High-affinity Binding of Basic Fibroblast Growth Factor and Platelet-derived Growth Factor-AA to the Core Protein of the NG2 Proteoglycan*

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NG2 is a transmembrane chondroitin sulfate proteoglycan that is expressed by immature progenitor cells in several developmental lineages and by some types of malignant cells. In vitro studies have suggested that NG2 participates in growth factor activation of the platelet-derived growth factor-α receptor. In this study the ability of recombinant NG2 core protein to interact with several different growth factors (epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF)-AA, PDGF-BB, vascular endothelial growth factor (VEGF)165 and transforming growth factor (TGF)-β1) was investigated using two different assay systems: enzyme-linked immunosorbent assay-type solid-phase binding and an optical biosensor (BIAcore) system. High-affinity binding of bFGF and PDGF-AA to the core protein of NG2 could be demonstrated with both types of assays. Using both the BIAcore software analysis program and nonlinear regression analysis of the solid phase binding data, K_D values in the low nanomolar range were obtained for binding of each of these growth factors to NG2. The results further indicate that NG2 contains at least two binding sites for each of these two growth factors. PDGF-BB, TGF-β1, VEGF, and EGF exhibited little or no binding to NG2 in either type of assay. These data suggest that NG2 can have an important role in organizing and presenting some types of mitogenic growth factors at the cell surface.

Due to their structural complexity, proteoglycans are highly interactive macromolecules that participate in a broad range of cell-cell and cell-matrix interactions, including regulation of cell adhesion, proliferation, motility, and differentiation (Refs. 1–3; for review, see Ref. 1). Proteoglycans are important mediators of growth factor binding. Several examples illustrate how proteoglycans can function both as extracellular reservoirs for growth factors and as facilitators of growth factor binding to signal transducing receptors on the cell surface (4–6).

Most research has focused on the interaction of growth factors with the glycosaminoglycan (GAG)1 chains of proteoglycans. bFGF (FGF-2) binds tightly to the heparan sulfate chains of extracellular proteoglycans, as well as to free heparin (7, 8). Since the bound growth factor is resistant to degradation by extracellular proteases, the complex serves as a reservoir of matrix-bound FGF. Active FGF is released by proteolysis of the proteoglycan core protein or by partial degradation of the heparan sulfate chains, processes that occur during tissue development and remodeling (9, 10).

bFGF also binds to cell-surface heparan sulfate proteoglycans such as syndecan, which present the bound FGF to its signaling receptor, a receptor tyrosine kinase whose activation leads to induction of a variety of cellular processes, including proliferation. Binding of bFGF has also been demonstrated to the core protein of the chondroitin sulfate proteoglycan phosphacan (11). TGF-β is another growth factor that can bind to proteoglycan core proteins. For example, betaglycan (also called the type III TGF-β1 receptor) is a transmembrane proteoglycan with multiple binding sites for TGF-β1 (12). Upon binding to betaglycan, TGF-β1 is presented to types I and II receptors, which are serine/threonine kinases that activate intracellular signaling cascades.

Results from our laboratory have suggested that the integral membrane chondroitin sulfate proteoglycan NG2 is another example of a proteoglycan that can influence growth factor activity. NG2 is found on the surface of several different types of immature progenitor cells, including oligodendrocyte progenitors, chondroblasts, and smooth muscle cells (13–17). Some types of neoplasms, such as melanomas, glioblastomas, osteosarcomas, chondrosarcomas, and lymphomas also express NG2 (18–21). NG2 thus appears to be a developmental marker that is expressed at high levels on mitotic cells but is down-regulated during terminal differentiation. Developmental studies in the central nervous system have shown that there is a close relationship between the expression of NG2 and PDGF-α receptor on oligodendrocyte progenitors (15). Moreover, antibodies against NG2 are capable of blocking the mitogenic effects of PDGF-AA on both oligodendrocyte progenitors and vascular smooth muscle cells, suggesting that NG2 is involved in some way in the operation of the PDGF-AA/PDGF-α receptor pathway (16, 17). Recently, direct comparisons of aortic smooth muscle cells from wild type and NG2 null mice have shown that the NG2-negative cells fail to respond to PDGF-AA in both proliferation and migration assays and that this lack of activity stems from failure of the PDGF-α receptor to be activated in the presence of PDGF-AA (22).

