Schwann Cell-conditioned Medium Inhibits Angiogenesis

Donghui Huang, J. Lynn Rutkowski, Garrett M. Brodeur, Pauline M. Chou, Janet L. Kwiatkowski, Angela Babbo, and Susan L. Cohn

Abstract

Neuroblastomas are biologically heterogeneous tumors that consist of two main cell populations: neuroblastic/ganglionic cells and Schwann cells. The amount of Schwannian stroma strongly impacts prognosis, and favorable outcome is associated with tumors that are Schwannian stroma rich/stroma dominant. At the present time, there is controversy regarding the origin of Schwann cells in neuroblastoma tumors. However, recent studies have suggested that the Schwann cells in mature neuroblastoma tumors may be normal cells that produce soluble substances that enhance the survival and differentiation of neuroblastoma cell lines. Previously, we reported that in neuroblastoma, high vascular index correlated with clinically aggressive disease. As a first step toward investigating whether Schwann cells also play a role in inhibiting angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors. In contrast, tumors with favorable histology and abundant Schwannian stroma had low tumor vascularity. As a first step toward investigating whether Schwann cells also play a role in inhibiting angiogenesis in neuroblastoma tumors, we examined the ability of conditioned medium collected from normal human Schwann cells to inhibit angiogenesis in neuroblastoma tumors. In vitro angiogenesis assays were also performed with conditioned medium collected from Schwann cells derived from a Schwannian stroma-dominant neuroblastoma tumor. Our results indicate that Schwann cells derived from either adult nerve or tumor tissue produce a potent inhibitor(s) of angiogenesis. Expression studies revealed tissue inhibitor of metalloproteinase (TIMP)-2 in conditioned medium collected from both normal and tumor-derived Schwann cells. In addition, TIMP-2 was detected in the cytoplasm of Schwann cells and ganglion cells in stroma-rich/stroma-dominant neuroblastoma tumors by immunohistochemistry studies. We postulate that the low level of vascularity and more benign clinical behavior of Schwannian stroma-rich/stroma-dominant neuroblastoma tumors result from the Schwann cell production of TIMP-2 and/or other inhibitors of angiogenesis.

Introduction

NB, a pediatric malignancy of neural crest origin, is characterized by a broad spectrum of clinical behavior. Almost all patients with localized tumors can be cured with surgery alone, and most infants with disseminated disease have favorable outcome (1, 2). In contrast, <30% of children older than 1 year of age with advanced-stage NB become long-term survivors (3, 4). Numerous factors including tumor stage, patient age (5), tumor histology (6), molecular markers (7, 8), and genetic abnormalities (9–11) have been shown to correlate with outcome. However, the biological mechanisms that underlie the clinical variability observed in NB remain largely unknown. NB tumors consist of two main cell populations, neuroblastic/ganglionic cells, and Schwann cells. At the present time, controversy exists as to whether both cell types arise from a neoplastic neuroblastic clone. Using laser-capture microdissection, Mora et al. (4) recently performed allelic analysis of chromosomes 1p, 11q, and 14q in stage 4 NB tumors. In these studies, similar genetic abnormalities in the neuroblastic and Schwannian cell components of the tumor were seen, suggesting that both cell types do arise from a common malignant clone. In contrast, a study by Ambros et al. (12) demonstrated that the Schwann cells in maturing NB tumors lacked genetic anomalies, indicating that the Schwann cells may be normal cells that infiltrate the tumor. On the basis of the known interactions between normal neuroblasts and Schwann cells (13), Ambros et al. (12) proposed that neoplastic neuroblasts produce mitogens and chemotactic factors that are important to the recruitment of Schwann cells, and that the Schwann cells within the tumor produce antiproliferative and differentiation-inducing factors crucial to neuronal differentiation. In support of this hypothesis, recent laboratory studies have indicated that normal Schwann cells produce soluble substances capable of supporting the survival and differentiation of NB cell lines (14). The prognostic impact of the presence of Schwannian stroma has been emphasized in the classification system of Shimada et al. (15) and has also been incorporated in the International Neuroblastoma Pathology Classification (6). All neuroblastic tumors that are Schwannian stroma rich and stroma dominant are classified as favorable, with the exception of those that are composite Schwannian stroma rich/stroma dominant and stroma poor (ganglioneuroblastoma, nodular).

