Fibroblast Growth Factor-binding Protein Is a Novel Partner for Perlecan Protein Core*

Received for publication, December 20, 2000
Published, JBC Papers in Press, January 8, 2001, DOI 10.1074/jbc.M011493200

Maurizio Mongiat‡‡, Juliet Otto‡‡, Rachel Oldershaw‡, Felix Ferrer††, J. Denry Sato, and Renato V. Iozzo **‡‡‡

From the ‡Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, the †Division of Cell, Molecular and Developmental Biology, American Type Culture Collection, Manassas, Virginia 20110, and the **Cellular Biology and Signaling Program, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Perlecan, a widespread heparan sulfate proteoglycan, functions as a bioactive reservoir for growth factors by stabilizing them against misfolding or proteolysis. These factors, chiefly members of the fibroblast growth factor (FGF) gene family, are coupled to the N-terminal heparan sulfate chains, which augment high affinity binding and receptor activation. However, rather little is known about biological partners of the protein core. The major goal of this study was to identify novel proteins that interact with the protein core of perlecan. Using the yeast two-hybrid system and domain III of perlecan as bait, we screened ~0.5 10^6 cDNA clones from a keratinocyte library and identified a strongly interactive clone. This cDNA corresponded to FGF-binding protein (FGF-BP), a secreted protein previously shown to bind acidic and basic FGF and to modulate their activities. Using a panel of deletion mutants, FGF-BP binding was localized to the second EGF repeat of domain III, a region very close to the binding site for FGF7. FGF-BP could be coimmunoprecipitated with an antibody against perlecan and bound in solution to recombinant domain III-alkaline phosphatase fusion protein. Immunohistochemical analyses revealed colocalization of FGF-BP and perlecan in the pericellular stroma of various squamous cell carcinomas suggesting a potential in vivo interaction. Thus, FGF-BP should be considered a novel biological ligand for perlecan, an interaction that could influence cancer growth and tissue remodeling.

Heparan sulfate proteoglycans are emerging as key molecules governing crucial events in embryonic development, pattern formation, inflammation, wound repair, and cancer (1–5). Perlecan is a major heparan sulfate proteoglycan of basement membranes (6), which is expressed in virtually all vascularized tissues (7). As demonstrated by sequence analysis (7–11), perlecan has a complex multidomain structure based on seven protein modules arranged in five distinct domains (12). The modules harbor protein motifs with similarities to proteins involved in nutrient uptake, cell growth, adhesion, and signaling (13, 14). Functional pleiotropism is inferred from its complex structure and broad expression (15). Perlecan can self-aggregate into dimeric or multimeric forms when incubated under neutral isotonic conditions (16) and is involved in heterotypic interactions with various macromolecules including laminin (17–19), nidogen (20), fibronectin (12, 21, 22), fibulin-2 (12), collagen type IV (12, 23), α-dystroglycan (24), and lipoproteins (25).

During mammalian development, its expression is detected quite early in tissues of vasculogenesis, being deposited along nearly all the endothelial-lined vascular beds (26, 27). Perlecan is present not only in the basement membranes but also within extracellular matrices (13, 28) and in close proximity to cell surfaces (29), where its binding is likely mediated by members of the integrin family (30–32). Targeted disruption of the perlecan gene causes embryonic lethality at day 10.5 with widespread cephalic and skeletal abnormalities (33, 34). Notably, the basement membranes are normally formed in the homoygous null animals, but vascular and cephalic abnormalities are generated in areas of increased pressure, suggesting that perlecan is required for maintaining basement membrane integrity (35). The abnormal cartilage structure and the disregulated endochondral ossification of the perlecan null animals are suggestive of a phenotype encountered with activating mutations of the fibroblast growth factor (FGF) receptor 3, thereby positioning perlecan as a negative regulator of this signaling pathway (33).

Perlecan affects cell proliferation, tumor invasion, angiogenesis, and thrombosis (13, 36–38), but the pathways by which it is able to influence such important events are not completely understood. Increased perlecan deposition is observed in breast and colon (13) carcinomas as well as in metastatic melanomas (39), and these changes correlate with enhanced metastatic potential (40). Perlecan functions as a ligand reservoir for angiogenic growth factors that become stabilized against misfolding or proteolysis (41, 42). For example, perlecan binds FGF2 (43, 44) and promotes receptor activation and mitogenesis (45). In a rabbit ear model of angiogenesis, perlecan–FGF2 complexes induce blood vessel formation at levels higher than those induced by heparin–FGF2 complexes (46). FGF2 binds to the heparan sulfate chains of perlecan, and its displacement by various proteolytic enzymes offers a plausible physiological

* This work was supported by National Institutes of Health Grants RO1 CA39481 and RO1 CA47282 (to R. V. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported in part by a fellowship from the American-Italian Cancer Foundation.