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1 The abbreviations used are: GAG, glycosaminoglycan; bFGF, basic fibroblast growth factor; BSA, bovine serum albumin; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; SPR, surface plasmon resonance; TGF-β1, transforming growth factor-β1; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

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In order to investigate the possibility that NG2 is involved in binding and presentation of PDGF-AA, we have studied the ability of NG2 to bind to a variety of different growth factors. Our laboratory has produced and purified several recombinant forms of NG2, including the entire 270-kDa extracellular domain (both with and without GAG chains) and several well-defined fragments of this ectodomain. Use of these species has allowed us to determine both the binding specificity and affinity of NG2 for growth factors. Here we report that the NG2 core protein binds with high affinity to PDGF-AA and bFGF (FGF-2). In addition we show that the large ectodomain of NG2 appears to contain at least two binding sites for each of these growth factors. These findings provide a molecular basis for understanding the role of NG2 in PDGF-α receptor activation and suggest that similar effects may be found for FGF receptors.

**EXPERIMENTAL PROCEDURES**

**Antibodies**

Goat polyclonal antibodies against human bFGF, EGF, PDGF-AA, PDGF-BB, TGF-β1, and VEGF were purchased from R&D Systems (Minneapolis, MN). Rabbit polyclonal antibodies against rat NG2 were affinity-purified from crude antisera using a matrix of recombinant NG2 fragments NG2/EC or NG2/D3 coupled to Sepharose CL-4B. Rabbit anti-goat IgG conjugated to horseradish peroxidase (HRP) was obtained from Calbiochem. Peroxidase-labeled goat anti-rabbit IgG was purchased from Bio-Rad.

**Purified Proteins**

Expression and structural features of the recombinant NG2 fragments have been previously described (23). Four different NG2 fragments were used in our experiments: NG2/EC (extracellular) without GAG chains, comprising the whole extracellular domain (residues 1–2223); NG2/EC with GAG chains; D2 (domain 2, residues 632–1450) and D3 (domain 3, residues 1585–2218). The D2 preparation used for our binding studies also contains GAG chains, while the D3 preparation does not. Recombinant human bFGF, EGF, PDGF-AA (long isofrom), PDGF-BB, TGF-β1, and VEGF (long isofrom) were purchased from R&D Systems.

**Reagents**

Research grade CMS SensorChips (carboxymethylated dextran matrix), amine-coupling kit (N-ethyl-N′-(dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide), and HBS buffer (10 mM Hepes with 0.15 M NaCl, 3.4 mM EDTA, and 0.005% surfactant P20 at pH 7.4) were all obtained from Pharmacia Biosensor AB (Uppsala, Sweden). Turbo TMB (3,3′,5,5′-tetramethylbenzidine) was purchased from Pierce.

**Surface Plasmon Resonance (SPR)**

A BIACore 2000 surface plasmon resonance-based biosensor (Pharmacia Biosensor AB) was used to measure kinetic parameters for the interaction between soluble NG2 fragments (analytes) and immobilized growth factors (ligands). Growth factors were immobilized to the sensor chip surface by the amine-coupling method (24) according to the manufacturer’s suggestion. Each ligand was immobilized at a concentration of approximately 1500 resonance units (1.5 ng/mm²). The reverse design, immobilizing NG2 and injection of growth factors as soluble analytes, could not be utilized due to high nonspecific binding of the basic growth factor proteins to the unmodified chip surface.

For all kinetic measurements, we used a flow path involving all four flow cells of the BIACore 2000. Simultaneous measurements were obtained from one flow cell containing the growth factor-coated sensor chip and a second flow cell containing an un derivatized chip. Parallel measurements of specific and background binding were thus obtained. The other two flow cells were not monitored. Solutions of NG2 fragments were injected into the flow cells using the KINJECT command specifying a 40-μl analyte volume and a 90-s dissolution time. Each assay cycle was performed with a constant flow of HBS at 20 μl/min. Between cycles, the immobilized ligands were regenerated by injecting 20 μl of 1 M NaCl and activating the EXTRACLEAN command. For each immobilized growth factor a complete set of sensorgrams was recorded at three different analyte concentrations in the range between 100 and 3,500 nM. This set of sensorgrams was analyzed using the BIACalculator version 3.0 software. To prepare the data for analysis, baselines were adjusted to zero for all curves, and injection start times were aligned. Background sensorgrams were then subtracted from the experimental sensorgrams to yield curves representing specific binding.

