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MATRIX BIOLOGY

Matrix Biology 18 (1999) 509–518

www.elsevier.com/locate/matbio

Expression and characterization of the IPM 150 gene (*IMPG1*) product, a novel human photoreceptor cell-associated chondroitin-sulfate proteoglycan

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Received 9 May 1999; received in revised form 29 July 1999; accepted 3 August 1999

Abstract

The interphotoreceptor matrix (IPM) occupies the extracellular space between the apical surface of the retinal pigmented epithelium and the external limiting membrane of the neural retina. This space contains two chondroitin sulfate proteoglycans, designated IPM 150 and IPM 200, which are likely to effect retinal adhesion and photoreceptor survival. In an effort to characterize human IPM 150, several cDNA clones encoding its core protein have been isolated from a human retinal cDNA library. Translation of overlapping cDNA sequences yields a novel core protein with a predicted molecular mass of 89.3 kDa. Northern and dot-blot analyses as well as the isolation of expressed sequence tags demonstrate that IPM 150 mRNA is expressed not only in the neural retina but also in several other non-ocular tissues. In situ hybridization analyses indicate that, in the eye, IPM 150 mRNA is expressed specifically by cone and rod photoreceptor cells. Characterization of IPM 150 proteoglycan core protein and identification of its site of synthesis are important steps towards understanding the architecture and biology of the IPM.

Keywords: cDNA; Chondroitin-sulfate proteoglycan; Interphotoreceptor matrix; Photoreceptor; Retina

1. Introduction

Invagination of the optic vesicle during vertebrate ocular morphogenesis results in the formation of a unique extracellular ('subretinal') space between the apical surfaces of the neural retina and the retinal pigmented epithelium (RPE). Photoreceptor cell outer segments project into this compartment which is occupied by the interphotoreceptor matrix (IPM), an extracellular matrix comprised of an array of proteins, glycoproteins, and proteoglycans (Hageman and Johnson, 1991a; Mieziewska, 1996; Hageman and Kuehn, 1998). There is general consensus that the IPM mediates significant functional interactions between the neural retina and the RPE, including the exchange of metabolites and catabolic byproducts, regulation of the ionic milieu, mediation of retinal adhesion, sequestration of growth factors, and maintenance of photoreceptor polarization, orientation, turnover and viability (Hewitt et al., 1990; Hageman and Johnson, 1991a,b; Hageman et al., 1991).

IPM glycoconjugates mediate the physical attachment of the neural retina to the RPE, and although the precise molecular mechanisms involved have not been elucidated, chondroitin sulfate proteoglycans appear to be crucial in this function (Hollyfield et al., 1989; Yao et al., 1990, 1992, 1994; Johnson and Hageman, 1991; Lazarus and Hageman, 1992; Marmor et al., 1994; Hageman et al., 1995). Cone photoreceptor cells are associated with cone matrix sheaths (CMS),

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0945-053X 99 $ - see front matter
PII: S0945-053X(99)00043-8

distinct domains of the IPM that contain chondroitin 6-sulfate proteoglycan and have firm attachments to both RPE cells and the neural retina (Johnson et al., 1986; Blanks et al., 1988). This adhesive system is sufficiently strong to detach the RPE or tear the CMS following manual separation of the neural retina from the RPE (Hageman et al., 1995). Furthermore, enzymatic cleavage of IPM-associated chondroitin sulfate glycosaminoglycans leads to a rapid decrease of retinal adhesiveness in both rabbits and primates in vivo (Yao et al., 1990, 1994). Adhesion is restored several days after injection, concomitant with the de novo biosynthesis of IPM chondroitin sulfate proteoglycans (Yao et al., 1992). In addition, disruption of proteoglycan synthesis in vivo leads to loss of CMS-associated chondroitin 6-sulfate proteoglycans, IPM disruption, localized retinal detachments and photoreceptor outer segment degeneration (Lazarus and Hageman, 1992).

Other studies have documented a correlation between changes in IPM composition and photoreceptor cell degeneration in various ocular diseases (LaVail et al., 1981; Blanks et al., 1987, 1993; Johnson et al., 1989; Hageman and Kuehn, 1998). In the RCS rat and the MPS VII mouse, for example, loss of IPM chondroitin 6-sulfate proteoglycan precedes detectable photoreceptor degeneration (Porrello and LaVail, 1986; Johnson et al., 1989; Chu et al., 1992; Lazarus et al., 1993).

Immunohistochemical and biochemical investigations have demonstrated that CMSs contain two major chondroitin 6-sulfate proteoglycans (Hageman and Johnson, 1986, 1987) which, upon digestion with chondroitinase ABC, migrate in SDS-polyacrylamide gels with apparent molecular weights of 150 kDa and 200 kDa (Hageman and Johnson, 1991a). These proteoglycans are herein referred to as IPM 150 and IPM 200, respectively. They comprise a significant portion of the IPM and they are the only IPM constituents bound by AC6S antibodies on Western blots of IPM preparations, suggesting that they represent the same molecules, which mediate retinal adhesion in vivo.