For a tumor to grow beyond a limited volume of 1–2 mm³, the tumor cells must be able to induce the growth of new capillary blood vessels (16). These new vessels also increase the opportunity for tumor cells to enter the circulation and metastasize (17). Brem et al. (18) were the first to propose that the intensity of intratumoral angiogenesis may correlate with tumor grade and aggressiveness. In the past 10 years, an overwhelming majority of published reports have shown a significant correlation between the density of intratumoral microvessels in various types of carcinomas and patient survival (19, 20). We found that in NB, high tumor vascularity correlated with widely disseminated disease, MYCN amplification, unfavorable histology, and poor outcome (21). In contrast, low tumor vascularity was associated with prognostically favorable features such as localized disease and favorable histology. These observations suggest that inhibited angiogenesis may contribute to the more benign clinical behavior associated with tumors with favorable prognostic features. To specifically investigate whether the Schwann cells in Schwannian...
stroma rich/stroma dominant NBs play a role in inhibiting tumor vascularity, we examined the ability of normal human Schwann cell-conditioned medium to affect angiogenesis in vitro and in vivo. Additional angiogenesis assays were performed with conditioned medium collected from tumor-derived Schwann cells to determine whether the biological behavior of tumor Schwann cells was similar. Our results indicate that Schwann cells derived from either adult nerve tissue or stroma-dominant NB tumor tissue produce a potent inhibitor(s) of angiogenesis.

Materials and Methods

Cell Culture. Primary human Schwann cells were isolated from adult nerves and from a Schwannian stroma-dominant NB tumor (ganglioneuroma) as described previously (22), except that some cultures were expanded in Opti-MEM I medium (Life Technologies, Inc., Rockville, MD) supplemented with 1% penicillin/streptomycin, 2.5 μg/ml of amphotericin, 50 ng/ml of recombinant human glial growth factor 2 (Cambridge NeuroScience, Cambridge, MA), and 2 μM forskolin. The BAECs, a kind gift from Dr. Gerald Soff (Northwestern University), the human fibroblast cell line MLK-1-FB (23), a gift from Dr. Steven Rosen (Northwestern University), and the human smooth muscle jejunum cells (American Type Culture Collection, Rockville, MD) were grown in DMEM containing 10% FBS, antibiotics, and glucose (Life Technologies, Inc.). The human lung microvascular endothelial cells, obtained from Clonetics Corp. (San Diego, CA), were cultured in Microvascular Endothelial Cell Growth Medium with EGM-MV Bullet kit, which contains FBS and growth factors (Clonetics).

Collection of Conditioned Media. Conditioned media were collected from normal human Schwann cells cultured in two different growth media, as described previously (14). Briefly, confluent 10-cm plates were washed with Dulbecco’s PBS to remove growth factors, and the cells were fed either with DMEM containing 1% FBS or with Opti-MEM I alone. After 24 h, the cells were refed with 4 ml of fresh basal medium, which was harvested 24 h later. The supernatant was collected and centrifuged for 5 min at 3000 g.

Proliferation Assays. The in vitro proliferation of endothelial cells was determined using the CellTiter 96 AQ nonradioactive cell proliferation assay (Promega Corp., Madison, WI). Briefly, BAECs were seeded into 96-well tissue culture plates at a concentration of 3 × 10^4 cells/well and incubated overnight in DMEM with 10% FBS and antibiotics at 37°C. Medium was then aspirated from each well, and the cells were refed with 0.1 ml of Schwann cell-conditioned medium containing 1% FBS with 9 ng/ml bFGF (R&D System, Minneapolis, MN) or 100 μg/ml VEGF (R&D Systems). In control studies, the cells were refed with DMEM containing 0.1% BSA, 1% FBS, antibiotics, and 9 ng/ml bFGF or 100 μg/ml VEGF. After 72 h, the absorbance at the 490-nm wavelength was measured using an automated microplate reader (Bio-Tek Instruments, Winooski, VT). The assays were performed at least three times with different batches of Schwann cell-conditioned medium. The assays were repeated with conditioned medium collected from normal human Schwann cells and tumor-derived Schwann cells cultured in Opti-MEM I lacking FBS. Opti-MEM I media with 9 ng/ml bFGF served as the positive control for the latter experiments. The CellTiter 96 AQ nonradioactive cell proliferation assay was also used to determine the effect of Schwann cell-conditioned medium on the proliferation of smooth muscle cells and fibroblasts.