The association and dissociation phases of the sensorgrams were fit simultaneously, assuming a simple bimolecular reaction model: A + B → AB. The analysis software allowed us to determine both the binding specificity and affinity of NG2 for growth factors. Here we report that the NG2 core protein binds with high affinity to PDGF-AA and bFGF (FGF-2). In addition we show that the large ectodomain of NG2 appears to contain at least two binding sites for each of these growth factors. These findings provide a molecular basis for understanding the role of NG2 in PDGF-α receptor activation and suggest that similar effects may be found for FGF receptors.

**Solid-Phase Binding Assays**

**Growth Factor Binding to Immobilized NG2—Enzyme-linked immunosorbent assays were used to evaluate the binding of growth factors to various NG2 fragments coated into microtiter wells, 96-well microtiter plates (Greiner, Nuertingen, Germany) were coated overnight at 4 °C with proteins at 3 μg/ml in 100 μl of coating buffer (50 mM sodium carbonate, pH 9.6). After washing twice with PBST (10 mM phosphate buffer, pH 7.4, containing 2.7 mM KC1, 137 mM NaCl and 0.05% Tween 20), residual protein binding sites in the wells were saturated by incubating for 1 h at room temperature with 200 μl of blocking solution (PBS, 1% BSA). Growth factor samples diluted in PBS, 1% BSA were then incubated in the wells either at 4 °C overnight or for 2 h at room temperature. Both incubation protocols yielded similar results. After four washes with PBST, 100 μl of the growth factor-specific detection antibody (goat polyclonal antibody/2 μg/ml in PBS, 1% BSA) was added and incubated for 1 h at room temperature. Wells were then washed and bound HRP was detected by addition of 100 μl of TMB as a peroxidase substrate.

The reaction was terminated after 10 min by addition of 50 μl of 0.5 M H₂SO₄. The absorbance of the yellow reaction product was then measured at 450 nm on a Titertek microtiter plate reader.

**NG2 Binding to Immobilized Growth Factors—**Wells were coated with the growth factor of interest (NG2 fragments were expressed in forms of NG2, including the entire 270-kDa extracellular domain and the subdomains D2 and D3. PDGF-AA and bFGF bound strongly to each of these NG2 species in a concentration-dependent manner (Fig. 1, A and B). In each case the levels of binding to BSA-coated wells were quite low. Nonlinear regression analysis yielded apparent dissociation constants (K_d) between 10 and 20 nM for bFGF and K_d values between 10 and 20 nM for PDGF-AA (Table I). bFGF has a somewhat higher affinity for NG2/EC with GAG chains than for NG2/EC without GAG chains, whereas PDGF-AA binds slightly better to the species without GAG chains.

To further address the specificity of the interaction between...
bFGF or PDGF-AA and NG2, we asked whether the binding of these growth factors to immobilized NG2 could be blocked by increasing concentrations of soluble NG2. Fig. 2 shows that soluble D2 and D3 inhibited the binding of either bFGF or PDGF-AA to the respective NG2 subdomains in a concentration-dependent fashion. A 300-fold molar excess of soluble D2 or D3 almost completely blocked the respective interaction with bFGF or PDGF-AA.

Neither EGF nor VEGF exhibited binding to any of the NG2 fragments that was significantly above the low background levels observed with BSA-coated wells, whereas PDGF-BB and TGF-β1 bound strongly to both NG2-coated wells and BSA-coated wells (data not shown). Although the binding of these two growth factors to NG2-coated wells often appeared to be slightly higher than that to BSA-coated wells, the extremely high background makes it difficult to draw definitive conclusions from these results.

**TABLE I**

Solid-phase binding data

Dissociation constants (apparent $K_D$ values) for the interaction between plastic-immobilized NG2 fragments and bFGF or PDGF-AA were calculated by nonlinear regression analysis.

|       | D2  | D3  | EC with GAG chains | EC without GAG chains |
|-------|-----|-----|--------------------|-----------------------|
| bFGF  | 9.8 | 6.2 | 5.2 ± 0.5          | 10.5 ± 3.4            |
| PDGF-AA | 13.2 | 9.3 | 15.3 ± 4.9         | 10.0 ± 3.5            |

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**Binding of NG2 to Immobilized Growth Factors**—As an additional assessment of the specificity of growth factor binding to NG2, we examined the interactions in assays in which the roles of the binding partners were reversed: i.e. the growth factors were immobilized in plastic wells, and the coated wells were incubated with soluble NG2. Fig. 3 shows that when bFGF or PDGF-AA-coated wells were incubated with NG2/EC without GAGs or with NG2/D3 (inset of Fig. 3) at two different concentrations, each of these NG2 fragments bound effectively to both growth factors. Little background binding of NG2 species was observed to BSA-coated wells.