In order to characterize further the structure and function of IPM 150, we have determined the cDNA sequence encoding its core protein. The results presented herein show that IPM 150 is expressed by rod and cone photoreceptor cells in the retina as well as various other tissues and possesses a unique sequence, bearing no significant homology to any other protein. It is anticipated that this information will not only aid in subsequent studies of the role of IPM constituents in the retina but during molecular characterization of other tissues expressing this proteoglycan as well.

2. Materials and methods

2.1. Tissues

Human eyes were obtained from MidAmerica Transplant Services (St. Louis, MO, USA) and the University of Iowa Lion's Eye Bank (Iowa City, IA, USA) and processed within 3 h of cardiac cessation under approved Institutional Review Board protocols. Eyes and other organs were obtained from cynomolgus macaque monkeys (*Macaca fascicularis*) immediately after barbiturate-induced euthanasia. All animals were treated in conformity with the NIH 'Guide for the Care and Use of Laboratory Animals', and the established guidelines of St. Louis University and the University of Iowa.

2.2. Isolation of IPM

Human and monkey neural retinas were separated from the RPE and incubated in a 10-mM phosphate buffered saline (PBS), pH 7.4, containing a cocktail of protease inhibitors as described earlier (Hageman et al., 1991). The isolated retinas were then placed in 4 M urea solution in PBS containing 0.5% NP-40 and protease inhibitors until the IPM dissociated from the photoreceptor cells. The resulting sheets of insoluble IPM were isolated, pelleted by centrifugation, resuspended in PBS and repelleted (7–10 times) to remove any remaining soluble components.

2.3. Amino acid sequencing

Aqueous-insoluble human and monkey IPM preparations were homogenized and digested with chondroitin ABC lyase (E.C. 4.2.2.4) (Seikagaku) in the presence of protease inhibitors, for 2 h at 37°C. Proteins were separated on SDS-polyacrylamide gels, transferred to PVDF membranes and briefly stained with 0.1% Coomassie blue. The appropriate bands were excised and NH_2-terminal amino acid sequences for IPM 150 were determined by Edman degradation (Stone et al., 1992).

Other amino acid sequences were obtained from fragments of IPM 150 generated by an 'in-gel' trypsin digestion protocol. Briefly, chondroitinase ABC-treated IPM preparations were separated by SDS-PAGE, the gels were stained with 0.1% Coomassie and IPM 150 was identified and excised. Gel strips were incubated at 37°C for 24 h in a 1:25 (w w) ratio of trypsin to protein. Following incubation, the peptides were extracted (Stone and Williams, 1993) and fractionated by reverse phase HPLC in 0.2 ml of 2 M urea solution. Separated peptides were subjected to amino acid sequencing.

2.4. Re erse transcription-polymerase chain reaction (RT-PCR)

Total retinal RNA was isolated from monkey and human retinas using RNAStat-60 reagent (Tel-Test) and 100 ng tube were reverse-transcribed using random hexamer primers and the GeneAmp® RNA PCR Kit (Perkin Elmer Cetus). For the initial experiments, monkey retinal cDNA was amplified for 25 cycles using a degenerate sense PCR primer (5′-TATTAGGAAT TCCATYTTYT TYCCIAAYGG-3′), designed using the amino acid sequence of the monkey IPM 150 amino-terminus and a degenerate antisense primer (5′-TTICCIGCIA GYTCYTGRTA RTAIGG-3′) which was designed based on the sequence of tryptic peptide #70, derived from human IPM 150. During the synthesis of these primers, inosine residues were used in positions of complete degeneracy. An amplification product of 580 bp was isolated and cloned. The resulting clone was designated 70-1.

Another clone, designated 9-5#3, was generated by RT-PCR. Human retinal RNA was reverse transcribed, using a primer complementary to nucleotides 2934–2950 of the human IPM 150 sequence. The resulting cDNA was amplified using sense (nucleotides #111–126) and antisense (nucleotides #2681–2696) primers corresponding to sequences flanking the predicted open reading frame of IPM 150. The resulting PCR fragment was subcloned into the vector pCRII (InVitrogen).

2.5. Isolation of human IPM 150 cDNA clones

A human retinal cDNA library in λgt11 (Clontech Laboratories Inc.) was screened by plaque hybridization using probes derived from clone 70-1. Plaques were plated to a density of approximately 20 000 pfu 150 mm Petri dish on *E. coli* Y1090 host cells. Duplicate plaque lifts were prepared using nitrocellulose filters. cDNA probes were labeled with ^{32}P-dCTP in a random-primed reaction and hybridized to the plaques for 12–20 h at 65°C. After hybridization, the filters were washed at room temperature for 10 min in $2 \times$ SSC (0.3 M NaCl and 30 mM sodium citrate) containing 0.3% SDS, and two additional times, for 10 min each in $0.2 \times$ SSC containing 0.1% SDS at 60°C. Plaques giving signals on autoradiograms derived from both filters were isolated, eluted in λ-buffer (10 mM Tris, pH 7.5 and 10 mM MgCl), replated at a lower density, and rescreened until isolated, positive plaques were obtained. cDNA inserts of purified λgt11 clones were PCR-amplified using primers to the flanking regions of λgt11 and ligated into pCRII using the TA cloning kit (InVitrogen).

