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Versican in human fetal skin development

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Abstract The extracellular matrix of human fetal skin differs substantially from that of adult skin. Fetal skin contains sparse amounts of fibrillar collagen enmeshed in a highly hydrated amorphous matrix composed of hyaluronan and sulfated proteoglycans. Both fetal and adult skin contain two major interstitial proteoglycans that are extracted by chaotrophic agents and detergents. These are the large chondroitin sulfate proteoglycan versican and the small dermatan sulfate proteoglycan decorin. For this study, proteoglycans extracted from fetal and adult skin were compared on Western blots to determine the relative amounts of versican. Decorin present in the same samples provided an internal standard for these studies. Fetal skin differed from adult skin in that it contained a significantly higher proportion of versican than did adult skin. Immunohistochemical studies compared early-fetal with mid-fetal skin and found that versican was a significant component of the interstitial extracellular matrix at both of these stages of skin development. However, by the mid-fetal period, interstitial versican became restricted to the upper half of the dermis, although versican also continued to be highly expressed around hair follicles, glands, and vasculature in the lower half of the dermis. Fetal skin extracts differed from an adult skin extract by the presence of a 66-kDa protein immunologically related to versican and by the absence of a 17-kDa core protein of a proteoglycan related to decorin. Both of these molecular species may represent degradation products of their respective proteoglycans. Monoclonal antibodies which detect epitopes in native chondroitin sulfate glycosaminoglycan chains recognized versican extracted from fetal skin. However, the tissue distribution of these antigens did not entirely conform to that for versican core protein, suggesting that versican in different regions of the skin may be substituted with glycosaminoglycan chains with different microchemistries. The results of these studies indicate that human fetal skin is structurally different from adult skin in terms of both the distribution and the composition of the large, aggregating chondroitin sulfate proteoglycan versican.

Key words Skin · Proteoglycan · Development · Human · Fetal

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Introduction

In fetal skin, the sparse amounts of fibrillar collagen are enmeshed in an amorphous, highly hydrated matrix composed principally of hyaluronan and sulfated proteoglycans, creating a matrix that confers special characteristics on this developing organ (Breen et al. 1970; Holbrook et al. 1993; Kaplan et al. 1994). These matrix components support the active cellular proliferation, migration, and differentiation events that are required for growth and development (Goetinck 1991; Knudson and Knudson 1993; Ellis and Schor 1995). It has also been proposed that the hyaluronan component of this amorphous matrix facilitates the scar-free healing characteristics of fetal skin (Longaker et al. 1991; Adzick and Lorenz 1994).

Hyaluronan, an atypical, non-sulfated glycosaminoglycan, differs from other glycosaminoglycans in that it binds to a specific group of proteins, sometimes termed matrix hyaladherens (Knudson and Knudson 1993), through non-covalent interactions (Rodén 1980; Carney and Muir 1988). Some of these hyaluronan-binding molecules, like CD44, concentrate and organize hyaluronan in pericellular domains (Knudson and Knudson 1993). In addition, a substantial amount of hyaluronan resides in the interstitial spaces where it interacts with and is stabilized by sulfated proteoglycans (Lever-Fischer and Goetinck 1988; LeBaron et al. 1992; Knudson and Knudson 1993).

Versican, like aggrecan, neurocan, and brevican, is a chondroitin sulfate proteoglycan that consists of a core protein that contains various globular and chondroitin

sulfate-attachment domains (Zimmermann and Ruoslahti 1989; Margolis and Margolis 1993; Dours-Zimmermann and Zimmermann 1994; Yamada et al. 1994; Miller et al. 1995). One of these domains, that which is located near the N-terminus, specifically recognizes and binds hyaluronan with relatively high affinity (Johansson et al. 1985; Lever-Fischer and Goetinck 1988; Goetinck 1991; LeBaron et al. 1992). Thus, versican has the potential to form multimeric aggregate structures with hyaluronan.

Little is currently known about the content and distribution of versican in human fetal skin. Breen et al. (1970) reported that chondroitin sulfate and hyaluronan, but not dermatan sulfate, were the principal glycosaminoglycans found in extracts of human fetal skin. These data are consistent with the view that versican is a major constituent of human fetal skin. However, studies of fetal and neonatal rodent skin suggested that versican was not the principal interstitial proteoglycan. Immunohistochemical studies of developing mouse skin failed to detect versican in interstitial regions of the dermis, but found this molecule in regions surrounding hair follicles, glands, and perivascular regions (du Cros et al. 1995). It has also been reported that in developing rat skin a small dermatan sulfate proteoglycan, presumably decorin, is the major sulfated proteoglycan (Habuchi et al. 1986).

The studies undertaken here were designed to compare the relative contents of versican extracted from human fetal and adult skin and to study the immunohistochemical distribution of this proteoglycan during early and mid-fetal periods of skin development. Proteoglycans extracted from fetal and adult skin were compared on Western blots to determine the relative amounts of versican. Decorin present in the same samples provided an internal standard for these studies. Fetal skin differed from adult skin in that it contained a significantly higher relative proportion of versican than did adult skin. Immunohistochemical studies compared early-fetal with mid-fetal skin and found that versican was a significant component of the interstitial extracellular matrix at both of these stages of skin development. However, by the mid-fetal period, interstitial versican became restricted to the upper half of the dermis, although versican also continued to be highly expressed around hair follicles, glands, and vasculature in the lower half of the dermis. Fetal skin extracts differed from an adult skin extract by the presence of a 66-kDa protein immunologically related to versican and by the absence of a 17-kDa core protein of a proteoglycan related to decorin. Both of these molecular species may represent degradation products of their respective proteoglycans. Monoclonal antibodies that detect epitopes in native chondroitin sulfate glycosaminoglycan chains recognized versican extracted from fetal skin. However, the tissue distribution of these antigens did not entirely conform to that for versican core protein, suggesting that versican in different regions of the skin may be substituted with glycosaminoglycan chains with different microchemistries. The results of these studies indicate that human fetal skin is structurally different from adult skin with respect to both the distribution and the composition of the large, aggregating chondroitin sulfate proteoglycan versican.

Materials and methods

Subjects

Human fetal skin samples were obtained from the Central Laboratory for Human Embryology at the University of Washington, Seattle, Wash. in accordance with policies established by the Institutional Review Board at Case Western Reserve University and, therefore, have been performed as established by the 1964 Declaration of Helsinki. All human tissues used for these studies were classified as discarded tissue; thus, informed consent of the donors was not required. Fetal scalp and trunk skin samples in cold, sterile saline solution were shipped by overnight delivery. These samples were prepared for further study immediately upon receipt. Those samples that were used for histology were cut into small pieces and embedded in Tissue-Tek, O.C.T. embedding medium (Sakura Finetek, Torrance, Calif.) without prior fixation. Proteoglycans were extracted from other pieces of tissue (as described below), and some pieces of tissue were used to establish fetal dermal cell cultures. Adult human skin in cold, sterile saline solution was obtained from surgical breast reductions and was provided by the Human Tissue Cooperative, Department of Pathology, School of Medicine, Case Western Reserve University, Cleveland, Ohio in accordance with policies established by the Institutional Review Board of this University. Adult papillary and reticular dermal fibroblasts were obtained from Dr. Irwin A. Schafer, MetroHealth Medical Center, Cleveland, Ohio (Schafer et al. 1985). The adult papillary and reticular fibroblasts used for this study were grown from the same piece of volar arm skin from a 36-year-old donor.

Antibodies

Anti-chondroitin sulfate antibodies 4C3, 6C3, and 7D4 (produced in this laboratory) and antibody CS-56 (Sigma Chemical, St. Louis, Mo.) were used as previously described on tissue sections without prior chondroitinase digestion (Avnur and Geiger 1984; Sorrell et al. 1990, 1993). Antibody 2B6 was a gift from Dr. Bruce Caterson, University of Cardiff, Wales (Couchman et al. 1984; Caterson et al. 1985). Anti-human versican antibody 12C5 was obtained from the Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, Iowa (Asher et al. 1991, 1995), and monoclonal antibodies against human versican, 2B1, and decorin, 6B6, (Yamagata et al. 1987; Sobue et al. 1989; Isogai et al. 1996) were obtained from Seikagaku America (Rockville, Md.). Anti-decorin polyclonal antibodies LF-30 (Bianco et al. 1990) and LF-122 (Fisher et al. 1995) were a generous gift from Dr. Larry W. Fisher, National Institutes of Health, Bethesda, Md. Antibody S103L against chick aggrecan was generously provided by Dr. N. Schwartz, University of Chicago, Chicago, Ill.

Immunohistochemistry

Frozen tissue sections, 8-µm thick, were mounted onto glass slides for immunohistochemical analyses (Sorrell et al. 1996). Following incubation with the primary antibody at room temperature, the sections were washed and incubated with secondary antibody (goat, anti-mouse Ig) conjugated to fluorescein isothiocyanate (Organon Teknika, West Chester, Pa.). Control sections were prepared for each tissue. For these sections, the tissue was incubated with buffer only or with hybridoma culture medium containing no antibody. No immunoreactivity was observed on these sections. The sections were viewed on an Olympus BH-2 microscope and photographed using Kodak Tmax ASA 400 film.