For statistical comparison, raw data from these and other experiments were converted to percentiles where necessary and analyzed using the Student’s two-tailed t test. Plotted values represent the mean ± SE.

Endothelial Cell Migration Assay. The endothelial cell migration assays were performed as described previously (24). Briefly, human lung microvascular endothelial cells were suspended in Endothelial Cell Growth Medium containing 0.1% BSA at a concentration of 1.5 × 10^6 cells/ml. Cells were seeded into the lower wells of a Boyden chamber (Neuro Probe, Inc., Cabin John, MD). The chamber was assembled and inverted, and the cells were incubated for 2 h at 37°C to allow adherence to the surface of a 0.01% gelatinized polycarbonate chemotaxis membrane (5-μm pore size; Corning, Acton, MA). After the chamber was reinserted, 50 μl of Schwann cell-conditioned medium with 1% FBS and 10 ng/ml bFGF, Microvascular Endothelial Cell Growth Medium with 0.1% BSA, 1% FBS, and 10 ng/ml bFGF (positive control), or Microvascular Endothelial Cell Growth Medium with 0.1% BSA and 1% FBS (negative control) was added to the wells of the upper chamber in quadruplicate, and the apparatus was incubated for 4 h at 37°C. The membranes were removed from the chamber, fixed, and stained with the Diff-Quick Stain kit (Baxter, McGaw Park, IL). The number of cells that had migrated to the upper surface of the membrane per three fields at ×400 for each well was counted. The assays were repeated with conditioned medium collected from normal human Schwann cells and tumor-derived Schwann cells cultured in Opti-MEM I lacking FBS. Opti-MEM I media with 10 ng/ml bFGF served as the positive control for the latter experiments.

Corneal Angiogenesis Assay. Conditioned medium containing 1% FBS collected from normal human Schwann cells was concentrated 20-fold using a centrifugal filter device (Millipore, Bedford, MA). The corneal assay was performed as described previously (25). Briefly, 5 μl of Hydron pellets (Hydro Med Sciences, Cranbury, NJ) containing bFGF (2 μg/ml) plus concentrated Schwann cell-conditioned medium, control medium (DMEM with 1% FBS), or PBS were implanted into the cornea of anesthetized female rats (7–8 weeks of age; Harlan Sprague Dawley, Indianapolis, IN). After 7 days, the animals were sacrificed and perfused with water or formalin (Sanford, Bellwood, IL) by intracardiac injection. The eyes were fixed in 10% neutralized buffered formalin overnight. The corneas were then excised and examined for angiogenic activity.

Western Blot Analysis and ELISA. Schwann cell-conditioned medium lacking FBS was used in the expression studies. The level of expression of the following well-established inhibitors of angiogenesis was examined by Western blot analysis: anti-thrombin, thrombospondin-1, angiostatin, TIMP-1, TIMP-2, and PEDF (26–28). Media samples were concentrated 20-fold using the centrifugal filter devices (Millipore), electrophoresed in a 10% SDS-PAGE gel, and transferred to a nitrocellulose membrane (Bio-Rad, Richmond, VA), using standard techniques (29). After transfer, the blots were stained with Ponceau S (Sigma Chemical Co., St. Louis, MO) to confirm equal loading. Membranes were blocked with 5% nonfat dry milk for 1 h and then incubated for 2 h with anti-angiostatin (1:1000 dilution; Calbiochem, Cambridge, MA), anti-thrombospondin-1 (1:400 dilution; clone A4.1; Calbiochem), anti-angiostatin (generous gift from Dr. Gerald Soff, Northwestern University), anti-TIMP-1 (1:400 dilution), and anti-TIMP-2 (1:200 dilution). The membranes were washed three times with PBS (0.05% Tween) and then incubated for 2 h with a 1:5000 dilution of horseradish peroxidase-conjugated secondary antibody specific for the proper isotypes (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The bound antibody complexes were detected using the LumiGLO chemiluminescence reagent (KPL). TIMP-2 expression in conditioned medium collected from tumor-derived Schwann cells was also examined by Western blot analysis using the same methods. PEDF expression studies were performed using an antibody specific for recombinant PEDF in the laboratory of Dr. Patricia Becerra (NIH, Bethesda, MD) as described previously (30). ELISA kits were used to measure human endostatin (Cytimmune Sciences, Inc., College Park, MD), TGF-β1 (R&D Systems Inc.), and human IFN-α (EndoGen, Inc., Woburn, MA) expression.