2.6. cDNA sequencing

Subcloned cDNA fragments were manually sequenced by dideoxy nucleotide chain termination (Sanger et al., 1977) using the Sequenase 2.0 sequencing Kit (Amersham). Both strands were analyzed at least twice using either vector-specific primers or custom oligonucleotide primers. Comparisons between the obtained sequences and those in the NCBI databases were executed using the BLAST algorithm (Altschul et al., 1990) in the MacVector software package (Kodak International Biotechnologies) or the NCBI web server.

2.7. Northern blot analyses

Total RNA from various ocular and non-ocular tissues was isolated as described above and separated on agarose gels containing formaldehyde. The membranes were hybridized with a specific ^{32}P-labeled cDNA (corresponding to nucleotides 817–3160 of human IPM 150) for detection of the corresponding transcripts. Hybridization and rinse conditions were exactly as described above. The blot was then stripped and hybridized with a human GAPDH probe to ensure the integrity of the mRNA.

2.8. Tissue expression

A commercially available RNA dot-blot containing 100–500 ng poly A RNA from various human tissue sources (Clontech Laboratories Inc.) was probed as described under Northern blot analyses.

2.9. In situ hybridization

Human retinas were fixed in 4% paraformaldehyde in 10 mM phosphate buffered saline, embedded in acrylamide, frozen, and sectioned to a thickness of 6 μm as described previously (Hageman and Johnson, 1986). A 605-bp fragment of the human IPM 150 cDNA, spanning from bases 1636 to 2241 was used in the generation of α^{35}S-UTP labeled riboprobes. Tissue sections were incubated overnight in a moist chamber, at 45°C, in a buffer containing 50% formamide, 500 μg ml yeast tRNA, 10% dextran sulfate, 100-mM DTT, 0.3-M NaCl, 1-mM EDTA, 10-mM Tris–HCl, pH 8.0, and 5×10^5 cpm section of antisense probe or, as a control, sense RNA derived from the same sequence. Following hybridization the sections were rinsed, digested with RNase A (10 μg ml) and washed in $2 \times$ SSC at 50°C. The sections were then dehydrated in increasing concentrations of ethanol and dipped in Kodak NTB-2 emulsion. Slides

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Human	X	A	F	F	P	T	G	V	K	V	X	P	Q	E	(S)	M	K	Q	I	L											
Monkey	X	I	F	F	P	D	G	V	K	V	X	P	Q	E	(S)	M	K	Q	I	L	A	S	X	Q	A	Y	Y	R	X	R	V

Fig. 1. Amino-terminal sequences obtained by direct amino acid sequencing of IPM 150 isolated from native human and monkey IPM. 'X' represents positions for which an amino acid could not be identified conclusively.

were developed after 4–5 days and photographed on an Olympus Vanox light microscope using darkfield optics.

3. Results

3.1. Amino acid sequence

Direct amino-terminal amino acid sequencing resolved 31 and 20 amino acids of the amino-termini of monkey and human IPM 150, respectively (Fig. 1). Alignment of these two sequences reveals a high degree of sequence conservation between the two species; differences are present at positions 2 and 6. Comparison of these sequences to those in the NCBI database indicates that IPM 150 is not homologous to other proteins.

In addition, the amino acid sequences of six distinct tryptic peptides of human IPM 150 have been determined (Fig. 2). The sequences of two of these peptides (peptides #68 and #81) are homologous to those of the amino-terminus. The sequences of the four remaining IPM 150 peptides are novel and not homologous to the amino-terminus.

3.2. Isolation of monkey IPM 150 cDNA

RT-PCR of monkey retina total RNA, employing a sense primer designed according to amino acids 2–7 of the monkey amino-terminal sequence (Fig. 1) and an antisense primer based on amino acids 9–17 of tryptic peptide #70, repeatedly yielded a 580-bp amplification product. This fragment, designated 70-1, was isolated and subcloned. Nucleotide sequence

Peptide	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
# 67	X	X	Q	E	A	V	X	E	A	Y									
# 68	X	A	F	F	P	T	G	V	K										
# 70	F	K	A	E	L	A	D	S	Q	S	P	Y	Y	Q	E	L	A	G	K
# 81	X	I	L	D	S	L	Q	A	Y	Y									
# 95	X	P	A	S	D	L	L	S	F										
# 130	X	L	X	E	M	D	L	S	D	(V)	P	A	P	X	G	V	P		

Fig. 2. Amino acid sequences of tryptic peptides derived from human IPM 150. 'X' represents positions for which an amino acid could not be identified conclusively.

analyses confirm that clone 70-1 contains an uninterrupted open reading frame encoding a protein which contains both the entire amino-terminal sequence of monkey IPM 150 and the full amino acid sequence obtained from human tryptic peptide #70 (Fig. 3).

3.3. Isolation of human IPM 150 cDNA

In order to obtain the complete cDNA sequence of human IPM 150, clone 70-1 was used as a probe to screen a human retinal cDNA library. Three cDNA clones, designated 8.1.2, 11.1.1 and 12.3.1, have been isolated, sequenced and assembled into one contiguous sequence (Fig. 4). The assembled nucleotide sequence of 3261 bp contains an uninterrupted open reading frame of 2313 bp and several hundred base pairs of untranslated 5′ and 3′ regions (Fig. 5). Verification that the assembled sequence encodes human IPM 150 is provided by the presence of the complete amino-terminal amino acid sequence (Fig. 1), as well as all internal peptides (Fig. 2), within the deduced amino acid sequence.