¶ These two authors contributed equally to this work.

†† Their correspondence should be addressed: Dept. of Pathology, Anatomy and Cell Biology, Rm. 249, Jefferson Alumni Hall, Thomas Jefferson University, 1020 Locust St., Philadelphia, PA 19107. E-mail: iozzo@lac.jci.tju.edu.

1 The abbreviations used are: FGF, fibroblast growth factor; FGF-BP, FGF-binding protein (also known as HBp17); FGF7, fibroblast growth factor-7 (also known as keratinocyte growth factor); EGFR, epidermal growth factor; AP, alkaline phosphatase; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.
mechanism whereby a powerful angiogenic stimulus becomes operational at the site of active tumor invasion (42). Thus, we hypothesized that perlecan deposition in the newly formed tumor stroma may act as a scaffold on which capillaries proliferate to generate new vascular anastomoses (14). Suppression of perlecan expression blocks autocrine and paracrine activities of FGF2 in human melanoma cells (46) and halts melanoma cell proliferation and invasion (47). In fibrosarcoma cells, however, perlecan appears to act as a negative regulator of growth and invasion (48), suggesting that the specific cellular context may play a cardinal role in perlecan’s biological function. We have recently discovered (49) that antisense targeting of the perlecan gene correlates with a reduced colon carcinoma cell growth and a markedly attenuated responsiveness to mitogenic FGF7. FGF7 binds specifically to domains III and V of perlecan (50), and exogenous perlecan efficiently reconstitutes FGF7 mitogenic activity in perlecan-deficient cells (49).

The major goal of this study was to identify novel proteins that interact with the protein core of perlecan. Using the yeast two-hybrid system and domain III of perlecan as bait, we identified a strongly interactive clone that corresponded to HBp17 (51), also known as FGF-BP (52), a protein previously shown to modulate the activity of FGF1 and FGF2 to enhance the tumorigenicity of A431 squamous carcinoma cells (51) and to act as a potent angiogenic stimulus (52). FGF-BP bound specifically to domain III within the second EGF repeat, a region very close to the major binding site for FGF7 (50). FGF-BP could be coimmunoprecipitated with an antibody against perlecan and bound in solution to recombinant domain III-alkaline phosphatase (AP) fusion protein. Immunohistochemical studies revealed a significant up-regulation of FGF-BP in various squamous cell carcinomas with a distribution similar to that of...
perlecan, suggesting a potential in vivo interaction. Therefore, FGF-BP should be considered a novel biological ligand for perlecan, an interaction that could influence cancer growth and tissue remodeling.

**EXPERIMENTAL PROCEDURES**

**Materials and Cell Cultures**—Media and fetal bovine serum were obtained from Hyclone Laboratories (Logan, UT). 125I and Hybond ECL media and fetal bovine serum were used for transfections. Media and cell cultures were prepared and combined with 50 mg each of the library DNA and transcription/translation system (Promega) was used for in vitro transcription and translation of FGF-BP and the positive luciferase control. FGF-BP was subcloned from pGAD424 into pcDNA3.1 downstream from the T7 promoter by EcoRI/SalI digestion followed by ligation into an EcoRI/SalI-digested pKG10. The plasmid DNA was isolated and sequenced by DNA sequencing. The yeast reporter strain strain SPS226 with all the combinations of hybrid constructs, using FGF-BP and perlecan domain III as both prey and bait expressing interacting hybrid proteins. The plates were incubated at 30 °C for 8 days. The His+ colonies were isolated and screened for the inserts in the activation domain vector using primers specific for the GAL4 activation domain plasmid.

