The Protein Core of the Proteoglycan Perlecan Binds Specifically to Fibroblast Growth Factor-7*

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Perlecan is a multifaceted heparan sulfate proteoglycan that is expressed not only as an intrinsic constituent of basement membranes but also as a cell-surface and pericellular proteoglycan. Perlecan functions as a ligand reservoir for various growth factors that become stabilized against misfolding or proteolysis and acts as a co-receptor for basic fibroblast growth factor by augmenting high affinity binding and receptor activation. These biological properties are mediated by the heparan sulfate moiety. Rather little is known about the protein core's mediation of functions. We have recently discovered that fibroblast growth factor-7 (FGF7) binds to perlecan protein core and that exogenous perlecan efficiently reconstitutes FGF7 mitogenic activity in perlecan-deficient cells. In this report we examined the specific binding of FGF7 to various domains and subdomains of perlecan protein core. Using several experimental approaches including overlay protein assays, radioligand binding experiments, and the yeast two-hybrid system, we demonstrate that FGF7 binds specifically to the N-terminal half of domain III and to a lesser extent to domain V, with affinity constants in the range of 60 nM. Thus, perlecan protein core should be considered a novel biological ligand for FGF7, an interaction that could influence cancer growth and tissue remodeling.

Fibroblast growth factors (FGFs)1 are potent growth-promoting and angiogenic proteins that are abundant at the site of active tissue remodeling and tumor invasion (1, 2). The best characterized members of this family, namely FGF1 and FGF2, also known as acidic and basic FGF, respectively, interact with distinct transmembrane tyrosine kinase receptors (FGFRs) and heparan sulfate chains linked to various cell-surface and basement membrane proteoglycans. These specific interactions among the FGFs, their receptors, and heparan sulfate activate the FGF-mediated signal transduction process through FGFR dimerization. An unusual FGF member is FGF7, also known as keratinocyte growth factor, insofar as it displays a unique cell specificity. In vivo, FGF7 is a product of stromal cells but acts in a paracrine fashion on adjacent epithelial cells that express an isoform of FGFR, the FGFR2(IIIb) (3). An example of this unique interaction occurs in prostate and prostate carcinoma where FGF7 is limited strictly to the stromal cells and its expression is androgen-dependent, although the stromal cells do not express FGFR2(IIIb) (4). Therefore, this represents a directionally specific paracrine signal system between the mesenchymal and epithelial compartments (4). Notably, perlecan, a ubiquitous modular proteoglycan that is expressed primarily as a heparan sulfate-carrying proteoglycan at the cell surfaces and basement membranes (5, 6), is up-regulated in the stroma of tumor xenografts of PC3 human prostate carcinoma cells and is also induced in various tumors (7, 8). Perlecan is directly involved in the binding of FGF2 within the basement membrane (9) and induces high affinity binding of FGF2 to heparan sulfate-deficient cells (10). Purified perlecan is angiogenic in a rabbit ear model, and when complexed with FGF2, it can induce blood vessel formation at levels higher than those induced by heparin-FGF2 complexes (10). Suppression of perlecan expression blocks autocrine and paracrine activities of FGF2 in human melanoma cells (11) and halts melanoma cell proliferation and invasion (12). FGF2 binds to the heparan sulfate chains located in domain I of perlecan, and its displacement by various proteolytic enzymes represents a plausible physiological mechanism whereby a powerful angiogenic stimulus can be made available at the site of active changes (13).

Because of the strategic location of perlecan at the epithelial/mesenchymal zone and because of its interaction with members of the FGF family, we reasoned that perlecan protein core might interact with other members of the FGF family, primarily those acting on the epithelial cell compartment. This hypothesis was further strengthened by our observation that antisense targeting of the perlecan gene correlated with a reduced colon carcinoma cell growth, both in vivo and in vitro, and to a markedly reduced responsiveness to mitogenic FGF7 (14).

In this report we investigated in more depth the interaction between FGF7 and various domains of human perlecan. Using a combination of overlay protein assays, radioligand binding experiments, and the yeast two-hybrid system, we demonstrate a specific interaction between FGF7 and domain III, homologous to the short arm of laminin α1 chain, and to a lesser degree between FGF7 and domain V, homologous to the globular C-terminal end of the laminin α1 chain. The affinity constants for both sites are in the nanomolar range (∼60 nM). Using deletion mutants we mapped the most active binding site...
to the N-terminal half of domain III. Thus, not only the heparan sulfate chains but also the perlecan protein core should be considered as potential biological ligands for FGF7, an interaction that could influence cancer growth and remodeling.