Immunohistochemistry. Primary NB tumor tissue was fixed in 10% buffered formalin and embedded in paraffin. Four-μm-thick histological sections were obtained from three Schwannian stroma-rich/stroma-dominant NB tumors and one Schwannian stroma-poor tumor. Immunohistochemical staining with anti-TIMP-2 antibody (Ab-4; Oncogene Research Products, Boston, MA) was performed on the tumor sections using an automated stainer (Ventana; NEXES, Tucson, AZ) according to the manufacturer’s instructions.

Results

Schwann Cell-conditioned Medium Inhibits Endothelial Cell Proliferation and Migration. As a first step toward investigating whether Schwann cells produce soluble factors that inhibit angiogenesis, conditioned medium was obtained from normal human Schwann cells cultured in DMEM and 1% FBS and tested for its effect on endothelial cell proliferation and migration. We found that Schwann cell-conditioned medium inhibited bFGF-induced BAEC proliferation.
in a dose-dependent manner (Fig. 1A). Complete inhibition of bFGF-induced BAEC cell proliferation was seen in experiments performed with undiluted Schwann cell-conditioned medium ($P < 0.0001$). However, even when the Schwann cell-conditioned medium was diluted 1:36, endothelial cell proliferation was significantly inhibited ($P = 0.0193$), suggesting that low levels of these inhibitory factor(s) are sufficient to induce a biological response. Schwann cell-conditioned medium also completely inhibited VEGF-induced BAEC proliferation ($P < 0.001$; Fig. 1B). To investigate whether this biological effect was specific to endothelial cells, the rate of proliferation of fibroblast and smooth muscle cells cultured in Schwann cell-conditioned medium was examined. In contrast to the inhibition in proliferation observed in the endothelial cell studies, fibroblast cell proliferation was significantly increased with Schwann cell-conditioned medium ($P < 0.001$), whereas no significant difference in the rate of smooth muscle cell proliferation was observed (Fig. 1C).

The results of the endothelial cell migration studies paralleled the endothelial cell proliferation assays. Schwann cell-conditioned medium significantly inhibited bFGF-induced endothelial cell migration ($P < 0.001$; Fig. 1D). The number of migrating endothelial cells in wells containing Schwann cell-conditioned medium with bFGF was similar to that seen with the control medium lacking the angiogenesis activators. bFGF-induced endothelial cell proliferation and migration were also inhibited with conditioned medium collected from Schwann cells cultured in Opti-MEM I medium lacking FBS. To specifically examine the ability of tumor-derived Schwann cells to affect angiogenesis, endothelial cell proliferation and migration assays were repeated using conditioned medium collected from Schwann cells purified from a Schwannian stroma-dominant NB tumor. Significant inhibition of bFGF-induced endothelial cell proliferation (Fig. 1A) and migration (data not shown) was seen with conditioned medium collected from tumor-derived Schwann cells.