**Generation of A431 Cells Expressing Domain III-AP Fusion Protein and Co-immunoprecipitation Studies**—Perlecan domain III was sub-
cloned by PCR (Expand™ Template PCR kit, Roche Molecular Biochemicals) using the oligonucleotides 5'-CGCAATGGCCCTGCCCTGACGGCC-3' and 5'-CGGGATCCAATTGTGGGGCTTGGTTTCTCG-3', which introduced an MfeI site. The purified digested fragment was ligated into an engineered pcDNA3.1 vector linearized with EcoRI containing the sequence of the human placental AP preceded by the BM-40 signal peptide. The construct was sequenced, and 10 μg of the purified plasmid were used to transfect A431 cells by electroporation (375 V, 490 microfarads, 1 ms). Cells were cultured in G418 (400 μg/ml), and clones were isolated by ring cloning and expanded. Conditioned media from confluent cells were collected after 2 days and assayed for AP enzymatic activity using a SEAP (secreted alkaline phosphatase) Chemiluminescence Detection Kit (CLONTECH). The expression of the recombinant RNA was analyzed by Northern blotting and the chimeric protein detected in Western immunoblotting employing a specific antibody directed against domain III. For immunoprecipitation, aliquots of the media containing domain III-AP fusion proteins were brought to 50 mM of perlecan domain III/AP and FGF-BP, both cloned into pcDNA3.1 (Invitrogen) containing the sequence of the BM-40 signal peptide. After 72 h of incubation, the serum-free conditioned medium was collected and filtered, and various protease inhibitors were added (see above). Two μg of either anti-domain III or anti-FGF-BP antibody were added to 1 ml of medium of transfected and untransfected COS-1 cells, and the proteins were immunoprecipitated at 4 °C for 18 h with continuous rocking. The immunocomplexes were

**Fig. 4.** FGF-BP can be coimmunoprecipitated with perlecan’s domain III-AP fusion protein. Aliquots of serum-free media conditioned for 24–36 h by A431 squamous carcinoma cells were precipitated with 6 volumes of ethanol-0.1% potassium acetate at −20 °C, separated on a 8.5% SDS-PAGE, and analyzed by Western immunoblotting with either anti-domain III (αdIII) or anti-FGF-BP (αFGF-BP). Notice, in the top panel, the presence of a ~190-kDa immunoreactive protein in clone 1 (lanes 1 and 2) or clone 11 (lanes 5 and 6), in contrast to clone 4 or the wild type cells (lanes 3 and 4 and 7 and 8, respectively). The bottom panel, reacted with αFGF-BP, shows a significant amount of FGF-BP migrating with an average mass of ~23 kDa. B, alkaline phosphatase assays of various clones as indicated. C, immunoprecipitation (ip) followed by Western immunoblotting (wb) as indicated. Notice the presence of domain III-AP fusion protein and FGF-BP in both immunoprecipitates. The reactive ~85 kDa band (lanes 1 and 2, top panel) corresponds to IgG homo- or heterodimers since the gel was run under nonreducing conditions because the C9 antibody does not work well following reduction. Lane 3 shows immunoblotting of the total medium alone without any immunoprecipitation.

In Vivo Interaction of Human Perlecan Domain III and FGF-BP in Transiently Transfected Cells—To establish the in vivo interaction of perlecan’s domain III and FGF-BP, the two full-length cDNAs were subcloned into pcDNA3.1 containing the signal peptide of BM-40 and cotransfected into COS-1 cells. The 72-h conditioned media were immunoprecipitated with the two antibodies as indicated and subjected to Western immunoblotting (wb). The bottom panel represents the IgG to show equal loading. Molecular mass markers in kDa are shown in the right margin. Notice the presence of the predicted ~190- and ~23-kDa proteins corresponding to perlecan’s domain III-AP and -FGF-BP, respectively. Captured with protein A/G-agarose beads (Pierce). Samples were analyzed in a 3–15% SDS-PAGE gradient gel under nonreducing conditions and transferred into nitrocellulose. The membranes were incubated in 5% nonfat dry milk at room temperature for 1 h. The lower portion of the membrane (proteins <40 kDa) was then incubated with the anti-FGF-BP antibody and the upper portion with the anti-domain III antibody for 1 h. Membranes were washed three times in Tris-buffered saline containing 0.1% Tween 20 and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h. After three washes, the immunocomplexes were visualized by enhanced chemiluminescence (Pierce).