**EXPERIMENTAL PROCEDURES**

**Materials and Cell Cultures—**Media and fetal bovine serum were obtained from Hyclone Laboratories (Logan, UT). 125I and Hybond ECL membranes were purchased from Amersham Pharmacia Biotech. Carrier-free FGF7 was purchased from R & D Systems. Antibodies used in this study include: the C19 monoclonal against FGF7, the 6D3 mAb against FGF7 (Dr. R. L. Moscatelli, University of California, San Francisco), an affinity-purified goat polyclonal anti-biotin antibody raised against the C terminus residues 176–194, and monoclonal antibodies against either domain III (15) or other domains of human perlecan (13). Heparitinase I, thrombin, plasmin, and collagenase were from Sigma.

**Purification of Perlecan and Overlay Assays—**Perlecan was purified to homogeneity from the medium conditioned by WiDr human colon carcinoma (16) or umbilical artery endothelial cells by immunofinity chromatography using A71 monoclonal antibody coupled to Sepharose beads (13). The purity of the final perlecan product was determined by SDS-PAGE and Western immunoblotting using specific antibodies (13, 14). To detect specific interactions between perlecan and FGF7, we immobilized scalar concentrations of perlecan or collagen type I onto nitrocellulose membranes. Membranes were washed twice for 20 min in Tris-buffered saline, 25 mM Tris-HCl, 150 mM NaCl and blocked overnight at 4 °C with 5% fetal bovine serum, 5% non-fat milk in TBS-T (1% Tween 20). Following this 3-5 min washes, the membranes were incubated with human recombinant FGF7 (100 ng/ml) for 1 h, washed three times, and incubated with the anti-FGF7 antibody. After an additional three 5-min washes, the membranes were incubated for 1 h with secondary antibody (1:15000), washed again, and developed using chemiluminescence ECL Substrates (Pierce). In separate experiments, -20 pg of immunopurified human perlecan were digested for 2–16 h with heparitinase I (100 units/ml) in 10 mM Hepes, 3 mM CaCl2, pH 7.0. Further digestion with various proteases, including plasmin (30 units/ml), thrombin (7.5 units/ml), and collagenase (625 units/ml) was performed as described previously (13). The products were then separated on 8% reducing SDS-PAGE, transferred onto nitrocellulose, and incubated with recombinant FGF7 followed by immune detection with anti-FGF7 antibody.

**Expression of Various Domains of Human Perlecan using the pMAL System and Radioligand Binding Assays—**The pMAL expression vector system (New England Biolabs) was used to express various regions of perlecan as MBP-fusion proteins (15). For radioligand binding assays (15). For radioligand binding assays. Carrier-free FGF7 was labeled with 125I in borosilicate-lined tubes precoated with 50 μg of the IODO-GEN (Pierce) and allowed to bind various domains of perlecan coated onto Immulon 4 HBX wells (Dynex Technologies). Binding was measured in TBS supplemented with 2 M CaCl2, 2 M MgCl2, 0.02% NaN3, and 1 mg/ml heparin inactivated bovine serum albumin. Following incubation under gentle shaking (60 rpm) for 4–14 h, reversible binding was demonstrated by incubation with 100-fold molar excess of unlabeled FGF7. After incubation the wells were washed three times with ice-cold TBS-T and then counted in toto. Scatchard plots were generated using the Ligand program (17).