Clone 8.1.2 lacks a 234-bp segment within its 5′ region that encodes the amino-terminus of IPM 150 (see Fig. 5). This clone may represent a cloning artifact or it may be indicate the existence of IPM 150

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1   ATTTTCTTTCCGAACGGGGTTAAAGTCTGTCCACAGGAATCCATGAAACAGATTTTAGCCAGTCTTCAAGCTTATTATAGATTGAGAGTGTGTCAGGAAGCAGTATGGGAAGCATATCGGATCTTTCTGGATCGCATCCCTGACACAGGG
    I  F  F  P  N  G  V  K  V  C  P  Q  E  S  M  K  Q  I  L  A  S  L  Q  A  Y  Y  R  L  R  V  C  Q  E  A  V  W  E  A  Y  R  I  F  L  D  R  I  P  D  T  G

151 GAATATCAGGACTGGGTCAGCTTCTGCCAGCAGGAGACCTTCTGCCTCTTTGACATCGGACAAAACTTCAGCAATTCCCAGGAGCACCTGGATCTTCTCTCCAGCAGAGAATAAAACAGAGAAGTTTCCCTGAGAGAAAAGATGAAGTATCT
    E  Y  Q  D  W  V  S  F  C  Q  Q  E  T  F  C  L  F  D  I  G  Q  N  F  S  N  S  Q  E  H  L  D  L  L  Q  Q  R  I  K  Q  R  S  F  P  E  R  K  D  E  V  S

301 ACAGAGAAGACATTGGGAGAGCCTAGTGAAACCATTGTGGTTTCAACAGATGTTGCCAGCGTCTCACTTGGGCCTTTCCCTGTCACTCCTGATGACACCCTCCTCAATGAAATTCTCGATAATGCACTCAACGACACCAAGATGCCTACA
    T  E  K  T  L  G  E  P  S  E  T  I  V  V  S  T  D  V  A  S  V  S  L  G  P  F  P  V  T  P  D  D  T  L  L  N  E  I  L  D  N  A  L  N  D  T  K  M  P  T