This reversed assay also provided an opportunity to re-examine the interaction of NG2 with PDGF-BB and TGF-β1. As shown in Fig. 3, NG2/EC exhibited no significant binding to plastic-immobilized PDGF-BB or TGF-β1.

**SPR Measurements of NG2/Growth Factor Interaction**

Initial experiments indicated that injecting bFGF or PDGF-AA over a blank sensor chip yielded a high nonspecific binding response, probably due to electrostatic interaction of the cationic growth factors with the negatively charged carboxymethyl dextran layer on the chip surface. In contrast, injection of NG2 fragments onto surfaces coated with bFGF or PDGF-AA had good signal to noise ratios and exhibited concentration-dependent increases in both the rate and extent of binding. Bound NG2 could be eluted by injection of 1M NaCl, suggesting that ionic interactions play an important part in the association of NG2 with these growth factors. Curve fitting of the sensorgrams yielded on and off rates and apparent dissociation constants as presented in Table II. These values represent the mean of triplicate measurements made on the same sensor chip. The $K_D$ values obtained from SPR measurements are in good agreement with those obtained from solid-phase assays (Table I).

BIACore analysis was also used to examine the interaction of soluble NG2 species with sensor chips coated with EGF, VEGF, PDGF-BB, and TGF-β1. In agreement with the results of the ELISA-type binding assays, none of the four NG2 fragments exhibited significant affinity for this set of growth factors. The inset of the NG2/EC− sensorgram in Fig. 5 shows an example of sensorgrams obtained for injection of different concentrations of NG2/EC without GAGs over a PDGF-BB-coated chip. The blank chip surface responses are subtracted. Injection of the other NG2 fragments over immobilized PDGF-BB also failed to yield positive signals (sensorgrams not shown).
DISCUSSION

Several pieces of information obtained previously in our laboratory have suggested that NG2 plays an important role in cellular responsiveness to PDGF-AA. After noting the close co-localization of NG2 and PDGF-α receptor on oligodendrocyte progenitor cells during neural development (15), we found that antibodies against NG2 could block PDGF-induced proliferation of oligodendrocyte progenitors in vitro (16). Extension of this work to rat aortic smooth muscle cells showed that these antibodies blocked both proliferation and migration in response to PDGF-AA (17). Responses to PDGF-BB were unaffected by the antibodies, specifically implicating NG2 in PDGF-AA-mediated activation of the α-receptor. Recent work with aortic smooth muscle cells from the NG2 knockout mouse has allowed us to confirm these findings independent of the use of antibodies. While smooth muscle cells from both wild type and NG2 knockout mice are able to proliferate and migrate in equivalent fashion in response to PDGF-BB, only the wild type cells are able to respond to PDGF-αA (22).

More detailed analysis of growth factor-activated signaling pathways in the wild type and knockout cells showed that while PDGF-AA did not activate the MAP kinase pathway in knockout cells, the pathway was still fully operative in response to other effectors such as PDGF-BB and PMA. This suggested that the absence of NG2 probably affected a mechanism upstream of the MAP kinase pathway. In support of this idea we found that in NG2 null cells PDGF-AA was unable to induce autophosphorylation, and thus activation, of the PDGF-α receptor. We hypothesized that NG2 could affect α-receptor activation in one of two ways: 1) by participating in growth factor binding and/or presentation to the signaling receptor or 2) by interacting with the α-receptor in such a way as to facilitate either growth factor binding or growth factor-mediated changes in receptor structure (such as dimerization).

While the findings presented in this report do not address the possibilities suggested by the second model, they nevertheless demonstrate a clear role for NG2 in the binding of two growth factors, PDGF-AA and bFGF, as suggested in model 1 above. The specificity of these interactions is emphasized by the failure of NG2 to bind several other growth factors, including PDGF-BB, TGF-β1, or BSA at a concentration of 2 μg/ml. Bound NG2 fragments were detected with NG2/EC-specific or NG2/D3-specific polyclonal antibodies. The data represent the mean ± S.D. of duplicate values from four separate experiments.