** Yeast Two-hybrid System and β-Galactosidase Assay—**We utilized either previously cloned perlecan cDNAs (18) or cDNAs generated by reverse transcription-polymerase chain reaction. Total RNA was extracted from human fibroblasts with the Tri-Resagent. Reverse transcriptase was performed for 2 h at 42 °C in the presence of 25 units of avian myeloblastosis virus-reverse transcriptase. For perlecan domain III, two polymerase chain reaction fragments were generated. A 1555-bp fragment, spanning the 5’ region, was primed with 5’-CGGGATCCCTGCTGCTATGGCCG-3’ (containing a BamHI site) and 3’-CGGTTGATATGGGCGCT-5’ (arrows). A 486-bp fragment, spanning the 3’ region, was originated using 5’-GCGGATAAATGCGGCTCC-3’ and 3’-GTCCGGACTGGTCTGTGGTGTC-5’ (containing a BamHI site). Fragments were digested with BamHI/Dral and Xhol/BamHI, respectively, purified, and ligated together with a Dral/Xhol fragment into BamHI-linearized pGBT9 and pGAD424 plasmids. Further deletion fragments were generated by endonuclease digestion of the construct. Domain IV was cloned in both pGBT9 and pGAD424 plasmids. All constructs were analyzed by DNA sequencing. For the two-hybrid system assay, 1 μl of ATR-1 and ATR-2 reagent was added to 100 μl of YRG-2 competent cells (Strategene) as described before (19) and incubated for 30 min at 30 °C in the presence of 1 μg of plasmid. The cells were then heat-shocked for 5 min at 42 °C and incubated at 30 °C in the presence of 0.9 M trp /Leu medium for 3 h with constant shaking at 30°C. The transformed cells were plated in Trp /Leu /His or Trp /Leu /Ade agar plates to check for interactions. The colonies were grown for 4 days at 30 °C. For β-galactosidase assays, cells grown in Trp /Leu -plates were transferred onto Whatman No. 3MM paper filters, soaked in 2 buffer/X-gal solution (0.1 mM NaHPO4, 45 mM NaHPO4, 10 mM KCl, 2 mM MgSO4, 0.3% β-mercaptoethanol, 3.3 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Yeast cells were frozen in liquid nitrogen for 10 s, thawed, transferred onto a filter, and incubated at 30 °C for ~8 h. Additional details are provided in the text and legends to figures.

**RESULTS**

** Perlecan Protein Core Binds to FGF7 in Overlay Assays—**Perlecan was immunopurified to homogeneity from the medium conditioned by either human colon carcinoma (16) or umbilical artery endothelial (13) cells using A71 monoclonal antibody coupled to Sepharose beads (20). As reported before (14), the final product was essentially pure proteoglycan with an estimated molecular mass of ~500 kDa carrying heparan sulfate side chains (not shown). To detect specific interactions between perlecan and FGF7, we immobilized scalar concentrations of perlecan or collagen type I onto nitrocellulose membranes by slot blotting, incubated the membranes with recombinant FGF7, and detected specific binding by monoclonal antibodies against FGF7 and chemiluminescence. In these overlay assays both proteins (the immobilized and the soluble ligand) are under native conditions. The results showed a significant FGF7 binding to perlecan preparations purified from both colon carcinoma and endothelial cells (Fig. 1A), whereas collagen type I and DMEM remained unreactive. The endothelial cell-derived perlecan showed more binding than the colon carcinoma perlecan based on a protein weight basis. We attribute this difference to a differential contribution of the heparan sulfate side chains, which are significantly undersulfated in the colon carcinoma perlecan (21). Soluble perlecan also bound to immobilized FGF7 as detected by overlay assay and anti-domain III antibodies (Fig. 1B).

To further investigate this interaction, we subjected equal amounts (~5 μg) of endothelial cell perlecan to heparitinase I followed by incubation with various proteases known to release fragments of perlecan protein core (13). Notably, FGF7 interacted specifically with two fragments generated by plasmin, a 50-kDa and a 34–36-kDa doublet (Fig. 1C, lane 2). In addition, it reacted with an ~30-kDa thrombin fragment (Fig. 1C, lane 3). When the blot was stripped and reacted with a monoclonal antibody against domain III (15), the 50-kDa plasmin (double arrowheads) and the 30-kDa thrombin (arrow) fragments reacted specifically with anti-domain III antibodies (Fig. 1D). In contrast, the 34–36-kDa plasmin doublet did not react, suggesting that it might represent a domain other than domain III. Moreover, the heparitinase- and the plasmin-digested perlecan revealed high molecular mass bands (Fig. 1D, lanes 1 and 2, respectively) consistent with the size of perlecan protein core. The lack of binding of FGF7 to high molecular mass species of perlecan generated by heparitinase alone or plus plasmin or thrombin suggests that the binding site(s) may be cryptic or, more likely, that the denaturing conditions used in these experiments may have altered or hindered the binding site for FGF7. As expected, the intact perlecan (molecular mass >500 kDa) did not penetrate the gel (Fig. 1, C and D, lanes 5), whereas collagenase degraded the protein core quite extensively (Fig. 1D, lane 4). Immunoblotting with monoclonal antibodies directed against domain I did not show any reactivity with any band (not shown). Therefore, we believe that the 34–36-kDa plasmin doublet likely belongs to domain V, insofar...
as experiments to be discussed below showed an interaction between domain V and FGF7. Unfortunately, the available monoclonal antibody against domain V does not work well in immunoblotting (13), thus precluding precise identification of these reactive bands.