Extraction and purification of proteoglycans

For illustrative purposes in this manuscript, human fetal skin was obtained from the pooled trunk and scalp skin from four fetuses ranging in age from day 76 to day 88 estimated gestational age (EGA), which will be referred to as day 80 EGA in the text, and from pooled trunk and scalp skin of a single day 127 EGA fetus. Adult human breast skin was obtained from a 33-year-old individual. However, additional specimens have been studied; altogether, two different sets of day 80 and day 127 fetal samples have been studied. Additional adult breast samples from 26-, 31-, 78-, and 82-year-old individuals have also been studied. These tissues were cut into small pieces and were placed into cold extraction buffer consisting of 0.05 M sodium acetate, pH 5.8 containing 4 M guanidine hydrochloride, detergent (0.5% Triton X-100 for the fetal samples and 0.5% 3-[(3-choloamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) for the adult samples), and protease inhibitors (5 mM benzamidine, 0.1 M 6-aminocaproic acid, 0.01 M sodium ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonyl fluoride, and 0.01 M N-ethylmaleimide). The tissues were extracted for 24 h at 4 C with constant stirring (DeLuca et al. 1977; Carrino et al. 1994). The extraction buffer was removed and fresh extraction buffer was added for an additional 24-h period, at which time the two sets of extracts were combined. These extracts were clarified by centrifugation at 15,000×g for 20 min and were then filtered through 1.0-µm filters.

The extraction buffer for both the fetal and adult samples was replaced with a 0.05 M sodium acetate buffer, pH 7.0 containing 8 M urea, 0.15 M NaCl, and 0.5% CHAPS. Proteoglycans were partially purified from skin extracts by ion exchange chromatography on a diethylaminoethyl (DEAE)-Sephacel column; after loading the sample, the column was washed sequentially with five column volumes of the above buffer and then the same buffer containing 0.25 M NaCl. The proteoglycans were eluted from the column with the same buffer containing 1.0 M NaCl (Carrino et al. 1994).

Cell culture

Fetal skin samples were cut into small pieces under sterile conditions and treated with a 1:1 mixture of 0.25% trypsin-1 mM EDTA (Life Technologies, Gaithersburg, Md.) and 1 mg/ml collagenase P (Sigma Chemical, St. Louis, Mo.) at 37 C for 3 to 4 h. The tissue was repeatedly pipetted using a 5-ml pipet, and the released cells and tissue pieces were plated separately in Dulbecco's modified Eagle's Medium (DMEM) (Sigma Chemical, St. Louis, Mo.) supplemented with 10% fetal calf serum (FCS). Fibroblasts were removed from the culture plates by brief treatment with 0.5% trypsin-1 mM EDTA; keratinocyte colonies tended to remain on the plates under these conditions. The released cells were replated at low density in DMEM containing 10% FCS. Fetal dermal cells, 5th passage, day 57 EGA and 4th passage, day 127 EGA, were used for these studies. Additional studies, not reported here, were performed using day 70, 105, and 132 fetal dermal cell samples. Adult 4th passage papillary and 4th passage reticular dermal cells from a 36-year-old donor (Schafer et al. 1985) were cultured in DMEM containing 10% FCS. Additional studies, not reported here, were also performed with papillary and reticular cells from a 51-year-old donor.

The $[^{35}S]SO_4$ incorporation studies were performed by adding radioisotope to dermal cell cultures that had attained 80–90% of confluence. Radiosotope was added to sulfate-free medium containing 10% FCS, and cultures were labelled for 5 h at 37 C. The cell culture medium was removed and the cell layer fractions were washed twice with Tyrode's balanced salt solution; this wash was added to the medium. The cell layer fractions were extracted with 4 M guanidine hydrochloride, 0.5% Triton X-100, and protease inhibitors as described above. The unincorporated isotope was removed and the guanidine hydrochloride was exchanged for 8 M urea by gel filtration chromatography on Sephadex G-50 columns. The proteoglycans were isolated by ion exchange chromatography on a DEAE-Sephacel column as described above. Following dialysis against water and lyophilization, the samples were dissolved in chondroitinase buffer containing protease inhibitors (Oike et al. 1980). Chondroitinase ABC (Seikagaku America, Rockville, Md.) was added to half of the sample to give a final concentration of 0.05 U/ml; an equal volume of chondroitinase buffer was added to the other half of the sample. Both samples were incubated at 37 C for 1 h.

Gel filtration chromatography

Lyophilized samples were dissolved in 0.05 M sodium acetate, pH 6.0, containing 4 M guanidine hydrochloride and 0.5% CHAPS and applied to a Sepharose CL-2B column (110 cm×0.6 cm) that was eluted with the same buffer. Fractions collected from the column were analyzed on a liquid scintillation counter for the amount of $[^{35}S]SO_4$ or by dot-blot assays for the presence of specific proteoglycan epitopes. Proteoglycans obtained from the medium fractions by cold ethanol precipitation (Schmidtchen et al. 1990) were dissolved in 8 M urea and treated as described above.

Gel electrophoresis

Immunoblotting was performed essentially as described previously (Carrino et al. 1994). Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred to an Immobilon-P membrane. The transfer buffer consisted of 25 mM TRIS, 192 mM glycine, pH 8.3. After transfer, the membrane was blocked with powdered, non-fat dry milk in 0.05 M TRIS(dydroxymethyl)aminomethane buffered saline containing 0.05% Tween 20 (TBS-Tween) and then incubated in primary antibody diluted into this same solution. After three rinses with TBS-Tween, the blot was incubated in secondary antibody as described above for dot-blots. Secondary antibody was alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Promega, Madison, Wis.) or alkaline phosphatase-conjugated anti-rabbit immunoglobulin (Sigma Chemical, St. Louis, Mo.) for antibodies LF-30 and LF-122. These studies have also been repeated using monoclonal antibody 6B6 (Seikagaku America, Rockville, Md.). After three rinses with TBS-Tween, the blot was incubated in alkaline phosphatase substrate (as described above) until the bands appeared, rinsed with distilled water, and allowed to dry. Negative controls were performed by incubating lanes, containing the proteoglycan samples, with buffer in place of primary antibody. No immunoreactivity was observed for these lanes. The apparent molecular weights for versican were established using the core protein for embryonic chick aggrecan as a standard (Carrino et al. 1994). All other apparent molecular determinations were based upon the use of mixed prestained high and low molecular weight standards provided by Bio-Rad Laboratories (Hercules, Calif.).

Composite agarose/polyacrylamide gel electrophoresis

Composite agarose/polyacrylamide gels are prepared so that the final concentration of agarose, type HSC (Park Scientific, Northampton, UK), is 0.6% and the final concentration of polyacrylamide is 1.2%. The agarose is placed in a buffer containing 40 mM TRIS and 1 mM Na_2SO_4, pH 6.8, and heated to 95–100 C. Once this solution has cooled to 65 C, it is mixed with the polyacrylamide solution prepared according to the directions of Carney et al. (1986). This mixture is carefully poured into casting plates prewarmed to 37 C. For analyses of aggregating proteoglycans, the gel is equilibrated in running buffer, 10 mM TRIS and 0.25 mM Na_2SO_4, containing 4 M urea. This is washed from the gel prior to loading the sample. Proteoglycan samples are dissolved in sample buffer containing 8 M urea, 0.25 mM Na_2SO_4, 0.001% bromophenol blue, and 10 mM TRIS, pH 6.8. The samples are electrophore-

sed in running buffer at 50 volts until they have entered the gel (approximately 5 min) and then they are electrophoresed at 150 volts until the bromophenol blue dye has migrated 3 cm. Longer running does not improve resolution. The gels are removed from the plates and electrotransferred onto either Immobilon-P or nitrocellulose. The nitrocellulose is preferred for immunoblotting with glycosaminoglycan-specific monoclonal antibodies since there is less background. Immobilon-P is used for all other antibodies.

Results

Versican is the principal interstitial proteoglycan in fetal skin

Proteoglycans extracted from fetal and adult skin were compared to determine the relative amounts of the large, aggregating chondroitin sulfate proteoglycan versican. In this study, decorin present in the same samples provided an internal standard for comparing the relative amounts of versican in fetal and adult skin. The relative amounts of versican in fetal and adult skin extracts was determined on Western blots using two different monoclonal antibodies specific for the versican core protein, although the results are shown for only one of these antibodies, 12C5 (Asher et al. 1991; 1995; Isogai et al. 1996). The intact versican molecule, at about 900 kDa (Zimmermann and Ruoslahti 1989), remained in the stacking gel, and, although detectable, was difficult to visualize. However, chondroitinase ABC-treated samples migrated into the gel. Antibody 12C5 detected two bands with apparent molecular weights of 570 and 490 kDa (Fig. 1); these conform in size to the two major splicing variants of human versican (Dours-Zimmermann and Zimmermann 1994; Zimmermann et al. 1994). These molecular weights were established using the core protein from embryonic chick aggrecan, with an estimated molecular weight of 440 kDa, as a standard. This core protein was identified on Western blots using antibody S103L (Carrino et al. 1994). The intensities of the bands in the fetal sample were significantly higher than that for the adult sample, even though equal dry weight amounts of proteoglycan purified by ion exchange chromatography were applied to each lane. This strongly suggests that fetal skin contains a proportionally higher content of this proteoglycan. A similar study (not shown) was also performed on proteoglycans extracted from day 80 EGA fetal skin. These results were identical to those shown in Figs. 1 and *2*.