451 ACAGAAAGAGAAACAGAACTCGCTGTGTCTGAGGAGCAGAGGGTGGAGCTCAGCATCTCTCTGATAAACCAGAGGTTCAAGGCAGAGCTCGCTGACTCTCAGTCA
    T  E  R  E  T  E  L  A  V  S  E  E  Q  R  V  E  L  S  I  S  L  I  N  Q  R  F  K  A  E  L  A  D  S  Q  S
```

Fig. 3. Corresponding nucleotide and deduced amino acid sequences of clone 70-1 encoding the NH_2-terminal region of monkey IPM 150. The sequences of the primers employed in the RT-PCR reactions are underlined. Regions confirmed by direct amino acid sequencing are boxed. Putative *N*-glycosylation sites are designated by open triangles.

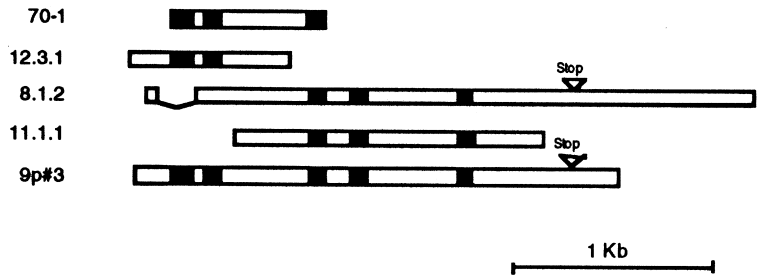

Fig. 4. Alignment of IPM 150 cDNA clones. Clone 70-1 was generated by RT-PCR of monkey retinal RNA and was employed as a probe to screen a human cDNA library from which clones 12.3.1, 8.12, 11.1.1 and 9p#3 were isolated. Filled boxes represent the locations of IPM 150 amino acid sequences obtained from tryptic peptides.

isoforms. In an attempt to resolve this issue and to demonstrate that the isolated clones in fact represent the IPM 150 cDNA, a PCR product encompassing the entire open reading frame of IPM 150 was cloned. PCR amplification of reverse-transcribed human retinal RNA yields a 2585 bp fragment which has been subcloned and designated 9p#3. This clone is completely homologous to the assembled sequence and includes the 234-bp region absent in clone 8.1.2 (see Fig. 5).

Database searches of the compiled nucleotide and amino acid sequences show that they are novel. A few expressed sequence tags (ESTs), derived from human retina, vein endothelial cells, and brain, however, do share homology with IPM 150. Translation of the open reading frame of the assembled cDNA sequences encodes a protein of 797 amino acids with an isoelectric point of 4.60 and a predicted molecular weight of 89.4 kDa. The protein is generally hydrophilic (Kyte and Doolittle, 1982) except for the

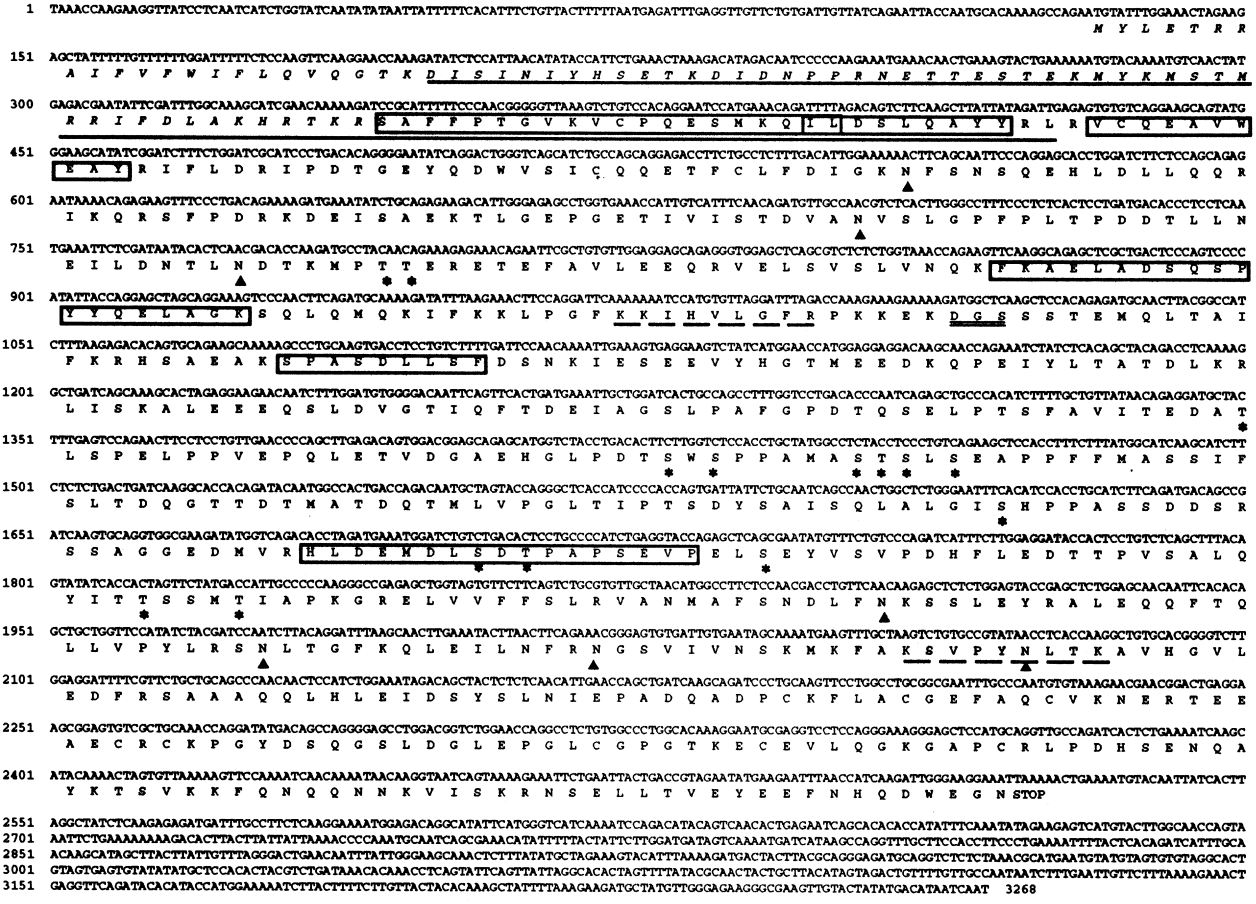

Fig. 5. Assembled nucleotide and predicted amino acid sequences of human IPM 150. The fragment that is absent in clone 8.1.2 is underlined by a solid line, the reading frame remains unchanged. Regions of the translated sequence which were confirmed by direct amino acid sequencing of the native protein are boxed. The putative glycosaminoglycan attachment site is indicated by a double line. N-glycosylation sites are designated by triangles, O-glycosylation sites by asterisks and possible hyaluronan binding sites are indicated by a broken line.

first 18 amino acids which form a hydrophobic region flanked by charged amino acid residues; this region may represent a signal sequence.

A distinct distribution of consensus sequence sites for N- and O-linked glycosylation are present in IPM 150 (Fig. 5). There are several consensus sequences for N-linked glycosylation (Kornfeld and Kornfeld, 1985) sequestered in the amino-terminal (amino acids 71–217) and the carboxyl-terminal (beginning at amino acid 591) portions of the IPM 150 core protein. In contrast, the central domain of IPM 150 (between amino acids 220 and 565) features 17 sites which are suitable for O-linked glycosylation, as predicted by a proposed algorithm for the activity of polypeptide *N*-acetylgalactosaminyl-transferase (Hansen et al., 1995). There is almost no overlap between the regions containing potential O- and N-linked glycosylation sites. A single site for glycosaminoglycan attachment (Li et al., 1992) is located at amino acid 296. Hyaluronan binding consensus sequences (Yang et al., 1994) and several cysteine residues are also present in the amino- and carboxyl-terminal regions of the core protein. The distribution of the carboy-terminal cysteine residues closely resembles that of EGF-like domains (Rees et al., 1988), motifs that are present in many extracellular matrix proteins.

3.4. Distribution of IPM 150 transcripts

In order to determine the cellular source(s) of IPM 150, Northern and in situ hybridization analyses have been conducted. IPM 150 cDNA probes hybridize to a 3.9-kb transcript that is present in relatively high abundance in retinal RNA. No signal is detected on Northern blots of RNA isolated from RPE choroid, iris or cornea, suggesting that — in the human eye — IPM 150 is transcribed only in the neural retina (Fig. 6). Dot blot analyses of polyA RNA from 50 different adult and fetal human tissues indicate that IPM 150 mRNA, or a transcript with a similar nucleotide sequence, is present in adult lung, liver, kidney, thymus and small intestine (Fig. 7). Weak hybridization to fetal lung and thymus RNA as well as a number of additional adult tissues is also observed.

