast adhesion and invasion as well as hemostasis in the placenta. Nonetheless, detection of HIP in other tissues suggests a more global function.

Like human HIP, murine HIP/L29 selectively binds Hp but not other glycosaminoglycans. It appears that the affinity of murine HIP/L29 for Hp may be lower than that of human HIP, inasmuch as it elutes from Hp-agarose with slightly lower salt concentrations (0.75 M) than required to elute human HIP (0.75–1.00 M) (6). This decrease in apparent affinity may be due to the observed divergence in protein homology at the C terminus between human HIP and mouse HIP/L29. Previous work with human HIP suggested that a motif in the C terminus of alternating alanines and lysines (CRPKAKAKAKADQTK; HIP peptide 1) was responsible for human HIP's ability to bind Hp/HS and compete with antithrombin III for anticoagulant species of Hp/HS (9, 10). It was hypothesized that HIP peptide-1 could form an amphipathic α-helical structure that could comprise a Hp/HS binding motif. Murine HIP/L29 does not contain a peptide sequence that is identical to HIP peptide-1. The comparable sequence within murine HIP/L29, CQPKK-VKQTGAKAPAKAPA, contains prolines that are likely to disrupt any alpha-helical structures that might bind Hp/HS. Therefore, it is hypothesized that there may be additional domains within murine HIP/L29 that enable it to bind Hp/HS. Systematic studies of different HIP domains will be required to determine the roles that individual HIP/L29 domains play in the biological activities of this protein.

Future studies will be aimed at determining the function of murine HIP/L29 in vivo. Although murine HIP/L29 is restricted to certain cells within various tissues examined, its presence in all of these tissues suggests more general functions. Some of these activities may include modulation of blood coagulation (10), cell adhesion (9), or maintenance of extracellular matrix integrity (36). Thus, murine HIP/L29 may play different roles in different biological contexts.

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