To confirm this difference between the relative amounts of versican in fetal and adult skin, the relative amount of decorin, the other principal proteoglycan of skin, was analyzed in the same manner. The same amounts of the same samples used for versican analysis were also used for decorin analysis. Monoclonal antibody 2B6, which recognizes a well characterized carbohydrate epitope on chondroitin/dermatan sulfate proteoglycans that is generated by prior chondroitinase ABC-treatment (Couchman et al. 1984; Caterson et al. 1985), was used for the Western blot shown in Fig. 2. Because

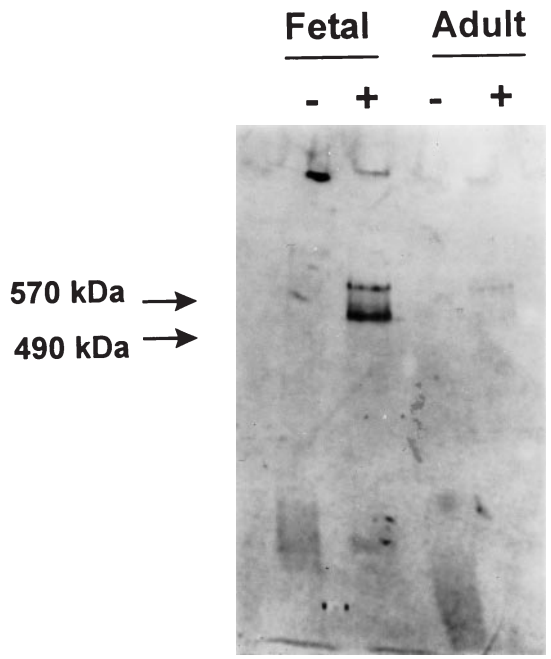


Fig. 1 Versican in fetal and adult skin. Proteoglycans extracted from fetal and adult human skin were electrophoresed on a 5% SDS-polyacrylamide gel and were electrotransferred onto blotting membrane. One set of samples was run as intact molecules (–) and the other set of samples was treated with chondroitinase ABC (+) prior to electrophoresis. The Western blot was probed with antibody 12C5. The chondroitinase ABC-treated core protein for embryonic chick aggrecan was used as a molecular weight standard for these assays (not shown)

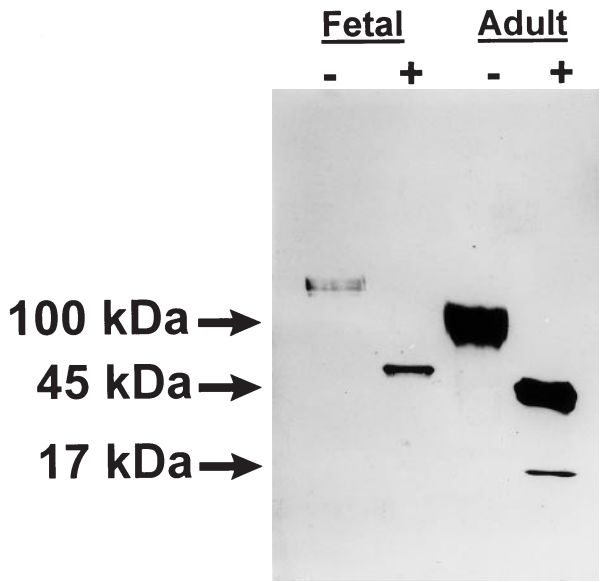


Fig. 2 Decorin in fetal and adult skin. Proteoglycans extracted from fetal and adult human skin were electrophoresed on a 5–17.5% linear gradient SDS-polyacrylamide gel and were electrotransferred onto blotting membrane. One set of samples was run as intact molecules (–) and the other set of samples was treated with chondroitinase ABC (+) prior to electrophoresis. A second treatment with chondroitinase ABC was performed following electrotransfer onto blotting membrane in order to create epitopes for antibody 2B6. Prestained high and low molecular weight standards from Bio-Rad laboratories were used as molecular weight standards (see below)

this antibody recognizes a carbohydrate epitope, it detects both decorin and biglycan in these skin extracts. However, as shown in Fig. 2, only the decorin bands appeared for the fetal and adults samples. These bands have the expected molecular weights for intact decorin and decorin core protein (Schmidtchen et al. 1990; Kresse and Schönherr 1993; Schönherr et al. 1993). In addition, the identity of these bands was also established through the use of anti-decorin core protein-specific antibodies (Yamagata et al. 1987; Bianco et al. 1990; Fisher et al. 1995). Although not visible in Fig. 2, extremely faint bands located at positions expected for biglycan could be discerned directly on the blot. The presence of biglycan was confirmed on other Western blots using antibodies specific for this core protein (Bianco et al. 1990; Fisher et al. 1995). As before, bands detected by the core protein-specific antibodies were faint and there was no discernible difference in the relative amounts of biglycan between fetal and adult skin extracts. Fetal and adult skin proteoglycan extracts contained substantially different relative amounts of decorin. Thus, fetal and adult skin proteoglycan extracts contained versican and decorin as their principal chondroitin/dermatan sulfate proteoglycans. These two proteoglycans appeared in relatively different proportion in fetal and adult skin extracts.

Adult decorin proteoglycan migrated on the SDS gel with an apparent molecular weight of just under 100 kDa (Fig. 2), which closely corresponds to the reported size of this molecule (Kresse and Schönherr 1993). In contrast, fetal decorin proteoglycan migrated more slowly (Fig. 2), indicative of a higher molecular weight for this molecular species. The size difference between these two proteoglycans appears to result from longer glycosaminoglycan chains being attached to the fetal decorin core protein, since the core proteins for the fetal and adult molecules, as shown in Fig. 2, were of approximately the same size. Adult skin also differed from fetal skin in that it contained another proteoglycan species, which, following chondroitinase ABC treatment, migrated with an apparent molecular weight of about 17 kDa. This molecule was also identified (not shown) by antibodies specific for decorin core protein. The fully glycosylated version of this molecule, not shown in Fig. 2, migrates on these gels as a polydisperse band with a molecular weight centering around 45 kDa. This 45-kDa material was detected on gels stained directly with toluidine blue. It was electrotransferred to the blotting membrane, but it was leached from the surface of this hydrophobic blotting membrane during the immunodetection procedure – hence its apparent absence in Fig. 2. Additional studies are in progress in this laboratory to more fully characterize this proteoglycan species related to or derived from decorin.

Fetal skin extracts also contain another molecule that was detected by anti-versican monoclonal antibody 12C5. This molecule bound weakly to an anion exchange column and required 0.25 M NaCl for its elution, which was a lower concentration than that required to elute proteoglycans from the column. Also, treatment of this

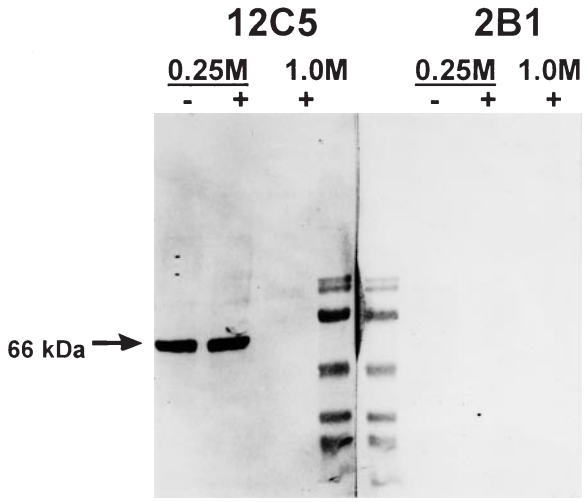


Fig. 3 A 66-kDa protein in fetal skin extracts. Molecules extracted from fetal skin were chromatographed over a DEAE-Sephacel anion exchange column. Molecules that eluted with 0.25 M NaCl and those which eluted with 1.0 M NaCl were treated with chondroitinase ABC (+) or remained untreated (–). All samples were electrophoresed on a 5–17.5% linear gradient SDS-polyacrylamide gel. Following electrotransfer onto blotting membrane the samples were immunoreacted with two different anti-versican antibodies, 12C5 and 2B1. The two center lanes (*arrows*) contain prestained molecular weight standards. The visible standards are 118 kDa, 107 kDa, 80 kDa, 47 kDa, 37 kDa, 27 kDa, and 19 kDa

molecule with chondroitinase ABC prior to analysis by SDS-PAGE did not alter its 66-kDa molecular weight (Fig. 3). These results indicate that it is not a chondroitin/dermatan sulfate proteoglycan. This 66-kDa molecule also appeared in extracts of adult skin in about the same proportion as did versican. Another anti-versican antibody, 2B1, failed to detect this molecular species. The location of the 2B1 epitope in the versican core protein has yet to be determined, but it is possible that it resides in the C-terminal region (Isogai et al. 1996), which could explain the absence of immunoreactivity with the 66-kDa band.