Collectively these results indicate that recombinant FGF7 reacts specifically with domain III of human perlecan in at least two fragments of 30 and 50 kDa, respectively. In addition, FGF7 binds to a doublet of 34–36 kDa that might represent a domain other than domain III.

Interaction of Domains III and V with Recombinant FGF7—To investigate in detail the precise location of FGF7 interaction with the protein core of perlecan, we generated all the domains of perlecan expressed in Escherichia coli as MBP-fusion proteins (Fig. 2, A and B) with the exception of domain IV, which was totally insoluble and required chaotropic reagents to be solubilized from the bacterial inclusion bodies. We were unable to properly refold domain IV, and therefore, no protein moiety bound to the affinity matrix. When equimolar amounts of each domain were bound to Immulon® wells, 125I-FGF7 bound specifically to domains III and V (Fig. 2C). In contrast, domains I and II and MBP failed to bind.

Incubation with radiolabeled FGF7 showed that the binding to either domain III (Fig. 3A) or V (Fig. 3C) became saturable at 0.25 pmol in contrast to MBP, which showed no significant binding at any concentrations of FGF7. Scatchard plots for the binding of domain III or V to FGF7 gave dissociation constants that were nearly identical, 62 ± 6 nM and 59 ± 7 nM, respectively.

FIG. 1. Perlecan binds to FGF7 in overlay assays. A, binding of soluble recombinant FGF7 (100 ng/ml) to immobilized perlecan purified from either human colon carcinoma (WiDr) or human umbilical artery endothelial cells (EC); type I collagen or DMEM served as negative controls. Proteins were slot-blotted unto nitrocellulose filters at the indicated concentrations, blocked in 5% milk, and incubated with soluble ligand. Detection was with a specific anti-FGF7 monoclonal antibody and chemiluminescence. B, binding of soluble human EC perlecan (5 μg/ml) to immobilized FGF7 or DMEM. The reaction was detected with specific monoclonal antibody against perlecan domain III. C, binding of soluble FGF7 to perlecan preparation treated with heparitinase I alone or following a sequential digestion with heparitinase I and plasmin, thrombin, or collagenase. The products were separated on an 8% SDS-PAGE, transferred onto nitrocellulose, and overlaid with soluble FGF7. Specific binding was detected with anti-FGF7 antibody. The migration of molecular mass markers is indicated in the left margin. D, the blot in C was stripped and reacted with a monoclonal antibody against domain III of perlecan. Notice the presence of domain III-specific epitopes in the 50-kDa plasmin digest (double arrowheads, lane 2) and in the 30-kDa thrombin-generated fragment (arrow, lane 3).

FIG. 2. Binding of various domains of perlecan to recombinant FGF7. A, schematic representation of the five domains of human perlecan indicated by Roman numerals. Each domain is color-coded. The number of the amino acid encoding the various constructs is shown in the bottom. B, SDS-PAGE of various domains following purification as MBP-fusion proteins. The migration of protein standard is indicated on the left margins. C, affinity of 125I-FGF7 to various domains of human perlecan. About 10 pmol of each domain was bound to Immulon® wells in triplicate and incubated with equal amounts of 125I-FGF7 (1–2 × 10^18 cpm/mol).
Perlecan-FGF-7 Interaction