Distinct hybridization of IPM 150 antisense riboprobes to the human retinal outer nuclear layer (ONL) is observed on sections of human retina, RPE and choroid (Fig. 8). The ONL contains the cell bodies of the photoreceptor cells, demonstrating that IPM 150 is synthesized by this cell type. IPM 150 transcripts are present within both rod and cone photoreceptor cells. No labeling of any other region is observed.

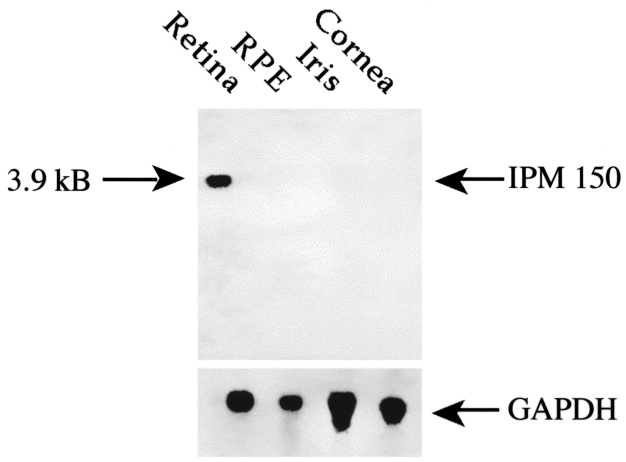

Fig. 6. Northern blot of RNA from several human ocular tissues, probed with IPM 150 cDNA. This probe hybridizes specifically to a retinal transcript of approximately 3.9 kb. The blot was subsequently stripped and reprobed with a probe to human GAPDH as a control for RNA integrity.

4. Discussion

We have cloned, sequenced and determined the cellular distribution of human IPM 150, a novel proteoglycan core protein which represents a major constituent of the IPM (Hageman and Johnson, 1987,

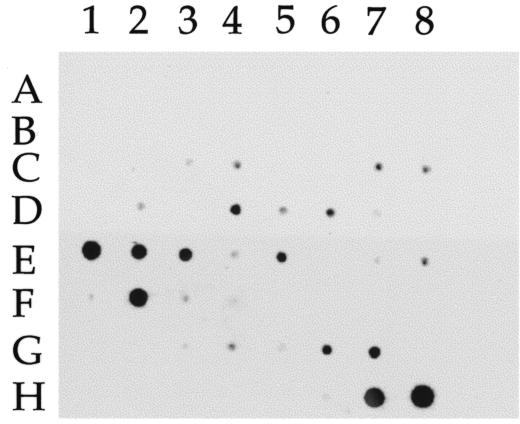

Fig. 7. Dot blot of polyA RNA from 50 adult and fetal human tissues probed with IPM 150 cDNA. Transcripts with a similar nucleotide sequence are present in adult kidney (E1), liver (E2), small intestine (E3), thymus (E5) and lung (F2). Weak hybridization to fetal thymus (G6) and lung (G7) RNA is also observed. Other adult human tissues represented on the blot include: whole brain (A1), various brain regions (A2–B6), spinal cord (B7), heart (C1), aorta (C2), skeletal muscle (C3), colon (C4), bladder (C5), uterus (C6), prostate (C7), stomach (C8), testis (D1), ovary (D2), pancreas (D3), pituitary gland (D4), adrenal gland (D5), thyroid gland (D6), salivary gland (D7), mammary gland (D8), spleen (E4), peripheral leukocyte (E6), lymph node (E7), bone marrow (E8), appendix (F1), trachea (F3), and placenta (F4). Fetal human brain (G1), heart (G2), kidney (G3), liver (G4), and spleen (G5) are also represented. Controls include *E. coli* DNA (H4), human ribosomal RNA (H5), human C_ot1 DNA (H6) and human genomic DNA (H7 and 8)

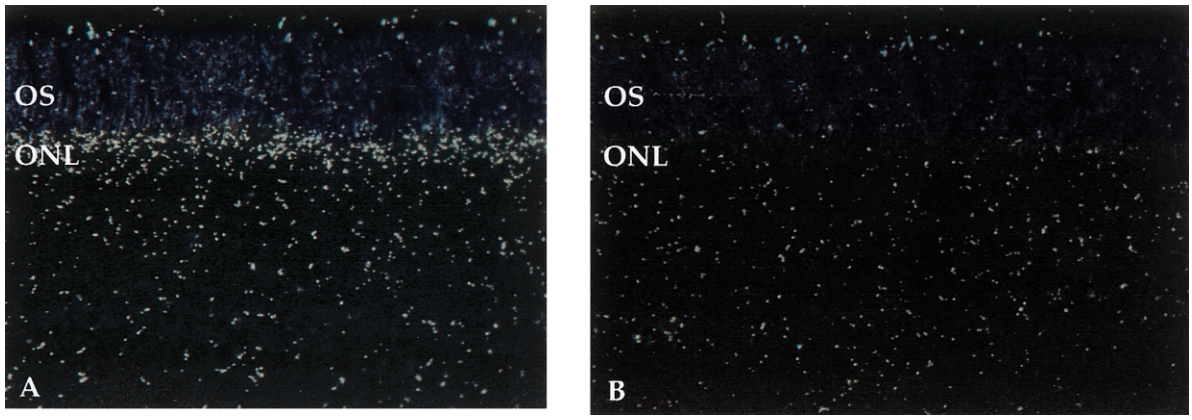

Fig. 8. In situ hybridization of an IPM 150 riboprobe to sections of human retina. ^{35}S-UTP labeled RNA antisense (A), but not sense (B), probes hybridize specifically to the outer nuclear layer (ONL), demonstrating that IPM 150 is synthesized specifically by photoreceptor cells. IPM 150 appears to be transcribed by both cone or rod photoreceptor cells. OS: outer segments of photoreceptor cells.

1991b; Hageman and Kuehn, 1998). The deduced amino acid sequence of human IPM 150 was verified by comparison to amino acid sequences of the amino-terminus and four internal tryptic peptides derived from native human IPM 150 protein. The apparent amino-terminus of the native protein begins with amino acid #70, based on direct sequencing from SDS-PAGE gels. Thus, either amino acids 1–69 are cleaved from the native protein in vivo or a sensitive proteolytic site exists and is cleaved during the purification of the protein.

The predicted molecular weight of the 'mature' IPM 150 core protein, as deduced from its cDNA sequence, is 80.8 kDa. The protein migrates at approximately 150 kDa on SDS-PAGE gels under denaturing conditions following digestion of native IPM with chondroitinase ABC. The discrepancy between the calculated and the observed molecular weights of IPM 150 is most likely due to the presence of N- and or O-glycosidically linked oligosaccharides that remain attached to the protein after chondroitinase treatment. This contention is supported by biochemical analyses demonstrating that IPM 150 migrates at 80 and 105 kDa following enzymatic or chemical deglycosylation, respectively (Hageman, unpublished data).