The ability to produce a high proportion of versican appears to be an innate characteristic of fetal dermal cells. This was demonstrated by comparing the size profiles of sulfated proteoglycans produced by fetal and adult dermal cells in monolayer culture. For illustrative purposes, cell layer proteoglycans produced by fetal cells from two donors, day 57 EGA and day 132 EGA, were compared with those produced by papillary and reticular dermal cells from the same piece of 36-year-old adult skin. Additional fetal and adult samples have been studied with results similar to those reported here. All four sets of cells were grown to 80–90% of confluence and metabolically labelled with $[^{35}S]SO_4$ for 5 h so that only newly synthesized proteoglycans would be analyzed. Following purification by CsCl density gradient centrifugation, the size profiles of the sulfated proteoglycans from the cell layer fractions were compared by gel filtration chromatography on a Sepharose CL-2B column. Proteoglycans from the medium fractions, obtained

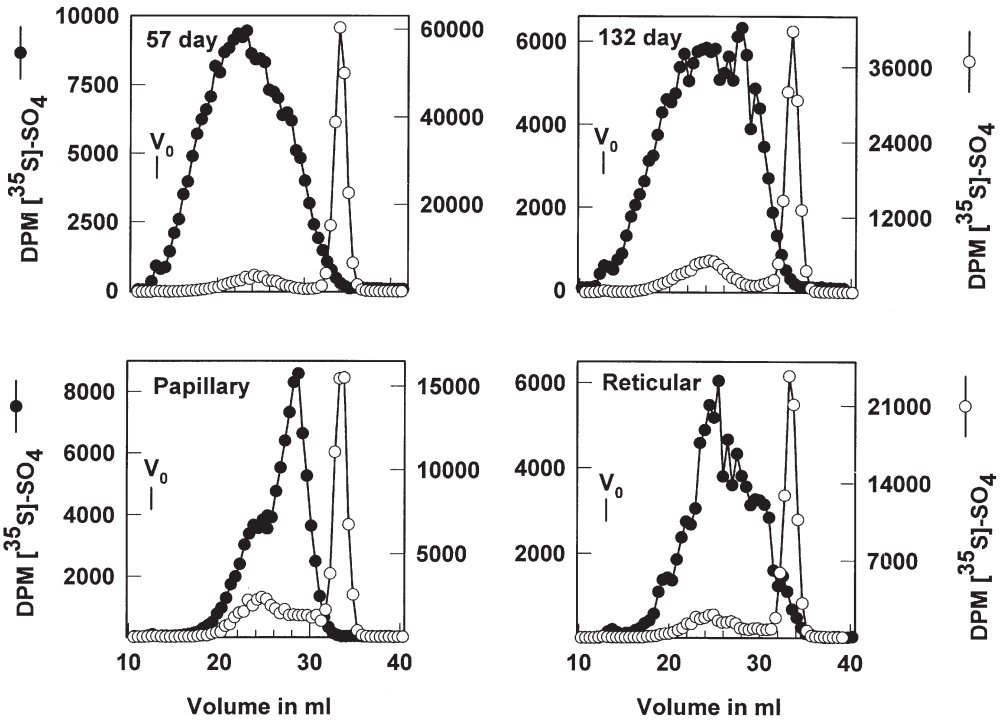


Fig. 4 Comparison of proteoglycans produced by cultured fetal and adult dermal cells. Proteoglycans produced by cultured dermal cells were metabolically labelled for 5 h with $[^{35}S]SO_4$ and were then extracted from the cell layer portions of these cultures for analyses by gel filtration chromatography on a Sepharose CL-2B column. Intact proteoglycans (–●–) and chondroitinase ABC treated proteoglycans (–○–) were compared for fetal day 57 EGA and fetal day 132 EGA dermal cells and for adult papillary and reticular dermal cells obtained from the same piece of 36-year skin. The void volume of the column, V_0, is indicated. The total volume of the column is indicated by the major peak of radioactivity for the chondroitinase ABC treated samples

by ethanol precipitation and ion exchange chromatography, were also studied (not shown). The medium fractions contained approximately one-third of the incorporated labelled sulfate. However, the relative amounts of small to large proteoglycan were the same as shown for the cell layer fraction. Schönherr et al. (1993) compared the expression of decorin in medium fractions of donor-matched human dermal papillary and reticular cell populations similar to those used in this study. As in this study, they found that decorin expression in the medium fraction of monolayer-cultured cells was significantly higher in the dermal papillary cells than in the donor-matched dermal reticular cells. In contrast, they found no significant difference in the expression of biglycan between donor-matched papillary and reticular cells.

The two sets of fetal proteoglycans showed different elution profiles than those obtained for the adult molecules (Fig. 4). Nearly half of the labelled fetal material, 49% for the day 57 sample and 44% for the day 132 sample, eluted with a k_{av} less than 0.5. In contrast, only 17% of the papillary and 38% of the reticular proteoglycans eluted with a K_{av} less than 0.5. These data indicated that fetal dermal cells in culture produce a significantly higher proportion of large proteoglycans than does either population of adult cells. Furthermore, the two adult populations of dermal cells also produced different proportions of large and small proteoglycans, a feature that indicates that adult dermal cells consist of physiologically distinct populations of cells. Dot-blot analyses of column fractions indicated that the population of large proteoglycans contains versican and that the population of small proteoglycans contains decorin (data not shown). Most of the sulfated proteoglycans, 90% or greater, were susceptible to treatment with chondroitinase ABC (Fig. 4). This was indicated by the shift of sulfated label from the included to the total volume (V_t). The remaining proteoglycans are probably heparan sulfate proteoglycans that are produced by cultured human dermal cells. No attempt was made to further characterize these latter molecules.

Immunohistochemical distribution of versican in fetal skin

The distribution of versican, for illustrative purposes, was compared for two time points – early fetal skin prior to the formation of hair follicles (day 76 EGA) and fetal skin with hair follicles (day 127 EGA). Although not shown, studies were also performed on numerous other fetal samples ranging in age from day 55 EGA to day 145 EGA and on adult skin samples. The distribution of versican in sections of fetal scalp skin was determined using two different monoclonal antibodies, both of which produced equivalent results. Fetal trunk skin was also studied; there were no significant differences from those described for scalp skin. In early fetal skin, day 76 EGA, versican appeared throughout the entire dermis, but the intensity of immunostain diminished abruptly at the vascular plane that demarcated the dermis from the underlying myogenic tissue (Fig. 5). Hair follicles and glands have not yet appeared at this stage of skin development. The distribution of versican within skin changed with fetal development. By day 127 EGA, versican appeared with high intensity in the upper half of the dermis, but the intensity dropped precipitously in the dermal region located below the level of the bulge region of hair follicles (Fig. 6a). However, the bulge region, which con-