In Vivo Interaction between Perlecan and FGF7—The interaction of FGF7 with perlecan was further investigated in vivo using the yeast two-hybrid technology. The cDNAs encoding domains III, IV, and V were cloned into the GAL4 binding domain vector, and the full-length cDNA encoding the human mature FGF7 was cloned into the GAL4 activating domain vector. As a control for possible interactions all constructs were transfected with the plasmids and assayed for growth both in triple minus (Trp−/Leu−/His+) and in double minus (Trp−/Leu−) media as a control for transfection efficiency. The assay showed that FGF7 bound domains III and V, although the growth of the yeast cells transfected with domain V was slower. This suggests that in vivo the interaction between FGF7 and domain V might be weaker than domain III. Domain IV showed no detectable interaction with FGF7 (Fig. 4C). To further map the binding site, we generated three deletion fragments of domain III, named III-A1 (aa 505–1371), III-A2 (aa 505–1019), and III-A3 (aa 1466–1676). Only the constructs spanning the N-terminal region (III-A1 and III-A2) allowed the yeast cells to grow in the triple minus media (Fig. 4, A and B). The predicted size of the protein encoded by mutant III-A2 (~50 kDa) is in good agreement with the overlay assays (cf. Fig. 1, C and D).

We also monitored perlecan/FGF7 interaction using β-galactosidase activity. In full support of the growth assays, the smallest N-terminal domain III deletion (III-A2) generated a blue reaction as strong as the whole domain III (Fig. 4E). Domain IV was unreactive, and the binding of domain V was weaker as in the growth assays. Furthermore, the color reaction triggered by domain III and FGF7 was detectable as fast as the positive control harboring the p53 and the SV40 T antigen genes, further stressing that this domain is the most interactive one.

Perlecan domains were also used in all combinations to check for potential self-interaction. The full-length domain III and mutant III-A2 were able to interact with themselves, in contrast to domains IV or V, which were essentially unreactive (Fig. 4F). These data are supportive of the reported homophilic interactions of perlecan in basement membranes.

Collectively, the results indicate that the first globular region and the three EGF repeats in domain III are the sites of the strongest interaction with FGF7.

DISCUSSION

Large multidomain proteoglycans, such as perlecan, not only provide a physical barrier to movement of cells into tissues but also tested as either bait or prey. The yeast cells were transfected with the plasmids and assayed for growth both in triple minus (Trp−/Leu−/His+) and in double minus (Trp−/Leu−) media as a control for transfection efficiency. The assay showed that FGF7 bound domains III and V, although the growth of the yeast cells transfected with domain V was slower. This suggests that in vivo the interaction between FGF7 and domain V might be weaker than domain III. Domain IV showed no detectable interaction with FGF7 (Fig. 4C). To further map the binding site, we generated three deletion fragments of domain III, named III-A1 (aa 505–1371), III-A2 (aa 505–1019), and III-A3 (aa 1466–1676). Only the constructs spanning the N-terminal region (III-A1 and III-A2) allowed the yeast cells to grow in the triple minus media (Fig. 4, A and B). The predicted size of the protein encoded by mutant III-A2 (~50 kDa) is in good agreement with the overlay assays (cf. Fig. 1, C and D).

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are also known to sequester a variety of bioactive proteins including growth factors, chemokines, cytokines, and enzymes (6). These molecules can be retained as supramolecular complexes protected against proteolysis or can be released when the protein core is degraded. This provides a plausible mechanism for induction of growth, chemotaxis, and extravasation of cells (13). Interaction of perlecan with various extracellular matrix proteins has been known for some time (23–25). However, little information is available regarding the direct binding of specific growth factors to perlecan protein core. In the present study we demonstrate that perlecan protein core is a relatively high-affinity (K_D ~ 60 nM) ligand for FGF7. Overlay assays and radioligand binding experiments demonstrated that the specific FGF7/perlecan interaction involves at least two distinct regions of the protein core within domains III and V. Domain III consists of three globular regions and eight EGF-like modules that follow an arrangement similar to that of the short arm of the laminin a1 chain (6). It is known that tandem arrays of EGF-like modules, highly enriched in disulfide-bonded cysteine residues, have a compact rod-like structure with tight interfaces (23), thereby explaining the resistance to pepsin (26) or V8 protease (27) digestion.