The pattern and specificity of growth factor binding to NG2 was confirmed using two separate experimental systems, a solid-phase ELISA-type assay and an optical biosensor assay. In addition to demonstrating that NG2 binds to a limited spectrum of growth factors, the two types of assays also yielded essentially identical values for the binding affinity of PDGF-AA and bFGF to NG2. In some cases the apparent dissociation constants obtained from surface plasmon resonance measurements are approximately 2–4-fold higher than the corresponding values obtained from solid-phase assays. Presumably these
differences are due to differences in the nature of the two assays. SPR measures the interactions directly in real time, while the solid-phase assays are lengthy and require the use of antibodies for indirect detection of the interaction. Also, differences between the electrostatic environments of the plastic wells and dextran-coated sensor chips may cause changes in the detailed conformation of adsorbed proteins, resulting in slight differences in ligand binding properties on the two sets of surfaces. Still, the two sets of values are in good agreement, and both sets reflect the same types of trends in the binding data. For example, in both sets of data bFGF displays a higher affinity for NG2/EC with GAG chains than for the same fragment without GAG chains. PDGF-AA, on the other hand, appears to bind slightly better in both assays to NG2 without GAG chains.

These latter observations may be indicative of subtle differences in the mechanism of binding of the two growth factors to NG2. It is known that negatively charged heparan sulfate chains are critical for the binding of bFGF to heparan sulfate proteoglycans, so interaction of bFGF with chondroitin sulfate chains may also contribute to the binding of bFGF to NG2. This interaction may be responsible for the very large plasmon resonance response of bFGF-coated chips exposed to soluble NG2 containing chondroitin sulfate chains (Fig. 4). While the binding affinities of NG2/EC+ and NG2/EC− differ by a factor of two in these experiments (Table II), the capacity of NG2/EC+ for binding of bFGF appears to be 5–6-fold greater than that of NG2/EC−. A similar, although much smaller trend can be seen in the ELISA-type binding assays (Fig. 1A). Whether electrostatic interactions between bFGF and the negatively charged chondroitin sulfate chains of NG2 are somehow magnified in the plasmon resonance experiments, and whether the large bFGF binding capacity of NG2 with chondroitin sulfate chains is preserved in a biological environment, are questions that remain to be resolved.

This large contribution of chondroitin sulfate is not seen in the case of PDGF-AA, even though both bFGF and PDGF-AA are basic proteins with isoelectric points between 8.5 and 9.5 (25–29). In fact, it is apparent that the major burden of binding both of these growth factors is borne by the NG2 core protein, independent of the presence of chondroitin sulfate chains. This therefore represents a major difference between the modes of action of NG2 and heparan sulfate proteoglycans and is reminiscent of the ability of the phosphacan core protein to interact with bFGF (11).

Even though their interaction with GAG chains may not be critical for binding of bFGF and PDGF-AA to NG2, the cationic nature of both growth factors is likely to be important for their interaction with the NG2 core protein. This is suggested by the sensitivity of both interactions to high ionic strength. While hydrophobic moieties are often thought to provide the bulk of free energy for binding between proteins and to help slow dissociation of the complex, hydrophilic interactions between polar residues are more likely to determine the specificity of protein-protein interactions and to increase the rate of association (30, 31). The core protein of NG2 has a net negative charge at physiologic pH (pI 5–6) and contains numerous clusters of acidic residues (32). In view of these multiple clusters of negative charges, it may be hypothesized that ligands that bind to NG2 would have properly positioned clusters of basic amino acids. The human PDGF-A chain contains two highly basic amino acid sequences, Arg-Lys-Lys-Pro-Lys (amino acids 73–77) and Lys-Lys-Arg-Lys-Arg-Lys-Arg (amino acids 114–120) (33). A somewhat less imposing sequence (Lys-Asp-Pro-Lys-...
Arg) is found in bFGF (28). Although PDGF-BB and VEGF are also highly basic proteins, having isoelectric points above 9 (34, 35), they fail to bind to NG2, underscoring the specificity of the interaction of NG2 with PDGF-AA and bFGF.

The above hypothesis might also predict the existence of multiple sites for interaction of NG2 with other molecules, depending on the number and positioning of charged clusters. Experimentally, we found that bFGF and PDGF-AA each could bind effectively to two distinct subdomains of NG2, the central domain 2 and the juxtamembrane domain 3, suggesting at least two separate sites of interaction for these growth factors. Consistent with this observation is the fact that domains 2 and 3 both have numerous clusters of acidic residues, including pairs and triplets of aspartic and glutamic acid (32). Multiple binding sites for PDGF isoforms have also been found in the large basement membrane proteoglycan perlecan (36), indicating that this phenomenon is not unique to NG2. The fact that our growth factor binding data can be fit most adequately by the use of a simple Langmuir adsorption isotherm suggests that the multiple binding sites on NG2 are noninteractive (i.e., not conformationally linked) and that they have similar binding affinities for the growth factors. This similarity in binding affinities can be observed in our comparisons of bFGF or PDGF-AA binding to the D2 and D3 fragments of NG2, each of which contains at least one binding site.