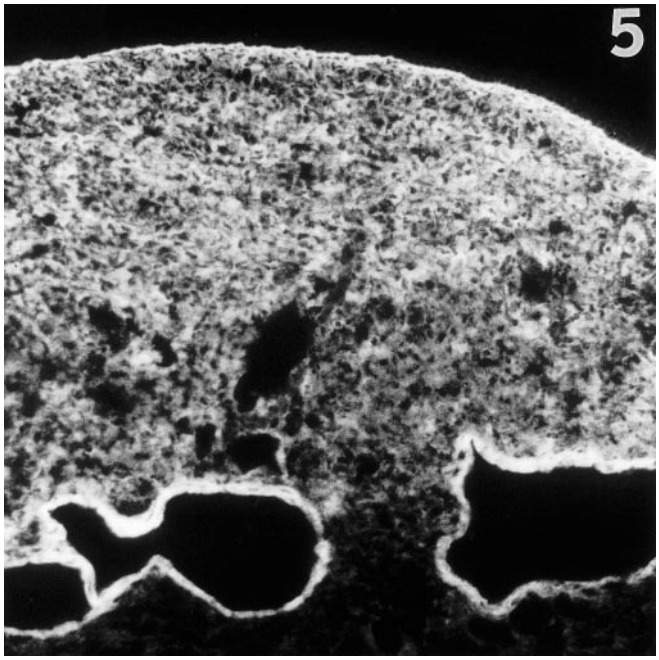


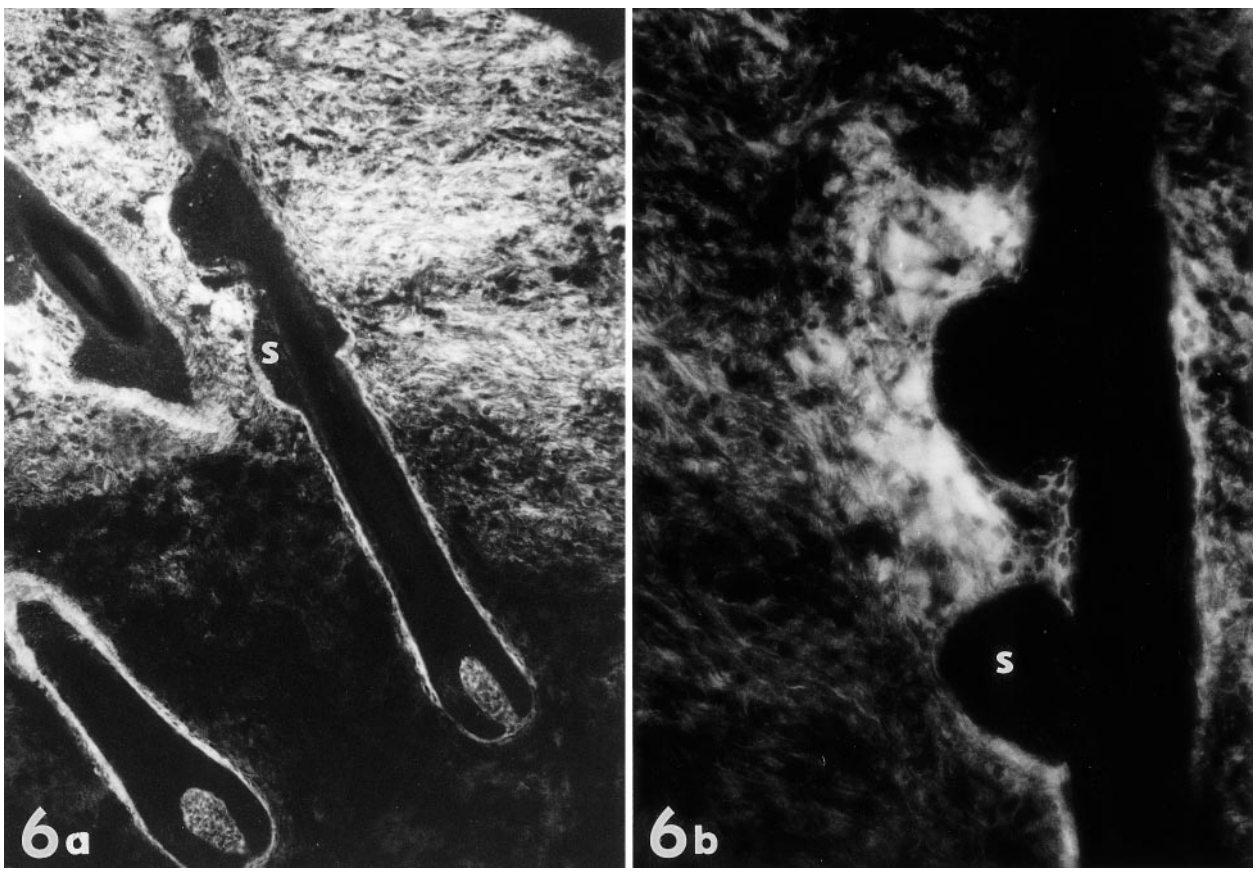
Fig. 5 Versican in day 76 EGA fetal skin. Versican, as detected by antibody 12C5, appears throughout the fetal dermis. The intensity of immunoreactivity diminishes abruptly at the vascular plexus that separates the dermis from the underlying myogenic tissue. ×120

tains the developing sebaceous gland (Akiyama et al. 1995), was surrounded by a mesenchyme that contained higher amounts of versican than did the nearby dermal matrix (Fig. 6b). Versican appeared in the dermal sheaths surrounding hair follicles and in the dermal papillae of these follicles (Fig. 6a).

Glycosaminoglycan-specific monoclonal antibodies

Monoclonal antibodies that identify native epitopes, that is, those epitopes located within the interior of intact chondroitin sulfate glycosaminoglycan chains (Avnur and Geiger 1984; Yamagata et al. 1987; Sorrell et al. 1990), recognized epitopes present in versican extracted from fetal skin, but not in decorin obtained from the same tissue. This was demonstrated on Western blots following the separation of intact versican and decorin extracted from human fetal skin by electrophoresis on com-

Fig. 6a, b Versican in day 127 EGA fetal skin. **a** Versican, as detected by antibody 12C5, appears in the upper half of the dermis. The intensity of immunoreactivity diminishes abruptly at the level of the bulge region, which contains the developing sebaceous gland (*s*). Versican appears in the dermal sheaths and in the dermal papillae of the hair follicles. ×150. **b** Versican, identified by antibody 12C5, appears more intensely in the dermal region immediately surrounding the bulge region than it does in the nearby dermis. The developing sebaceous gland is the bulge most distal from the epidermis (*s*). ×350



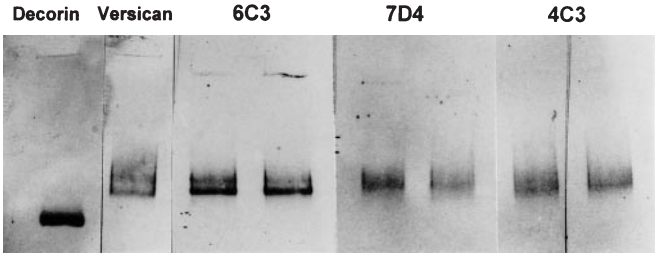


Fig. 7 Composite agarose/polyacrylamide gel electrophoresis of fetal skin proteoglycans. Intact proteoglycans extracted from day 80 and day 127 EGA fetal skin were electrophoresed on a composite agarose/polyacrylamide gel and electrotransferred onto blotting membrane. Lanes containing the day 127 EGA proteoglycans were immunostained with antibody LF-122 for decorin core protein and antibody 12C5 for versican core protein. Lanes containing both fetal proteoglycan samples were immunostained with anti-chondroitin sulfate monoclonal antibodies 6C3, 7D4, and 4C3

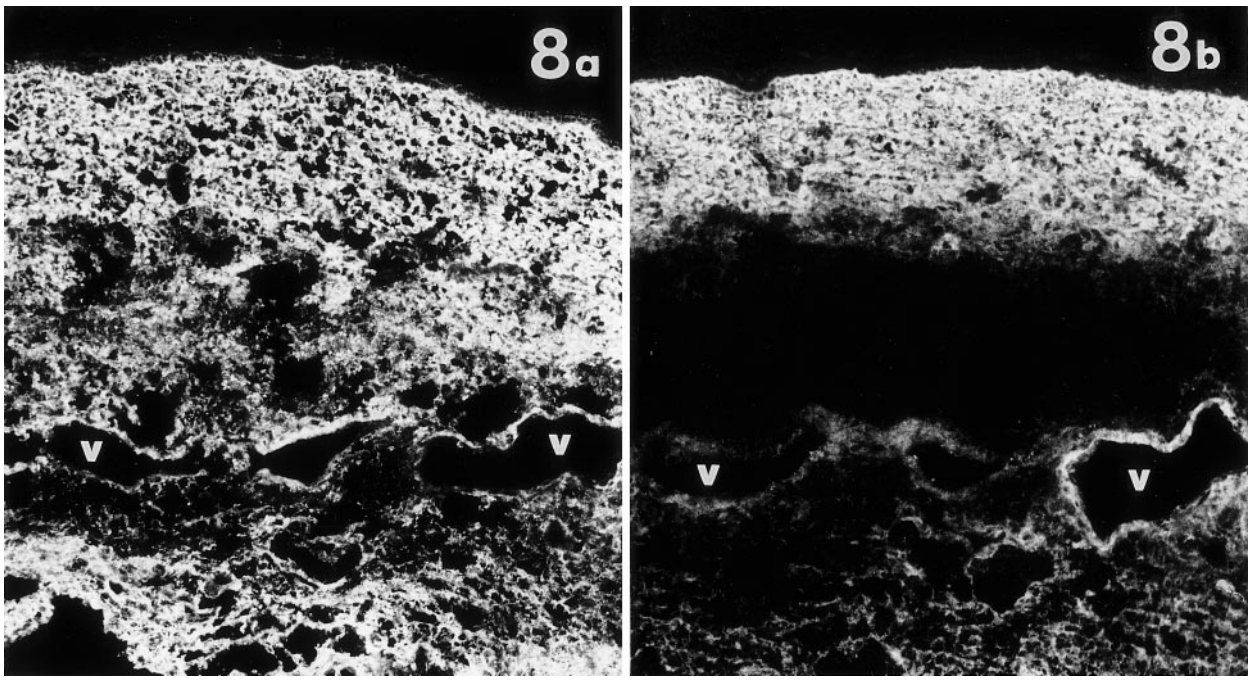
posite agarose/polyacrylamide gels (Carney et al. 1986). Two sets of fetal skin proteoglycans, from day 80 and day 127 EGA donors, were analyzed using monoclonal antibodies 6C3, 7D4, and 4C3 (Fig. 7). Antibodies that recognize versican and decorin core protein were used to identify the migration positions of these two proteoglycans in this system. Each of the anti-chondroitin sulfate monoclonal antibodies recognized proteoglycans that migrated identically with versican. There was no evidence for immunoreactivity of any of these antibodies with either decorin or biglycan, both of which are known to be present in these samples.