**Schwann Cell-conditioned Medium Inhibits Angiogenesis.** Schwann cell-conditioned medium was used in rat corneal assays to investigate its ability to block bFGF-induced angiogenesis in vivo. In control studies, pellets containing PBS and bFGF were implanted into six rat corneas, and angiogenesis was induced in all six experiments (Fig. 2; Table 1). Additional control studies were performed with pellets containing control medium and bFGF. In these animals, angiogenesis was induced in three of three corneas. However, when pellets containing Schwann cell-conditioned medium and bFGF were implanted in animals, the angiogenic response was completely inhibited in all four corneas tested.

**Expression of Angiogenesis Inhibitors.** In an effort to identify the angiogenesis inhibitor(s) produced by the Schwann cells, the expression of several known inhibitors of angiogenesis was examined in conditioned medium collected from normal Schwann cells. ELISA assays were used to measure endostatin, TGF-β1, and IFN-α expression. Anti-thrombin, thrombospondin 1, angiostatin, PEDF, TIMP-1, and TIMP-2 expression was examined by Western blot analyses. Because both activator and inhibitors of angiogenesis are known to be present in FBS (31), the expression studies were performed using conditioned medium collected from Schwann cells cultured in Opti-MEM I medium that lacked FBS. In these studies, Western blot analyses demonstrated high levels of TIMP-2 in Schwann cell-conditioned medium, whereas TIMP-2 was not detected in the control medium (Fig. 3). TIMP-2 was similarly detected in the conditioned media collected from tumor-derived Schwann cells. ELISA studies demonstrated low concentrations of endostatin (9 ng/ml) in the Opti-MEM I Schwann cell-conditioned medium. Anti-thrombin, thrombospondin 1, TIMP-1, TGF-β1, angiostatin, PEDF, and IFN-α were not detected in the Schwann cell-conditioned medium.

To further investigate TIMP-2 expression in tumor Schwann cells, histological tissue sections of Schwannian stroma-rich/stroma-dominant and stroma-poor tumors were immunohistochemically stained with anti-TIMP-2 antibody. TIMP-2 was expressed in the cytoplasm of Schwann cells and differentiated ganglion cells in all three stroma-rich/stroma-dominant tumors analyzed (Fig. 4). TIMP-2 was not detected in the stroma-poor tumor.
Discussion

In this study, we show that human Schwann cells derived from adult nerves and NB tumor tissue produce soluble substance(s) that inhibit angiogenesis. Endothelial cell proliferation induced by either bFGF or VEGF was significantly inhibited by Schwann cell-conditioned medium. This biological effect appears to be specific for endothelial cells, because Schwann cell-conditioned medium had little effect on smooth muscle cell growth and actually enhanced fibroblast proliferation. Similarly, NB cell line proliferation has been shown to be increased with Schwann cell-conditioned media (14). Schwann cell-conditioned media also inhibited endothelial cell migration toward a bFGF gradient in a Boyden chamber. Furthermore, Schwann cell-conditioned medium dramatically blocked bFGF-induced angiogenesis in in vivo rat corneal assays. Conditioned medium collected from tumor-derived Schwann cells also inhibited bFGF-induced endothelial cell proliferation and migration, indicating that the biological behavior of Schwann cells derived from adult nerve and NB tumor tissue is similar. At the present time, it remains unclear as to whether Schwann cells in NB tumors are malignant (12). However, recent studies suggest that the Schwann cells in NB tumors are capable of producing a number of chemotactic, mitogenic, and differentiating factors that affect tumor growth and differentiation (12, 14). Our results suggest that the Schwann cells in NB tumors also produce inhibitor(s) of angiogenesis.

Angiogenesis is thought to be regulated by the balance of inducers and inhibitors within a given microenvironment (32). Vascular quiescence is observed in most tissues because inhibitory influences predominate. In contrast, most malignant cells are potently angiogenic as a result of decreased production of inhibitors and increased secretion of inducers. Many tumors have a malignant compartment and a stromal compartment. Recent studies by Tomlinson et al. (33) have shown that the angiogenic profile of the stromal cells and malignant cells in breast cancer differ, indicating that both components probably contribute to the regulation of tumor angiogenesis. Similarly, blood vessel growth in Schwannian stroma-rich/stroma-dominant NB tumors is likely to be influenced by both the neuroblastic cells and the Schwannian stromal cells.