At least three distinct domains exist within the deduced protein sequence of human IPM 150. An amino terminal domain contains three consensus sites for potential N-linked glycosylation and one site which fits the proposed consensus sequence for hyaluronan binding (Yang et al., 1994). A central domain contains the likely glycosaminoglycan attachment site as well as 17 consensus sites for O-linked glycosylation (Hansen et al., 1995) as well as a possible site for GAG attachment. A carboxyl terminal domain features four possible N-glycosylation sites, a second site for hyaluronan attachment and a single EGF-like domain (Rees et al., 1988). EGF-like domains are present in a variety of extracellular matrix proteins. One reported function of the domain is to promote the survival of neighboring cells (Engel, 1992). By analogy, it is conceivable that this region of IPM 150 may promote photoreceptor survival in vivo. This contention is strengthened by previous studies suggesting that the IPM, and more specifically its proteoglycans, are important in maintaining photoreceptor cell viability (Lazarus and Hageman, 1992; Blanks et al., 1993).

With the exception of the amino-terminal signal sequence, which appears to be removed during the maturation of IPM 150, the core protein does not possess any other hydrophobic regions that might constitute transmembrane domains. This implies that IPM 150 is released into the interphotoreceptor space and is not membrane intercalated. Based on previous studies supporting a role for IPM proteoglycans in adhesion of the neural retina to the RPE (Hollyfield et al., 1989; Yao et al., 1992, 1994; Hageman et al., 1995), we propose that IPM 150 may act as a bridging element, between as yet unidentified membrane-associated molecules on the photoreceptor and RPE cell surfaces to effect retinal adhesion. The vitronectin receptor has recently been localized to the surfaces of photoreceptor outer segments and the apical microvilli of RPE cells (Anderson et al., 1995) and might serve as a receptor for IPM 150. Although IPM 150 does not possess any RGD consensus sequences, this does not preclude a potential interaction between IPM 150 and the vitronectin receptor. The chondroitin-sulfate glycosaminoglycans of IPM 150 might also participate in retinal adhesion through receptors like CD44 (Toyama-Sorimachi et al., 1995), an adhesion molecule associated with Müller cell apical microvilli in mice and humans (see Hageman and Kuehn, 1998). This is one interpretation of the data derived from in vivo studies documenting disruption of retinal

adhesion following subretinal administration of glycolytic enzymes or perturbation of chondroitin sulfate glycosaminoglycan biosynthesis (Yao et al., 1990, 1992, 1994; Lazarus and Hageman, 1992). Based on studies suggesting that hyaluronan is an IPM constituent (Tate et al., 1993; Hollyfield et al., 1998) and that IPM possesses two hyaluronan binding domains, it is also plausible that IPM 150 may interact with hyaluronan to effect retinal adhesion. Complete knowledge of the IPM molecules that elicit retinal adhesion will be of paramount importance for understanding the biology of retinal detachment and degeneration. Ongoing studies are being directed towards this end.

IPM proteins could be synthesized by any of the cell types that lie adjacent to it, including photoreceptor, RPE and or Müller cells. The in situ hybridization analyses presented herein provide convincing evidence that IPM 150 is synthesized by rod and cone photoreceptor cells. Based on previous immunohistochemical studies showing that IPM 150 is restricted to CMSs (Hageman and Johnson, 1987; Mieziewska et al., 1991), we predicted that IPM 150 would likely be synthesized solely by cone photoreceptor cells. These apparently disparate data obtained in this study might be explained as follows. It is conceivable that IPM 150 isoforms or spliced variants, which are synthesized by rod photoreceptors and do not contain chondroitin 6-sulfate, may exist. These isoforms could be produced either by differential glycosylation of the same core protein and or through small differences in the amino acid sequences of rod and cone IPM 150. The absence of 234 nucleotides in clone 8.1.2 suggests that splicing isoforms may indeed exist. However, the difference in overall length of the transcripts is too small to lead to two distinguishable bands on Northern blot analyses.

There are indications that IPM 150, or a molecule with a related cDNA sequence, may be transcribed in several non-ocular tissues. Dot-blot analyses of mRNA derived from a variety of human tissues indicate that IPM 150-derived probes hybridize to human lung, thymus, kidney, small intestine and perhaps several other tissues as well. In addition, ESTs homologous to the IPM 150 cDNA sequence have been identified from human endothelial cell and brain cDNA libraries. To date, however, we have been unable to demonstrate the presence of IPM 150 transcripts or proteins in the brain.