These anti-chondroitin sulfate monoclonal antibodies recognized antigen in human fetal skin; however, the different antibodies produced different profiles. Antibody 6C3, like the monoclonal antibodies to versican core protein (Fig. 5), detected antigen throughout the entire early fetal dermis (Fig. 8a). In contrast, antibody 7D4 detected antigen only in the upper half of the dermis (Fig. 8b). These results indicate that versican in different regions of human fetal skin may have different chondroitin sulfate glycosaminoglycan structures.

Fig. 8a, b Proteoglycans detected by monoclonal antibodies 6C3 and 7D4 in day 76 EGA Fetal Skin. **a** Antibody 6C3 detects proteoglycans that are distributed throughout the entire dermis and in the subdermal tissue. **b** Antibody 7D4 detects proteoglycans that appear in the upper half of the dermis and in the subdermal tissue. The subdermal vascular plexis (*v*) demarcates the dermis from the underlying myogenic tissue. ×100

Discussion

Versican is a large, hyaluronan-binding, chondroitin sulfate proteoglycan that appears in a wide variety of noncartilaginous connective tissues, including skin (Zimmermann and Ruoslahti 1989; Zimmermann et al. 1994). Although a number of functions have been ascribed to this proteoglycan (Krusius et al. 1987; Zimmermann and Ruoslahti 1989; Yamagata et al. 1993), little is as yet known about how it functions in skin. Consequently, obtaining information regarding the distribution and developmental regulation of this proteoglycan in human skin should provide basic information that could then be used to help to deduce its function in this organ. Much of the current information regarding versican in developing skin comes from studies of rodent skin where an immunohistochemical study of developing mouse skin revealed that versican is restricted specifically to sites of epithelial/mesenchymal interactions, such as around hair follicles and glands (du Cros et al. 1995). Habuchi et al. (1986), using metabolic labelling of fetal and neonatal



rat skin in organ culture, identified dermatan sulfate proteoglycan (decorin) as the principal labelled proteoglycan species. The studies of versican in human skin development reported here provide a different view of proteoglycans in skin development than those mentioned above for rodents.

In this study, versican appeared throughout the entire dermis of early fetal skin, indicating that, unlike in developing rodent skin, it is primarily an interstitial proteoglycan. The biochemical data, which assayed immunologically for proteoglycan, indicated that the chondroitin sulfate proteoglycan, versican, in relation to the dermatan sulfate proteoglycan, decorin, was the major proteoglycan species in human fetal skin. Proteoglycans in fetal and adult skin have not been quantified as has been reported elsewhere (Scott et al. 1996). However, these data are significant in that versican, decorin, and biglycan were each expressed at different levels in fetal and adult skin. These results are consistent with those of a previous study of human fetal skin in which acidic glycosaminoglycans, upon extraction and analysis from 3-month fetal skin, were found to contain primarily hyaluronan and chondroitin sulfate, with only a trace of dermatan sulfate (Breen et al. 1970). In the present study, no significant differences in the relative levels of versican to decorin were noted at two different fetal ages, day 80 and 120 EGA. However, subtle changes in the immunohistochemical distribution of versican occurred between these time points. By the mid-fetal period, i.e., day 120–140 EGA, interstitial versican disappeared from the lower half of the dermis, although, as in neonatal mouse skin, versican in these deep regions appeared around hair follicles and was especially prominent in the dermal papilla regions of these follicles and in perivascular connective tissue of the larger blood vessels. Significant changes in the distribution of versican occurred between fetal and adult periods. In adult skin, the distribution of versican becomes even more restrictive, as it now appears prominently with elastic fibers in the deeper regions of the dermis (Zimmermann et al. 1994; Bernstein et al. 1995). Our immunohistochemical studies of adult skin, not reported here, confirm these observations.

Additional evidence for developmental changes between the fetal and adult period occurred in two populations of small proteoglycans or proteoglycan-related molecules. Specifically, fetal skin contained a 66-kDa molecular species that was underrepresented in adult skin, and adult skin contained a 17-kDa core protein of a proteoglycan that was underrepresented in fetal skin. The 66-kDa protein, due to its recognition by anti-versican monoclonal antibody 12C5, is related to versican (Perides et al. 1993). This antibody recognizes an epitope located in the hyaluronan binding region of versican. The skin protein closely resembles a 60-kDa hyaluronan-binding molecule extracted from human brain and white matter called glial hyaluronan-binding protein or GHAP (Perides et al. 1989; Asher et al. 1991). The GHAP glycoprotein has an amino acid sequence that is virtually identical to the N-terminally located hyaluronan-binding region of versican and, at 60 kDa, GHAP is approximately the same size as the molecule identified in skin (Perides et al. 1989). Furthermore, the GHAP molecule, with an isoelectric point of 4.3–4.4, should, like the 66-kDa molecule, bind weakly to an anion exchange column (Perides et al. 1989). Thus, both GHAP and the 66-kDa molecule might be degradation products of versican (Perides et al. 1993). Alternatively, the 66-kDa molecule might be a newly identified splicing variant of human versican in which the two chondroitin sulfate-binding domains are missing (Zako et al. 1995). Further work will be required to more precisely identify this molecular species and to establish its significance in fetal skin.

Another difference between fetal and adult skin is the presence of a small chondroitin/dermatan sulfate proteoglycan that has a core protein of about 17-kDa and that is recognized by anti-decorin antibodies. The intact version of this molecule migrates on SDS-PAGE as a polydisperse species with a molecular weight centered at 45-kDa. In other studies, small dermatan sulfate proteoglycans with core proteins ranging in size from 14–21 kDa have been identified in human dermal scar tissue (Swann et al. 1988; Garg et al. 1990) and in neonatal calf skin (Matsunaga and Shinkai 1986). Unlike decorin, this material does not bind effectively to a hydrophobic support such as Immobilon-P (D.A. Carrino, J.M. Sorrell, A.I. Caplan, unpublished work); consequently, it has been dismissed in some studies as being free glycosaminoglycan chains (Scott et al. 1996). The results reported here indicate that this material is attached to a specific core protein. Such a truncated core protein might be generated by metalloproteolytic digestion of intact decorin. Matrix metalloproteases have been identified that are capable of generating such a catabolic species (Nakano and Scott 1988; Imai et al. 1997). The significance of this small proteoglycan species is uncertain and is currently under investigation in this laboratory.

The function of versican in fetal skin remains obscure. It has been proposed that its primary function in skin development is to help mediate intercellular signaling that occurs in epithelial/mesenchymal interactions, such as those associated with development of hair follicles and in the continued regulation of cyclic process of hair growth (du Cros et al. 1995). In adult skin, versican interacts specifically with the microfibrillar constituents of elastic fibers (Bernstein et al. 1995; Bode-Lesniewska et al. 1996). Increases in the content of elastic material, such as occurs with solar elastosis, also results in increased deposition of versican (Bernstein et al. 1995).

The present data do not refute the aforementioned functional hypotheses related to versican in either fetal or adult skin. However, they raise the additional possibility that in fetal skin a major function of versican is to interact with hyaluronan and thereby stabilize this developmentally important glycosaminoglycan. The versican core protein contains, near its N-terminal region, a globular domain that enables it to bind specifically to hyaluronan (Zimmermann and Ruoslahti 1989; LeBaron et al. 1992), much like the well characterized interaction of

the cartilage proteoglycan aggrecan with hyaluronan (Carney and Muir 1988; Heinegård and Oldberg 1993). Such interactions between versican from skin and hyaluronan have been documented and, as with proteoglycans from cartilaginous tissues, this interaction is stabilized by link protein (Binette et al. 1994). Aggregates consisting of proteoglycan and hyaluronan are retained within tissue more effectively than are the individual components, and these aggregates structure and retain water in the tissue (Carney and Muir 1988; Heinegård and Oldberg 1993). These versican/hyaluronan aggregates may then modulate cellular adhesion, migration, and proliferation, which are functions that have been ascribed to both of these molecules in fetal tissue, in healing skin wounds, and in stromal compartments in invasive tumors (Goetinck 1991; Yeo et al. 1991; Ellis and Schor 1995; Ellis et al. 1997; Nara et al. 1997). In addition, there is evidence that versican may retain hyaluronan in tissue through complex interactions with fibronectin and type I collagen (Yamagata et al. 1986).