In an effort to identify the factor(s) in the Schwann cell-conditioned medium responsible for the inhibition of angiogenesis, the expression pattern of well-established inhibitors of angiogenesis was examined by ELISA or Western blot analyses. In our studies, high levels of TIMP-2 were seen in conditioned medium collected from Schwann cells derived from adult nerve and NB tumor tissue by Western blot analyses.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>bFGF (2 μg/ml)</th>
<th>Positive corneas/Total implanted</th>
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<tbody>
<tr>
<td>PBS</td>
<td>+</td>
<td>6/6</td>
</tr>
<tr>
<td>Control media</td>
<td>+</td>
<td>3/3</td>
</tr>
<tr>
<td>Schwann cell-conditioned media</td>
<td>+</td>
<td>0/4</td>
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Fig. 2. In vivo antiangiogenic activity of Schwann cell-conditioned medium. Pellets containing bFGF plus PBS, control medium, or Schwann cell-conditioned media were implanted into the normally avascular cornea of a rat and neovascularization was assessed 7 days later.

Fig. 3. TIMP-2 expression. Western blot analysis was performed using control (CM) and Schwann cell-conditioned media (SCM) with a TIMP-2-specific monoclonal antibody.
expression is not likely to be of functional significance. Anti-thrombin, thrombospondin, TIMP-1, angiostatin, TGF-β1, PEDF, and IFN-α expression were not detected in the Schwann cell-conditioned medium lacking FBS.

TIMP-2, a member of a family of tissue inhibitors of metalloproteinases, has been shown to suppress tumor invasion, metastatic potential, and angiogenesis in many cell model systems (35). In NB tumors, enhanced expression of TIMP-2 is associated with localized disease and favorable outcome (36). The TIMPs are capable of inhibiting the activities of all known matrix metalloproteinase inhibitors and thereby play a key role in maintaining the balance between extracellular matrix deposition and degradation in different physiological processes (35). Disruption of this balance can result in various pathological processes including inflammation, chronic degenerative disease, and tumor growth and metastasis. Recent studies suggest that TIMP-1 and TIMP-2 are multifunctional proteins with diverse actions. TIMP-2, but not TIMP-1, inhibits bFGF-induced human endothelial cell growth (37), and both TIMP-1 and TIMP-2 have also been shown to have mitogenic activities on a number of cell types (38). Thus, it is possible that both the inhibition in angiogenesis as well as the increase in proliferation of fibroblasts observed with Schwann cell-conditioned medium are attributable to TIMP-2.

Numerous adult studies have demonstrated that enhanced intratumor vascularity is associated with various measures of tumor aggressiveness (19). Similarly, we found that high tumor vascularity in NB tumors associated with unfavorable biological features and poor outcome (21). Extending our studies, Erdeich-Epstein et al. (39) have recently demonstrated a significant association between high levels of expression of the integrins α5β1 and α6β4, which are markers of active angiogenesis, and high-risk NB. In contrast, Canete et al. (40) reported that vascular parameters were not predictive of survival in a cohort of NB patients that they analyzed. The conflicting results most likely reflect differences in techniques used to measure vessel number. Canete et al. (40) assessed angiogenesis parameters in the richest vascular area using a computerized system, whereas every vessel that resided within the tumor section was counted by two independent reviewers in our study to calculate a vascular index (21). Thus, in our study, the vascular index reflected the vascularity of the entire histological section. Similarly, Erdeich-Epstein et al. (39) analyzed the whole tissue section to determine the proportion of microvascular endothelium that expressed α5β1 and α6β4.

We postulate that the low level of vascularity and more benign clinical behavior associated with favorable histology Schwannian stroma-rich/stoma-dominant NB results from the Schwann cell production of TIMP-2 and/or other inhibitors of angiogenesis. Synthetic angiogenesis inhibitors have been shown to inhibit NB growth in preclinical studies (41). It is, therefore, tempting to speculate that future treatment approaches using TIMP-2 and/or other natural soluble angiogenesis inhibitors produced by Schwann cells may prove to be effective therapy for children with highly vascular, stroma-poor NBs.

Acknowledgments

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References

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