**Fig. 5.** Perlecan-FGF-BP interaction in transiently transfected cells. To establish the in vivo interaction of perlecan’s domain III and FGF-BP, the two full-length cDNAs were subcloned into pcDNA3.1 containing the signal peptide of BM-40 and cotransfected into COS-1 cells. The 72-h conditioned media were immunoprecipitated with the two antibodies as indicated and subjected to Western immunoblotting (wb). The bottom panel represents the IgG to show equal loading. Molecular mass markers in kDa are shown in the right margin. Notice the presence of the predicted ~190- and ~23-kDa proteins corresponding to perlecan’s domain III-AP and -FGF-BP, respectively.

In Vivo Interaction of Human Perlecan Domain III and FGF-BP in Transiently Transfected COS-1 Cells—Subconfluent cultures of COS-1 cells were transfected using LipofectAMINE Plus reagent (Life Technologies, Inc.) with 4 μg of perlecan domain II/AP and FGF-BP, both cloned into pcDNA3.1 (Invitrogen) containing the sequence of the BM-40 signal peptide. After 72 h of incubation, the serum-free conditioned medium was collected and filtered, and various protease inhibitors were added (see above). Two μg of either anti-domain III or anti-FGF-BP antibody were added to 1 ml of medium of transfected and untransfected COS-1 cells, and the proteins were immunoprecipitated at 4 °C for 18 h with continuous rocking. The immunocomplexes were
Perlecan-FGF-BP Interaction

RESULTS AND DISCUSSION

Discovery of FGF-BP as a Binding Partner for Perlecan Protein Core—The yeast two-hybrid system (53) was used to identify candidate proteins that interact with perlecan domain III in vivo. Before proceeding with the screening, the bait plasmid harboring domain III was tested for its inability to activate the prototrophic reporter gene HIS3. As a control for possible interactions, all constructs were also tested as either bait or prey. In addition, all of the constructs were assayed for growth in double minus (Trp /Leu ) media as a control for transfection efficiency. The screening of ~0.5 \times 10^6 cDNAs from a human keratinocyte library resulted in the isolation of 50 independent clones identified as large colonies growing in triple minus (Trp /Leu /His ) plates. Of these, 40 clones grew back and 38 clones showed inserts ranging between 0.4 and 1.6 kilobase pair. To minimize the number of false positives and to increase the likelihood of obtaining pure clones, the initial isolates were regrown in triple minus media and subjected to direct PCR screening using flanking primers specific for the pGAD plasmid. All of the bands were isolated and sequenced, and DNA homology searches using the NCBI BLAST program of the Genetics Computing Group package led to the elimination of many false positives, the majority of which were nuclear proteins that could themselves activate the transcription of the HIS3 reporter gene. A highly interacting clone contained a 1.2-kilobase pair insert (Fig. 1A) that encoded a small protein, named HBp17 (Fig. 1B) because it binds heparin-binding growth factors 1 and 2 (51) (also called FGF-BP, for FGF-binding protein (52)). The cDNA was in frame with the activating domain of the plasmid and coded for the full-length FGF-BP, further supporting the concept of a real protein-protein interaction.

The full-length FGF-BP cDNA is 1163 base pairs, and the primary structure of the encoded protein consists of 234 amino acids, including a 21-residue signal peptide sequence and a single putative N-linked glycosylation site. Computer searches revealed that homologues of human FGF-BP have been cloned previously from bovine, rat, and mouse tissues (54–56) and that FGF-BP is well conserved among species (Fig. 1B). Human FGF-BP showed 84, 57, and 49% identical amino acid sequence to the bovine, rat, and mouse species, respectively. Ten Cys residues are fully conserved among species, and there are two partial heparin-binding consensus sequences (57) (Fig. 1B) in addition to a highly basic region (residues 110–143) proposed to be the principal heparin binding domain in FGF-BP (58).

To establish the nature of FGF-BP cDNA, we cloned FGF-BP into the expression vector pcDNA3.1 and used in vitro transcription/translation to determine its molecular mass. The results showed a single band of the predicted size of ~23 kDa (Fig. 1C, lane 2) in contrast to the empty vector (Fig. 1C, lane 1), which showed no detectable bands. The positive control was provided by the luciferase cDNA, which produced the expected ~66 kDa protein (Fig. 1C, lane 3).