The amorphous matrix that dominates the fetal organ contains hyaluronan as a major constituent (Holbrook et al. 1993; Ågren et al. 1997). Hyaluronan, like versican, appears throughout the early fetal dermis, and, again like versican, gradually disappears from the deeper regions of the dermis, although the time course of this transition may occur slightly before that for versican (Ågren et al. 1997). The co-presence and co-distribution of versican and hyaluronan in fetal dermis suggest that these two molecules may have interrelated functions.

The small dermatan sulfate proteoglycan decorin is, in relation to versican, more abundant in adult than in fetal skin (Bianco et al. 1990; Kresse and Schönherr 1993). This may have structural significance for adult skin since one of the functions of decorin is to bind to, or decorate, type I collagen fibrils, and thereby prevent these structures from associating to form thick collagen bundles (Vogel et al. 1984; Kresse and Schönherr 1993). This role in regulating collagen fibrillogenesis is presumably not significant in fetal skin, since this tissue contains sparse amounts of fibrillar collagen (Holbrook et al. 1993). However, in adult skin, the situation is different. The lower, or reticular, region of the adult dermis is characterized by an interlacing network of thick collagen fiber bundles that do not appear in the upper, or papillary, region (Stenn 1983). A relatively low amount of decorin in the reticular dermis may, in part, account for the presence of thick collagen bundles.

Resident fibroblasts determine the composition and organization of their surrounding extracellular matrices. This is particularly evident in that fetal skin and the papillary and reticular regions of adult skin all display remarkably different histologic characteristics that in part result from the production of hyaluronan and sulfated proteoglycans. In this study, it was demonstrated that, when fetal fibroblasts and also adult fibroblasts from different regions of the same piece of skin are maintained in vitro, they produce significantly different proportions of large and small chondroitin/dermatan sulfate proteoglycans. In a related study, it was demonstrated that when matched sets of dermal fibroblasts were cultured, papillary dermal cells produced more decorin than did their reticular counterparts (Schönherr et al. 1993). Interestingly, the synthetic patterns exemplified by the different populations of dermal cells closely reflect the in vivo situation. For example, in fetal skin the large chondroitin sulfate proteoglycan versican predominates. Thus, these studies indicate the diversity that characterizes dermal fibroblasts and also help us to elucidate the functional significance of proteoglycans in skin.

Anti-chondroitin sulfate monoclonal antibodies that detect epitopes in native chondroitin sulfate glycosaminoglycan chains (Avnur and Geiger 1984; Sorrell et al. 1990; Yamagata et al. 1987) recognized versican, but not decorin, that had been extracted from human fetal skin. Consequently, the immunohistochemical staining pattern produced by these antibodies in fetal skin should be similar to that for antibodies specific for the versican core protein. Monoclonal antibody 6C3 and a closely related antibody, CS-56, both, like antibodies to versican core protein, recognized antigen throughout the entire dermal region of early fetal skin. However, antibody 7D4 and its closely related antibody 4C3 produced a substantially different immunohistochemical staining pattern. These antibodies recognized antigen that was confined to the upper region of skin. Thus, these data emphasize that caution must be used in interpreting results based upon the use of these carbohydrate-specific monoclonal antibodies. It also implies that the chondroitin sulfate glycosaminoglycan chains of versican from different regions of fetal skin may have different carbohydrate microchemistries that result in the differential recognition by these antibodies.

The data reported in this study must be viewed with some caution in that proteoglycans extracted from fetal skin were extracted essentially from whole-body skin, whereas proteoglycans used in this study were extracted exclusively from adult breast skin. The cellular and extracellular matrix composition of skin may vary at different anatomic sites, although in a previous immunohistochemical study of adult skin, no significant differences in chondroitin sulfate proteoglycans were observed in samples from different anatomic sites from the same individual (Willen et al. 1991). Factors, such as chronic sun exposure, affect the composition of proteoglycans in skin (Bernstein et al. 1995). Consequently, samples for this study were taken from a single anatomic site in order to minimize such effects. Taken together, the results reported in this study indicate that there are substantial changes in the composition and distribution of versican between fetal and adult skin. These changes correspond to reported changes in the content and distribution of hyaluronan (Breen et al. 1970; Ågren et al. 1997), which suggests that a major function for versican in human fetal skin is to interact with this important glycosaminoglycan.

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References

Adzick NS, Lorenz HP (1994) Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Ann Surg 220:10–18

Ågren UM, Tammi M, Ryynanen M, Tammi R (1997) Developmentally programmed expression of hyaluronan in human skin and its appendages. J Invest Dermatol 109:219–224

Akiyama M, Dale BA, Sun T-T, Holbrook KA (1995) Characterization of the hair follicle bulge in human fetal skin: the human fetal bulge is a pool of undifferentiated keratinocytes. J Invest Dermatol 105:844–850

Asher R, Perides G, Vanderhaeghen J-J, Bignami A (1991) Extracellular matrix of central nervous system white matter: demonstration of an hyaluronate-protein complex. J Neurosci Res 28:410–421

Asher RA, Scheibe RJ, Keiser HD, Bignami A (1995) On the existence of a cartilage-like proteoglycan and link proteins in the central nervous system. Glia 13:294–308

Avnur Z, Geiger B (1984) Immunocytochemical localization of native chondroitin-sulfate in tissues and cultured cells using specific monoclonal antibody. Cell 38:811–822

Bernstein EF, Fisher LW, Li K, LeBaron RG, Tan EML, Uitto J (1995) Differential expression of the versican and decorin genes in photoaged and sun-protected skin. Comparison by immunohistochemical and Northern analyses. Lab Invest 72:662–669

Bianco P, Fisher LW, Young MF, Termine JD, Robey PG (1990) Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. J Histochem Cytochem 38:1549–1563

Binette F, Cravens J, Kahoussi B, Haudenschild DR, Goetinck PF (1994) Link protein is ubiquitously expressed in non-cartilaginous tissues where it enhances and stabilizes the interaction of proteoglycans with hyaluronic acid. J Biol Chem 269:19116–19122

Bode-Lesniewska B, Dours-Zimmermann MT, Odermatt BF, Briner J, Heitz PU, Zimmermann DR (1996) Distribution of the large aggregating proteoglycan versican in adult human tissues. J Histochem Cytochem 44:303–312

Breen M, Weinstein HG, Johnson RL, Veis A, Marshall RT (1970) Acidic glycosaminoglycans in human skin during fetal development and adult life. Biochim Biophys Acta 201:54–60

Carney SL, Muir H (1988) The structure and function of cartilage proteoglycans. Physiol Rev 68:858–910

Carney SL, Bayliss MT, Collier JM, Muir H (1986) Electrophoresis of ^{35}S-labeled proteoglycans on polyacrylamide-agarose composite gels and their visualization by fluorography. Anal Biochem 156:38–44

Carrino DA, Dennis JE, Drushel RF, Haynesworth SE, Caplan AI (1994) Identity of the core proteins of the large chondroitin sulphate proteoglycans synthesized by skeletal muscle and prechondrogenic mesenchyme. Biochem J 298:51–60

Caterson B, Christner JE, Baker JR, Couchman JR (1985) Production and characterization of monoclonal antibodies directed against connective tissue proteoglycans. Fed Proc 44:386–393

Couchman JR, Caterson B, Christner JE, Baker JR (1984) Mapping by monoclonal antibody detection of glycosaminoglycans in connective tissues. Nature 307:650–652

DeLuca S, Heinegård D, Hascall VC, Kimura JH, Caplan AI (1977) Chemical and physical changes in proteoglycans during development of chick limb bud chondrocytes grown in vitro. J Biol Chem 252:6600–6609

Dours-Zimmermann MT, Zimmermann DR (1994) A novel glycosaminoglycan attachment domain identified in two alternative splice variants of human versican. J Biol Chem 269: 32992–32998

du Cros DL, LeBaron RG, Couchman JR (1995) Association of versican with dermal matrices and its potential role in hair follicle development and cycling. J Invest Dermatol 105:426–431

Ellis I, Schor SL (1995) The interdependent modulation of hyaluronan synthesis by TGF-β1 and extracellular matrix: consequences for the control of cell migration. Growth Factors 12:211–222

Ellis I, Banyard J, Schor SL (1997) Differential response of fetal and adult fibroblasts to cytokines: cell migration and hyaluronan synthesis. Development 124:1593–1600

Fisher LW, Stubbs JT, Young MF (1995) Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins. Acta Orthop Scand 66 [Suppl 266]: 61–65

Garg HG, Lippay EW, Burd AR, Neame PJ (1990) Purification and characterization of iduronic acid-rich and glucuronic acid-rich proteoglycans implicated in human post-burn keloid scar. Carbohydr Res 207:295–305