Biochemical studies conducted in this laboratory have clearly documented that chondroitin 6-sulfate moieties are associated with IPM 150 (Hageman and Johnson, 1987, 1991a). The deduced amino acid sequence for IPM 150 does not contain the more typical glycosaminoglycan attachment consensus sequence SGXG (Bourdon et al., 1987). However, a single glycosaminoglycan attachment site (DGS) that is associated with collagen type IX and chick decorin (Huber et al., 1988; Li et al., 1992), is present in the deduced IPM 150 protein. It is clear that future studies will need to be directed towards characterizing the glycosaminoglycan attachment site(s) associated with IPM 150.

A recent report indicates the presence of a 147 kDa IPM protein, referred to as SPACR, that migrates into SDS-PAGE gels without prior deglycosylation with chondroitinase ABC. The investigators interpret these data to suggest that this protein does not possess glycosaminoglycan moieties (Acharya et al., 1998). Curiously, the authors base the amino acid sequence of SPACR entirely on the IPM 150 cDNA sequence submitted to GenBank by this laboratory, yet they maintain that SPACR represents a 'new' IPM protein. If, indeed, SPAR is encoded by the *IMPG1* gene, then it is not a novel protein. It is plausible that SPACR may represent a non-glycosylated, or spliced, variant of IPM 150. Conversely, this 147-kDa protein might share only limited sequence homology with IPM 150. It is also conceivable that the protein identified by Acharya and coworkers is a proteolytic fragment of IPM 150. We have, on occasion, observed a 140–145-kDa protein on Western blots of non-deglycosylated IPM, but only when the human donor tissues employed were harvested after long post-mortem times. Until such time that the complete nucleotide and amino acid sequences of SPACR are determined and its relationship to IPM 150 is established, the nature of SPACR remains unclear. We propose that it is premature to refer to SPACR as a unique IPM glycoconjugate until such data are made available.

We reported earlier that the genomic location of IMPG1 is on chromosome 6 between markers D6S280 and D6S1659 (Felbor et al., 1997; Kuehn et al., 1997), placing it within the loci assigned for Stargardt-like dominant progressive macular dystrophy (STGD3) (Stone et al., 1994) and Salla disease, a lysosomal storage disorder (Leppanen et al., 1996). Using cDNA sequence information provided by this laboratory, Felbor et al. (1998) characterized the genomic organization of the *IMPG1* gene. Studies designed to identify sequence changes in the *IMPG1* gene of persons afflicted with dominant cone-rod dystrophy (CORD7), progressive bifocal chorioretinal atrophy (PBCRA), North Carolina macular dystrophy (MCDR1) and STGD3 did not reveal any disease causing mutations (Gehrig et al., 1998; Kelsell et al., 1998). Only one donor with STGD3 was included in these studies, however, and not enough clinical data were presented to determine whether this individual was afflicted with STGD3 as it was originally described (Stone et al., 1994). Thus, the issue as to whether IMPG1 mutations cause STGD3 remains largely unresolved.

Characterization of the IPM 150 core protein cDNA

represents a critical step towards expanding our knowledge of the interphotoreceptor matrix. Because the IPM mediates processes that are crucial to normal retinal function and survival, molecular analyses of novel IPM molecules will likely advance our understanding of this unique compartment within the central nervous system. Knowledge of the IPM 150-cDNA sequence will enable us to develop specific probes directed against it and to generate transgenic animal models to more closely examine the function of this unique proteoglycan in vivo. Together, these experiments should provide additional insight into the role of the IPM in the normal and diseased human retina as well as in non-retinal tissues that express IPM 150.

Acknowledgements

Supported in part by NIH EY06463, NIH EY15151, an RPB, Lew R. Wasserman Merit Award, and an unrestricted grant to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness, Inc.

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