Nature of FGF-BP—FGF-BP is a secreted, heparin-binding protein originally purified from media conditioned by A431 human squamous carcinoma cells (51). Notably, FGF-BP is localized to squamous epithelia (51) and to squamous cell carcinomas (59). FGF-BP binds FGF1 and FGF2 in a noncovalent reversible manner (51) and potentiates the activity of FGF7 (59), similar to the action of perlecan (49). In addition, A431 squamous carcinoma cells transfected with FGF-BP become more tumorigenic than wild type cells, and nontumorigenic A431–4 subclones, which do not express FGF-BP, become tumorigenic upon de novo expression of FGF-BP (51). Ectopic expression of FGF-BP in adrenal adenocarcinoma cells causes a release of FGF2 and formation of highly vascularized tumors.
FIG. 7. FGF-BP expression is elevated in dysplastic skin and codistributes with perlecan in the pericellular environment of squamous cell carcinomas. A, dysplastic skin, negative control (no primary antibody). B, dysplastic skin overlaying an invasive squamous cell carcinoma of the tongue. Notice the marked staining with anti-FGF-BP antibody of the expanded stratum spinosum. C, skin stained with anti-perlecan antibody. Notice the marked staining of the basement membrane (arrows) and of the upper dermis and blood vessels. D, squamous cell carcinoma of the esophagus, negative control (no primary antibody). E, parallel section stained with anti-FGF-BP. Notice the intense staining of the invasive cancer cells (Ca). F, dysplastic skin stained with anti-perlecan antibody. Notice the marked staining of the basement membrane (arrows) and of the upper dermis and blood vessels. G, invasive squamous cell carcinoma of the esophagus reacted with anti-FGF-BP antibody. H, parallel section stained with anti-perlecan antibody. Notice a distribution within the invasive cancer (Ca) nearly identical to that of FGF-BP. I, a different case of squamous cell carcinoma stained with anti-perlecan antibody showing similar pericellular distribution. A keratin pearl (Kp), nearly identical to that of FGF-BP, was observed by the positive control, further suggesting that the affinity between domain III and FGF-BP is relatively high. Preliminary solid phase binding experiments using purified FGF-BP and soluble domain III-AP demonstrated a saturable and high affinity ($K_d \approx 18 \text{ nM}$) binding (not shown).

FGF-BP Binds Specifically to Perlecan’s Domain III—To further verify that FGF-BP was a true interactive protein, we cloned the full-length FGF-BP cDNA into plasmids carrying both the binding (pGB) and activating (pGAD) domains and adopted them as either prey or bait with perlecan domain III using a different yeast host strain (SFY526). As positive and negative controls, we utilized the pTD1/pVA3 and pTD1/pLAM5’ plasmids, respectively (CLONTECH). The results showed a robust growth in triple minus media of clones coexpressing FGF-BP/perlecan domain III proteins, expressed as either bait or prey, with growth rates comparable with the positive control (Fig. 2A). To further prove this interaction, we performed $\beta$-galactosidase assays on the cotransfectants. In addition to growth in triple minus media, transcription of lacZ containing the upstream binding sites of GAL4 and the subsequent ability of cotransformant yeast strains to express functional $\beta$-galactosidase are additional strong proofs for a true protein-protein interaction (63, 64). Domain III, present as either bait or prey, showed a robust production of blue colonies (Fig. 2B), comparable in intensity with the positive control pTD1/pVA3 harboring the p53 and the SV40 T antigen (Fig. 2C). This suggests that the affinity between domain III and FGF-BP is relatively high. Preliminary solid phase binding experiments using purified FGF-BP and soluble domain III-AP demonstrated a saturable and high affinity ($K_d \approx 18 \text{ nM}$) binding (not shown).
FGF-BP expression is blocked by retinoic acid but highly induced by transformation and EGF. Released FGF-BP mobilizes FGF2 and FGF7 bound, in a presumably inactive status, to the heparan sulfate chains of various proteoglycans. In addition, FGF7 and FGF-BP can interact with the protein core of perlecan thereby providing an additional level of control. The liberated (active) FGFs can thus mediate various functions such as induction of angiogenesis and tumorigenesis. The model is modified from the work of Rak and Kerbel (75).