The yeast two-hybrid system has been used to study interactions occurring primarily between intracellular proteins (28), and only recently it has been used successfully to investigate interactions among extracellular proteins such as those involving collagen types VI and IV (29) or collagen type VII and thrombospondin (19). Because of its eukaryotic nature, the yeast-expressed proteins would be properly folded and glycosylated, thereby providing a strong validation for the cell-free experiments using prokaryotic fusion proteins. It is unclear whether the protein/protein interactions do actually take place inside or outside the nucleus (30). Consequently, it is plausible that the initial interactions do not take place in the nuclear environment, and yeasts are known to favor proper processing and disulfide bonding in recombinant proteins (30). Notably, we have designed vectors expressing specific domains of perlecan that have been shown before to fold independently as individual modules (25, 31–33). The most active construct in the two-hybrid system encompassed the N-terminal region of domain III, corresponding to residues 505–1019 (in the mouse being residues 503–923), also known as subdomain III-1 according to the nomenclature proposed by Timpl and co-workers (25). Using cell-free binding assays, a high affinity (K_D ~ 8–25 nM) binding site for platelet-derived growth factor (PDGF)-AA and -BB has been identified in subdomain III-2 (32). The flanking subdomains III-1 and III-3 did not bind at all. Thus, it appears that there are unique binding specificities for perlecan modules containing highly repetitive sequences. Binding of FGF7 to subdomain III-1 is the first extracellular ligand identified so far for this particular perlecan region. Moreover we show for the first time an in vitro self-interaction involving primarily domain III and the subdomain III-Δ2. This is interesting because the proposed homophilic interaction for perlecan has been previously assigned to domain V, i.e. the domain opposite the heparan sulfate-carrying end of the molecule (22). Future experiments need to elucidate whether domain III/ domain III interactions do actually occur with purified perlecan molecules.

Other protein cores of different proteoglycans bind members of the FGF and PDGF (17, 34) family. The high affinity (K_D ~ 6 nM) of FGF2 to the nervous tissue-specific phosphacan proteoglycan core (17) and its ability to potentiate the mitogenic effects of FGF2 to an extent comparable with heparin/heparan sulfate, indicate that protein/growth factor interactions may play biological roles of equal or comparable importance to the well established FGF/heparan sulfate interactions. The transmembrane proteoglycan NG2 binds to FGF2 and also to PDGF-AA with high affinity (K_D ~ 5–10 nM) (34). These findings underscore the concept that transmembrane protein cores may play important roles in organizing and presenting mitogenic factors at the cell surface. It is noteworthy that perlecan can be localized at the cell surface (16) probably bound via integrins (25, 27, 35). Thus, a much more complex scenario should be entertained in which members of the FGF family may interact with three distinct entities: (i) their own receptors, (ii) protein cores of tissue-specific proteoglycans, and (iii) heparan sulfate chains. We believe that the expression of a specific protein core and its glycosylation status (i.e. unique sequences in the heparan sulfate) may direct the activity of potent angiogenic and growth-promoting factors.

Previous studies have shown that low concentrations of heparin inhibit the binding of FGF7 to its receptor but stimulate the binding of FGF1, and the cell-surface heparan sulfate proteoglycan glypican has been involved in this differential modulation of FGF7/FGFR interaction (36). Our previous study has demonstrated a biological function of perlecan in reconstituting FGF7 mitogenic activity in perlecan-deficient tumor cells (14). Our current data expand these findings and further indicate that perlecan may function as an extracellular sink for FGF7, acting as a reservoir for this growth factor and thereby preventing the cellular changes associated with FGF7, such as epidermal growth and wound healing, until its release. Upon displacement by partial proteolysis of the protein core, FGF7 would become available to the surrounding cellular environment and could behave as a promoter of growth and differentiation.