Goetinck PF (1991) Proteoglycans in development. Curr Top Dev Biol 25:111–131

Habuchi H, Kimata K, Suzuki S (1986) Changes in proteoglycan composition during development of rat skin. The occurrence in fetal skin of a chondroitin sulfate proteoglycan with high turnover rate. J Biol Chem 261:1031–1040

Heinegård D, Oldberg Å (1993) Glycosylated matrix proteins. In: Royce PM, Steinmann B (eds) Connective tissue and its heritable disorders: molecular, genetic, and medical aspects. Wiley-Liss, New York, pp 189–209

Holbrook KA, Smith LT, Kaplan ED, Minami SA, Hebert GP, Underwood RA (1993) Expression of morphogens during human follicle development in vivo and a model for studying follicle morphogenesis in vitro. J Invest Dermatol 101:39s–49s

Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y (1997) Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-β1 release. Biochem J 322:809–814

Isogai Z, Shinomura T, Yamakawa N, Takeuchi J, Tsuji T, Heinegård D, Kimata K (1996) 2B1 antigen characteristically expressed on extracellular matrices of human malignant tumors is a large chondroitin sulfate proteoglycan, PG-M/versican. Cancer Res 56:3902–3908

Johansson S, Hedman K, Kjellen L, Christner J, Vaheri A, Höök M (1985) Structure and interactions of proteoglycans in the extracellular matrix produced by cultured human fibroblasts. Biochem J 232:161–168

Kaplan ED, Holbrook KA (1994) Dynamic expression patterns of tenascin, proteoglycans, and cell adhesion molecules during human hair follicle morphogenesis. Dev Dyn 199:141–155

Knudson CB, Knudson WB (1993) Hyaluronan-binding proteins in development, tissue homeostasis, and disease. FASEB J 7:1233–1241

Kresse H, Schönherr E (1993) Small proteoglycans. Experientia 49:403–416

Krusius T, Gehlsen KR, Ruoslahti E (1987) A fibroblast chondroitin sulfate proteoglycan core protein contains lectin-like and growth factor-like sequences. J Biol Chem 262:13120–13125

LeBaron RG, Zimmermann DR, Ruoslahti E (1992) Hyaluronate binding properties of versican. J Biol Chem 267:10003–10010

Lever-Fischer PL, Goetinck PF (1988) Identification and characterization of a proteoglycan in embryonic chicken skin that can interact with hyaluronic acid. Arch Biochem Biophys 263:45–58

Longaker MT, Chiu ES, Adzick NS, Stern M, Harrison MR, Stern R (1991) Studies in fetal wound healing. V. A prolonged expression of hyaluronic acid characterizes fetal wound healing. Ann Surg 213:292–296

Margolis RK, Margolis RU (1993) Nervous tissue proteoglycans. Experientia 49:429–446

Matsunga E, Shinkai H (1986) Two species of dermatan sulfate proteoglycans with different molecular sizes from newborn calf skin. J Invest Dermatol 87:221–226

Miller B, Sheppard AM, Bicknese AR, Pearlman AL (1995) Chondroitin sulfate proteoglycans in the developing cerebral cortex: the distribution of neurocan distinguishes forming afferent and efferent axonal pathways. J Comp Neurol 355:615–628

Nakano T, Scott PG (1988) Partial purification and characterization of proteodermatan sulphate-degrading proteinases produced by human gingival fibroblasts. Biomed Res 9:269–179

Nara Y, Kato Y, Torri Y, Tsuji Y, Nakagaki S, Goto S, Isobe H, Nakashima N, Takeuchi J (1997) Immunohistochemical localization of extracellular matrix components in human breast tumours with special reference to PG-M/versican. Histochem J 29:21–30

Oike Y, Kimata K, Shinomura T, Nakazawa K, Suzuki S (1980) Structural analysis of chick-embryo cartilage proteoglycans by selective degradation with chondroitin lyases (chondroitinases) and endo-β-D-galactosidase (keratanase). Biochem J 191:193–207

Perides G, Lane WS, Andrews D, Dahl D, Bignami A (1989) Isolation and partial characterization of a glial hyaluronate-binding protein. J Biol Chem 264:5981–5987

Perides G, Biviano F, Bignami A (1993) Interaction of a brain extracellular matrix protein with hyaluronic acid. Biochim Biophys Acta 1075:248–258

Rodén L (1980) Structure and metabolism of connective tissue proteoglycans. In: Lennarz WJ (ed) The biochemistry of glycoproteins and proteoglycans. Plenum Press, New York, pp 267–371

Schafer IA, Pandy M, Ferguson R, Davis BR (1985) Comparative observation of fibroblasts derived from the papillary and reticular dermis of infants and adults: growth kinetics, packing density ant confluence and surface morphology. Mech Ageing Dev 31:275–293

Schmidtchen A, Carlstedt I, Malmström M, Fransson L-Å (1990) Inventory of human skin fibroblast proteoglycans. Identification of multiple heparan and chondroitin/dermatan sulphate proteoglycans. Biochem J 265:289–300

Schönherr E, Beavan LA, Hausser H, Kresse H, Culp LA (1993) Differences in decorin expression by papillary and reticular fibroblasts in vivo and in vitro. Biochem J 290:893–899

Scott PG, Dodd CM, Tredget EE, Ghahary A, Rahemtulla F (1996) Chemical characterization and quantification of proteoglycans in human post-burn hypertrophic and mature scars. Clin Sci 90:417–425

Sobue M, Nakashima N, Fukatsu T, Nagasaka T, Fukata S, Ohiwa N, Nara Y, Ogura T, Katoh T, Takeuchi J (1989) Production and immunohistochemical characterization of a monoclonal antibody raised to proteoglycan purified from a human yolk sac tumour. Histochem J 21:455–459

Sorrell JM, Mahmoodian F, Schafer IA, Davis B, Caterson B (1990) Identification of monoclonal antibodies that recognize novel epitopes in native chondroitin/dermatan sulfate glycosaminoglycan chains: their use in mapping functionally distinct domains of human skin. J Histochem Cytochem 38: 393–402

Sorrell JM, Carrino DA, Caplan AI (1993) Structural domains in chondroitin sulfate identified by anti-chondroitin sulfate monoclonal antibodies. Immunosequencing of chondroitin sulfates. Matrix 13:351–361

Sorrell JM, Carrino DA, Caplan AI (1996) Regulated expression of chondroitin sulfates at sites of epithelial/mesenchymal interaction: spatio-temporal patterning identified with anti-chondroitin sulfate monoclonal antibodies. Int J Dev Neurosci 14:233–249

Stenn KS (1983) The skin. In: Weiss L (ed) Histology, cell and tissue biology. Elsevier, New York, pp 569–606

Swann DA, Garg HG, Hendry CJ, Hermann H, Siebert E, Sotman S, Stafford W (1988) Isolation and partial characterization of dermatan sulfate proteoglycans from human post-burn scar tissues. Collagen Relat Res 8:295–313

Vogel KG, Paulsson M, Heinegård D (1984) Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem J 223:587–597

Willen MD, Sorrell JM, Lekan CC, Davis BR, Caplan AI (1991) Patterns of glycosaminoglycan/proteoglycan immunostaining in human skin during aging. J Invest Dermatol 96:968–974

Yamada H, Watanabe K, Shimonaka M, Yamaguchi Y (1994) Molecular cloning of brevican, a novel brain proteoglycan of the aggrecan/versican family. J Biol Chem 269:10119–10126

Yamagata M, Yamada KM, Yoneda M, Suzuki S, Kimata K (1986) Chondroitin sulfate proteoglycan (PG-M-like proteoglycan) is involved in the binding of hyaluronic acid to cellular fibronectin. J Biol Chem 261:13526–13535

Yamagata M, Kimata K, Oike Y, Tani K, Maeda N, Yoshida K, Shimomura Y, Yoneda M, Suzuki S (1987) A monoclonal antibody that specifically recognizes a glucuronic acid 2-sulfate-containing determinant in intact chondroitin sulfate chain. J Biol Chem 262:4161–4152

Yamagata M, Shinomura T, Kimata K (1993) Tissue variation of two large chondroitin sulfate proteoglycans (PG-M/versican and PG-H/aggrecan) in chick embryos. Anat Embryol 187: 433–444

Yeo T-K, Brown L, Dvorak HF (1991) Alterations in proteoglycan synthesis common to healing wounds and tumors. Am J Pathol 138:1437–1450

Zako M, Shinomura T, Ujita M, Ito K, Kimata K (1995) Expression of PG-M(V3), an alternatively spliced form of PG-M without a chondroitin sulfate attachment region in mouse and human tissues. J Biol Chem 270:3914–3918

Zimmermann DR, Ruoslahti E (1989) Multiple domains of the large fibroblast proteoglycan, versican. EMBO J 8:2975–2981

Zimmermann DR, Dours-Zimmermann MT, Schubert M, Bruckner-Tuderman L (1994) Versican is expressed in the proliferating zone in the epidermis and in association with the elastic network of the dermis. J Cell Biol 124:817–825