Coimmunoprecipitation of FGF-BP and Domain III—To verify that the interaction detected using the yeast two-hybrid system could occur outside the yeast, and to show its relevance within the context of secreted proteins from mammalian cells, we studied the interaction of secreted FGF-BP and recombinant domain III. As mentioned above, FGF-BP was originally isolated from media conditioned by A431 squamous carcinoma cells (51). To investigate the potential interaction in solution between perlecan and FGF-BP, we generated stable transfec
tant clones of A431 secreting the full-length domain III fused to the human placental AP to serve as a marker. The generation of domain III-AP fusion protein would avoid the potential interference of the heparan sulfate chains, because this module has been shown to be synthesized without glycosaminoglycan substitution (66), and would facilitate a direct protein-protein interaction. Several clones were isolated, and two (clones 1 and 11) were synthesized and released into the medium of the A431 cells, the fusion protein of the correctly predicted mass of ~190 kDa. This protein was recognized by anti-domain III monoclonal antibodies (Fig. 4A, top panel) and by an antibody against alkaline phosphatase (not shown). Interestingly, FGF-BP was detected at high levels in these cells (Fig. 4A, bottom panel) and migrated as a ~23 kDa protein under reducing conditions, to a position identical to that obtained with in vitro transcription/translation (cf. Fig. 1C). Proper folding of domain III-AP fusion protein was verified by detection of strong AP activity in clones 1 and 11 (Fig. 4B). This assumption is based on the fact that the placental AP is positioned C-terminally, and thus we presume that domain III is also properly folded because the fusion protein expressed high enzymatic activity. In addition, domain III folds into an individual entity, as determined by rotary shadowed electron microscopy and biophysical studies (66, 67). Immunoprecipitation studies using anti-FGF-BP or anti-domain III monoclonal antibodies followed by Western immunoblotting showed the coimmunoprecipitation of domain III and FGF-BP (Fig. 4C).

We further confirmed the interaction identified in the two-hybrid screen system by coimmunoprecipitation of domain III and FGF-BP in transiently transfected kidney COS-1 (African green monkey) cells, which do not express FGF-BP. The media conditioned by COS-1 cells cotransfected with domain III and FGF-BP cDNAs showed that both proteins interacted in solution and could be identified by their respective antibodies (Fig. 5). Collectively, these data indicate that FGF-BP is a binding partner for perlecan domain III and that FGF-BP can bind perlecan protein core in solution.

FGF-BP Is Increased in Squamous Cell Carcinomas and Codistributes with Perlecan—To elucidate FGF-BP distribution in human tissues, we tested a number of normal and neoplastic frozen human tissues with the monoclonal antibody directed toward FGF-BP. In adult normal skin, FGF-BP was expressed at relatively low levels in the suprabasal region of the epidermis and focally in hair follicles (Fig. 6B). Interestingly, in a few cases of squamous epithelia, FGF-BP epitopes were clearly present along the basement membrane at the dermo-epidermal junction (Fig. 6C), occasionally extending into the basement membrane of dermal blood vessels (Fig. 6D). This is similar to the distribution of human perlecan using anti-domain III antibodies (15). All of the other normal tissues, including lung, brain cortex, kidney, uterus, breast, colon, and various fibro-adipose tissues, were essentially negative. In contrast, invasive squamous cell carcinoma showed a marked induction of FGF-BP, especially around the most undifferentiated cells (Fig. 6E). Notably, even at relatively high concentrations of the primary antibody (1:200) there was no detectable staining in the normal tissues and the nonsquamous cell carcinomas tested (see below), in contrast to skin and squamous cell carcinomas where the antibody had to be diluted significantly (1:1000) to achieve optimal staining. Overall, these data are in good agreement with in situ hybridization studies in the mouse, which have shown that FGF-BP expression starts at embryonic day 9 reaches its peak perinatally and is subsequently down-regulated during adult life (54). In concert with our findings, FGF-BP mRNA expression is dramatically increased upon induction of mouse skin papillomas and carcinomas (54).

We then investigated the expression of FGF-BP vis-à-vis that of perlecan in dysplastic skin and various tumors, including
Perlecan-FGF-BP Interaction