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REFERENCES
1. Faham, S., Linhardt, R. J., and Bees, D. C. (1998) Curr. Opin. Struct. Biol. 8, 578–586
2. Conrad, H. E. (1998) Heparin-binding Proteins, Academic Press, San Diego
3. Aaronson, S. A., Bottaro, D. P., Miki, T., Ron, D., Finch, P. W., Fleming, T. P., Ahn, J., Taylor, W. G., and Rubin, J. S. (1991) Ann. N. Y. Acad. Sci. 620, 62–77
4. McKeenan, W. L., Wang, F., and Kan, M. (1998) Prog. Nucleic Acid Res. Mol. Biol. 59, 135–176
5. Iozzo, R. V., and Murdoch, A. D. (1996) FASEB J. 10, 598–614
6. Iozzo, R. V. (1998) Annu. Rev. Biochem. 67, 609–652
7. Iozzo, R. V., Cohen, I. R., Grassel, S., and Murdoch, A. D. (1994) Biochem. J. 302, 625–639
8. Cohen, I. R., Murdoch, A. D., Nao, M. F., Marchetti, D., Berd, D., and Iozzo, R. V. (1994) Cancer Res. 54, 5771–5774
9. Vugry, M., Oliker-Hartmann, M. P., Lavigne, M., Fayein, N., Jeanne, J. C., Laurent, M., and Courtois, Y. (1988) J. Cell. Physiol. 137, 321–328
10. Aviezer, D., Hecht, D., Safran, M., Eisinger, M., David, G., and Yayon, A. (1994) Cell 79, 1005–1013
11. Aviezer, D., Iozzo, R. V., Noonan, D. M., and Yayon, A. (1997) Mol. Cell. Biol. 17, 1938–1946
12. Adatia, R., Albini, A., Carlone, S., Giancaglio, D., Benelli, R., Santi, L., and Noonan, D. M. (1998) Ann. Oncol. 9, 1257–1301
13. Whitelock, J. M., Graham, L. D., Melrose, J., Murdoch, A. D., Iozzo, R. V., and Underwood, P. A. (1999) J. Cell. Physiol. 176, 8544–8557
14. Milev, P., Monnerie, H., Popp, S., Margolis, R. K., and Margolis, R. U. (1998) J. Biol. Chem. 273, 1599–1608
15. Whitelock, J. M., Iozzo, R. V., and Underwood, P. A. (1996) J. Biol. Chem. 271, 10079–10086
16. Shimasaki, S., and Cockett, T. (1994) Cell 77, 1055–1013
17. Whitelock, J. M., Iozzo, R. V., Noonan, D. M., and Yayon, A. (1997) Mol. Cell. Biol. 17, 598–614
18. White, D., and Iozzo, R. V. (1999) Exp. Cell Res. 250, 17668–17676
19. Aho, S., and Uitto, J. (1998) Matrix Biol. 17, 854–867
20. Whitelock, J. M., Graham, L. D., Melrose, J., Murdoch, A. D., Iozzo, R. V., and Underwood, P. A. (1999) Matrix Biol. 18, 163–178
21. Iozzo, R. V. (1994) J. Biol. Chem. 269, 455–471
22. Milew, P., Monnerie, H., Popp, S., Margolis, R. K., and Margulis, R. U. (1998) J. Biol. Chem. 273, 21439–21442
23. Murdoch, A. D., Dode, G. R., Coh, I., Iozzo, R. V., and Noonan, D. M. (1998) J. Biol. Chem. 267, 8544–8552
24. Aho, S., and Uitto, J. (1998) Matrix Biol. 17, 401–412
25. White, D., and Iozzo, R. V. (1999) Matrix Biol. 18, 163–178
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26. Schulze, B., Mann, K., Battistutta, R., Wiedemann, H., and Timpl, R. (1995) \textit{Eur. J. Biochem.} 231, 551–556
27. Chakravarti, S., Horchar, T., Jefferson, B., Laurie, G. W., and Hassell, J. R. (1995) \textit{J. Biol. Chem.} 270, 404–409
28. Fields, S., and Song, O. (1989) \textit{Nature} 340, 245–246
29. Kuo, H.-J., Maslen, C. L., Keene, D. R., and Glanville, R. W. (1997) \textit{J. Biol. Chem.} 272, 26522–26529
30. Fields, S., and Sternglanz, R. (1994) \textit{Trends Genet.} 10, 286–292
31. Schulze, B., Sasaki, T., Costell, M., Mann, K., and Timpl, R. (1996) \textit{Matrix Biol.} 15, 349–357
32. Göhring, W., Sasaki, T., and Timpl, R. (1998) \textit{Eur. J. Biochem.} 255, 60–66
33. Hopf, M., Göhring, W., Kohfeldt, E., Yamada, Y., and Timpl, R. (1999) \textit{Eur. J. Biochem.} 259, 917–925
34. Goretzki, L., Burg, M. A., Grako, K. A., and Stallcup, W. B. (1999) \textit{J. Biol. Chem.} 274, 16831–16837
35. Battaglia, C., Aumailley, M., Mann, K., Mayer, U., and Timpl, R. (1993) \textit{Eur. J. Cell Biol.} 61, 92–99
36. Bonneh-Barkay, D., Shlissel, M., Berman, B., Shaoul, E., Admon, A., Vlodavsky, I., Carey, D. J., Asundi, V. K., Reich-Slotky, R., and Ron, D. (1997) \textit{J. Biol. Chem.} 272, 12415–12421