The high proportion of arginine and lysine residues in PDGF-AA and bFGF makes both proteins sensitive targets for proteolysis by plasmin and other proteases that cleave at basic amino acids. Thus the association of these growth factors with proteoglycans can be an important mechanism for protecting them from degradation (37–39). Information concerning such a role for NG2 remains to be obtained. It is of interest to note in this regard that, although NG2 is an integrated membrane protein, large soluble forms of the ectodomain are shed from the surface after proteolytic processing of the core protein (40). Therefore NG2 could be important not only for sequestering growth factors at the cell surface, but also in protecting them from degradation in the extracellular matrix and in body fluids. These possibilities will be addressed experimentally in future investigations.

Our current findings suggest that in addition to the previously observed effects of NG2 on PDGF-AA-mediated activation of the PDGF-α receptor, we might also expect to find similar effects of NG2 on bFGF-mediated receptor activation. PDGF-AA and bFGF are ubiquitous growth factors that have profound effects on proliferation, differentiation, and survival of cells from many different tissues and developmental lineages. NG2 is also widespread in a variety of developing tissues (13–17), thus providing a number of potential choices for study. One extremely interesting system involving NG2 and both of

**Fig. 5.** Analysis of PDGF-AA and PDGF-BB binding to NG2 fragments by surface plasmon resonance. Sensorgrams are shown for different concentrations of NG2/D2, NG2/D3, NG2/EC minus GAGs, and NG2/EC plus GAGs injected on immobilized PDGF-AA. The analysis and regeneration was performed as described in the legend to Fig. 4, and calculated dissociation constants are listed in Table II. The inset in the NG2/EC panel shows analysis of PDGF-BB interaction with NG2 by surface plasmon resonance. Sensorgrams were obtained from the injection of three different concentration of NG2/EC minus GAGs on immobilized PDGF-BB.

| Immobilized ligand | Soluble analyte | $k_a$ | $k_d$ | $K_D$ ($k_d/k_a$) |
|--------------------|----------------|-------|-------|------------------|
| bFGF               | D2             | $1.5 \times 10^5$ | $2.0 \times 10^{-4}$ | 12.7             |
| bFGF               | D3             | $3.9 \times 10^4$ | $1.7 \times 10^{-4}$ | 43.3             |
| bFGF               | EC+            | $2.9 \times 10^4$ | $1.9 \times 10^{-4}$ | 6.5              |
| bFGF               | EC−            | $1.5 \times 10^5$ | $2.0 \times 10^{-4}$ | 12.7             |
| PDGF-AA            | D2             | $8.5 \times 10^4$ | $2.2 \times 10^{-3}$ | 25.9             |
| PDGF-AA            | D3             | $2.5 \times 10^5$ | $5.7 \times 10^{-3}$ | 22.8             |
| PDGF-AA            | EC+            | $8.1 \times 10^4$ | $3.3 \times 10^{-3}$ | 40.1             |
| PDGF-AA            | EC−            | $3.3 \times 10^4$ | $1.0 \times 10^{-3}$ | 30.4             |
the relevant growth factors is that of developing oligodendrocyte progenitors. In the absence of bFGF and PDGF-AA, these progenitors differentiate through a series of intermediates into mature oligodendrocytes (41, 42). In contrast, the combination of bFGF and PDGF-AA, produced by neurons and astrocytes, delays this differentiation and promotes proliferation and expansion of the pool of progenitor cells (43–47). NG2 is present on the undifferentiated progenitors during the period in which they are sensitive to PDGF-AA and bFGF and therefore is in place to potentiate the effects of the growth factors (13, 15, and 16). NG2 is then down-regulated after progenitors progress past the pre-oligodendrocyte stage, at which time they have become insensitive to the effects of these two growth factors. This would appear to offer an excellent system for studying the functional importance of NG2 in events mediated by bFGF and PDGF-AA.

In summary, the current work shows that PDGF-AA and bFGF have the ability to bind directly and specifically to the NG2 proteoglycan. NG2 may thus be important for regulating the extracellular localization and levels of these two growth factors and possibly for presentation of these ligands to their respective signaling receptors. Further work will be required to ascertain the degree to which NG2 functions as a co-receptor for these growth factors and whether its involvement in receptor activation includes interaction with the receptor to induce structural transitions (such as dimerization) that facilitate signal transduction.

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