carcinomas of the lung, uterus, bladder, kidney, and squamous cell carcinomas of the esophagus, skin, tongue, pharynx, lung, and penis. A total of 38 samples were investigated. In dysplastic skin overlaying an infiltrating squamous cell carcinoma of the tongue, FGF-BP staining was markedly induced (Fig. 7B). Perlecan distribution was primarily along the basement membrane and blood vessels of the upper dermis (Fig. 7, C and F) in agreement with previous studies (15, 68). Again, there was a marked expression of FGF-BP in the invasive squamous cell carcinoma cells of the esophagus (Fig. 7E), and consecutive sections showed a significant codistribution of FGF-BP (Fig. 7G) and perlecan (Fig. 7, H and I). Keratin pearls, considered to represent a sign of cellular differentiation, did not contain either FGF-BP or perlecan epitopes (Figs. 6E and 7I). No significant FGF-BP immunoreactivity was observed in all the other nonsquamous carcinoma tumors (not shown) with the exception of focal positivity in colon and lung carcinomas. The latter finding is in agreement with the reports that FGF-BP is expressed by some colon carcinoma cell lines (52) and that FGF-BP transcript can be detected in the developing mouse intestine and lung (54).

Overall, our data indicate that under normal conditions, the distributions of FGF-BP and perlecan overlap only focally. The former is located in the suprabasal layer (stratum spinosum) of the epidermis, whereas the latter is located primarily in the dermis and the basement membrane zone at the dermo-epidermal junction and along the vasculature. When the squamous epithelium becomes transformed, regardless of its site of origin, FGF-BP is deposited in the pericellular space in close proximity to the cell surface of the most aggressive squamous carcinoma cells. This distribution clearly overlaps with that of perlecan, suggesting a potential in vivo interaction between these two proteins.

Conclusions—Because of its eukaryotic nature, the yeast two-hybrid system has been widely used to study protein-protein interactions, primarily those occurring among intracellular proteins (69). Only recently, however, has it been successfully used to investigate interactions among extracellular proteins such as those involving collagen types VI and IV (70), thrombospondin (71), EMILIN (72), or matrix metalloproteinase 2 (73). Binding sites for FGF7 and platelet-derived growth factor-α and -β have been identified in subdomains III-1 (50) and III-2 (74), indicating that there are unique binding specificities for perlecan modules containing highly repetitive sequences. The results of this study indicate that the second EGF-like repeat (LE2) of domain III-1 interacts specifically with the protein core, FGF7 and FGF-BP would become available to the surrounding cellular environment and could behave as a promotor of growth and differentiation. An alternate pathway would involve an FGF-BP-mediated displacement of FGF2 and FGF7 stored in the cell surface heparan sulfate proteoglycans. This pathway does not require the need of active proteolysis or glycolytic enzymes and could be operational at the site of tumor growth. According to this working model (Fig. 8), overproduction of FGF-BP in malignant cells or induction by EGF would liberate FGF-BP in the microenvironment where it would displace FGF2 or FGF7 bound to heparan sulfate chains of syndecan, glypicain, or perlecan proteoglycans. In addition, FGF-BP could interact and displace FGF7 bound to perlecan’s domain III, without the need of proteolytic activity. Various FGFs could then be released into the microenvironment where they would stimulate angiogenesis and tumorigenesis. Hence, perlecan would play a central role not only as molecular storage of growth factors but also as repository for FGF-BP, and possibly related proteins, that would act as angiogenic modulators.

Acknowledgments—We thank I. Eichstetter for excellent technical assistance, C. Munsey for help with the immunohistochemistry, and Jim San Antonio for helpful advice.

REFERENCES

1. Lindahl, U., Kusche-Gullberg, M., and Kjellén, L. (1998) J. Biol. Chem. 273, 24979–24982
2. Perrinon, N., and Bernfield, M. (2000) Nature 404, 725–728
3. Bernfield, M., Götte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Lincecum, J., and Zako, M. (1999) Annu. Rev. Biochem. 68, 799–777
4. Lander, A. D., and Selleck, S. B. (2000) J. Cell Biol. 148, 227–232
5. Selleck, S. B. (2000) Trends Genet. 16, 206–212
6. Noonan, D. M., and Hassell, J. R. (1993) Kidney Int. 43, 53–60
7. Dunlevy, J. R., and Hassell, J. R. (2000) in Proteoglycans: Structure, Biology and Molecular Interactions (Iozzo, R. V., ed) pp. 275–326, Marcel Dekker, Inc., New York
8. Noonan, D. M., Fulle, A., Valente, P., Cai, S., Horigan, E., Sasaki, M., Yamada, Y., and Hassell, J. R. (1991) J. Biol. Chem. 266, 22839–22847
9. Murdoch, A. D., Dodge, G. R., Cohen, I. T., Yuan, R. S., and Iozzo, R. V. (1992) J. Biol. Chem. 267, 8544–8557
10. Kallunki, P., and Tryggvason, K. (1992) J. Cell Biol. 116, 559–571
11. Rogalski, T. M., Williams, B. D., Mullen, G. P., and Meerman, D. G. (1993) Genes Dev. 7, 1471–1484
12. Hofp, M., Göhring, W., Kohfeldt, E., Yamada, Y., and Timpl, R. (1999) Eur. J. Biochem. 259, 917–925
13. Iozzo, R. V., Cohen, I. R., Grassel, S., and Murdoch, A. D. (1994) Biochem. J. 302, 625–639
14. Iozzo, R. V. (1999) Annu. Rev. Biochem. 67, 609–652
15. Murdoch, A. D., Liu, B., Schwarting, R., Tuan, R. S., and Iozzo, R. V. (1994) J. Biol. Chem. 269, 22939–22947
16. Reinhardt, D., Mann, K., Nischt, R., Fox, J. W., Chu, M.-L., Krieg, T., and Fox, J. W. (1997) J. Biol. Chem. 272, 9955–9962
17. Heremans, A., De Cock, B., Cassiman, J. J., Van Den Berghe, H., and David, G. W. (1987) J. Biol. Chem. 262, 17669–17676
18. Yurchenco, P. D., Cheng, Y.-S., and Ruben, G. C. (1987) J. Biol. Chem. 262, 8544–8553
19. Yurchenco, P. D., and Schittny, J. (1990) J. Cell. Biochem. 42, 239–249
20. Yurchenco, P. D., and Schittny, J. (1990) J. Cell. Biochem. 42, 239–249
21. Isemura, M., Sato, N., Yamaguchi, Y., Aikawa, J., Munakata, H., Hayashi, N., Yosizawa, Z., Nakamura, T., Kubota, A., Arakawa, M., and Hsu, C.-C. (1991) J. Biol. Chem. 266, 10881–10887
22. Handler, M., Yurchenco, P. D., and Iozzo, R. V. (1997) J. Biol. Chem. 272, 6595–6602
23. Villar, M. J., Hassell, J. R., and Brandan, E. (1999) J. Cell. Biochem. 75, 130–145
24. Peng, H. B., Xie, H., Rossi, S. G., and Rotundo, R. L. (1999) J. Cell Biol. 145, 911–921
25. Kaki, I., Iozzo, R. V., and Williams, K. J. (2000) J. Biol. Chem. 275, 25742–25750
26. Handler, M., Yurchenco, P. D., and Iozzo, R. V. (1997) Dev. Dyn. 209, 130–145
27. Couchman, J. R., Kapoor, R., Shihaman, M., and Wu, R.-R. (1996) J. Biol. Chem. 271, 9955–9962
28. SundarNaj, F., Fite, D., Liedeberger, S., Chakravarti, S., and Hassell, J. R. (1995) J. Cell Sci. 108, 2663–2672
29. Addicks, K., Timpl, R., and Faßler, R. (1999) J. Biol. Chem. 274, 655–674
30. Hayashi, K., Madri, J. A., and Yurchenco, P. D. (1992) J. Biol. Chem. 267, 8716–8724
31. Battaglia, C., Aumailley, M., Mann, K., and Timpl, R. (1999) J. Biol. Chem. 274, 945–959
32. Battaglia, C., Aumailley, M., Mann, K., Meyer, U., and Timpl, R. (1999) J. Cell Sci. 112, 99–112
33. Arikawa-Hirasawa, E., Watanabe, E., Takami, H., Hassell, J. R., and Yamada, Y. (1999) Nat. Genet. 23, 354–358
34. Costello, M., Gustafsson, E., Azozdi, A., Morgelin, M., Bloch, W., Hunziker, E., Addicks, K., Timpl, R., and Faßler, R. (1999) J. Cell Biol. 147, 1169–1172
Fibroblast Growth Factor-binding Protein Is a Novel Partner for Perlecan Protein Core
Maurizio Mongiat, Juliet Otto, Rachel Oldershaw, Felix Ferrer, J. Denry Sato and Renato V. Iozzo

J. Biol. Chem. 2001, 276:10263-10271. doi: 10.1074/jbc.M011493200 originally published online January 8, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011493200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 74 references, 39 of which can be accessed free at http://www.jbc.org/content/276/13/10263.full.html#ref-list-1