A Novel Animal Model for Hemangiomas: Inhibition of Hemangioma Development by the Angiogenesis Inhibitor TNP-470

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ABSTRACT

Hemangiomas represent the most frequent tumors of infancy. However, the pathogenesis of these tumors is still largely unknown, and current treatment of juvenile hemangiomas remains unsatisfactory. Here we present a novel animal model to study proliferating hemangiomas and to evaluate the effect of angiostatic compounds on their growth. Intraperitoneal (i.p.) infection of 4-day-old rats with murine polyomavirus resulted in the development of multiple cutaneous, intramuscular (i.m.), and cerebral hemangiomas with 100% frequency. Histological examination of the brain revealed the formation of immature lesions as soon as 4 days postinfection (p.i.). The subsequent exponential growth of the hemangiomas, both in number and size, was associated with severe hemorrhage and anemia. The cerebral, cutaneous, and i.m. lesions consisted of blood-filled cysts, histologically similar to human cavernous hemangiomas and stained positive for proliferating cell nuclear antigen, urokinase-type plasminogen activator, and vascular endothelial growth factor. Mature cerebral hemangiomas also expressed von Willebrand factor. Cerebral lesions caused death of the untreated animals within 19.2 ± 1.1 days p.i. Remarkably fewer and smaller hemangiomas developed in animals that had been treated s.c. with the angiogenesis inhibitor TNP-470. Accordingly, TNP-470 (50 mg/kg), administered twice a week from 3 days p.i., significantly delayed tumor-associated mortality [mean day of death, 28.2 ± 3.3 (P < 0.001)]. Even if therapy was initiated when cerebral hemangiomas were already macroscopically visible (i.e., 9 days p.i.), a significant delay in hemangioma-associated mortality was observed. Also, the IFN-inducer polyinosinic-polycytidylic acid caused a delay of 9 days (P < 0.005) in tumor-associated mortality when administered i.p. at 5 mg/kg, twice a week, starting at day 3 p.i. The model described here may be useful for investigating (a) the angiogenic mechanism(s) underlying hemangioma progression; and (b) the effect of anti-angiogenic compounds on vascular tumor growth.

INTRODUCTION

One of the most frequently occurring angiogenic diseases of childhood are hemangiomas, appearing in 2.5–12% of all newborns (1, 2). They occur more frequently in females than males (ratio of 3:1). Hemangiomas are characterized by rapid neonatal growth (proliferating phase). By the age of 6–10 months, the hemangioma's growth rate becomes proportional to the growth rate of the child, followed by a very slow regression for the next 5–8 years (involuting phase). Of all the hemangiomas, 80% occur as single tumors, whereas about 20% of the affected infants have multiple tumors, which may appear at any body site. Approximately 5% produce life-, sight-, or limb-threatening complications, with high mortality rates. Treatment is necessary for these life-endangering hemangiomas; however, only 30% respond to high-dose corticosteroids (3, 4). There are no safe and effective treatment alternatives, although some reports have been published on favorable responses to surgery (3), irradiation (3, 5), or IFN α-2a therapy (6–9). Because current treatment of life-threatening hemangiomas with these drugs remains unsatisfactory, future studies should focus on the identification of novel inhibitors of vascular tumor growth.

Hemangiomas are characterized by increased turnover of endothelial cells, mast cells, fibroblasts, and macrophages. The pathogenesis of hemangiomas has not yet been elucidated. However, several immunohistochemical studies have provided insight into the histopathology of these lesions. Endothelial cells of hemangiomas were found to express different endothelial markers, including CD31, vWF³ and VE-cadherin (10, 11). Moreover, the different stages in hemangioma evolution can be distinguished by specific cellular markers. In particular, the proliferating phase of juvenile hemangiomas was correlated with the expression of vWF, PCNA (a marker for cells in the S phase), and the endothelial-specific angiogenic factor VEGF (10).

On the basis of the definition given by Folkman and Klagsbrun (12), hemangiomas may be regarded as angiogenic diseases and should, therefore, be considered for treatment with angiostatic compounds. However, at present, there is no adequate animal model available to evaluate the effect of angiostatic compounds on hemangioma development and progression. Different animal models of induced vascular tumors have been reported. Hemangiomas or endotheliomas have been shown to develop in mice after s.c. inoculation of spontaneously immortalized (13) or PymT-transformed endothelioma cells (14, 15) or endothelial cells overexpressing bFGF (16). However, host cell recruitment rather than proliferation of the initial cell population was found to be the main mechanism responsible for tumor development in these animals (14–16).

The fumagillin analogue TNP-470 is an inhibitor of angiogenesis that inhibits endothelial cell proliferation and migration in vitro (17). In animal models, TNP-470 is effective in the treatment of a wide variety of tumors and their metastases (18–21). Its antitumor activity together with its moderate side effects has led to Phase I–III clinical trials for a variety of solid tumors (22, 23). O'Reilly and colleagues have described an inhibitory effect of locally administered TNP-470 on the growth of hemangiendotheliomas after s.c. inoculation of endothelioma cells in adult mice (13). Thus far, TNP-470 has not been evaluated clinically for the treatment of hemangiomas.

Infection of 3–4-day-old rats with a high titer of PyV results in the development of multiple cutaneous, i.m., and cerebral hemangiomas with 100% frequency. We have recently shown by means of titration for infectious virus content, semiquantitative PCR for VP1 and a DNA-DNA hybridization assay for VP1 that there is no replication of PyV in infected rats, which indicates that transformation of the endothelial cells by the viral middle T protein may be sufficient to induce the formation of hemangiomas (24).

The present study was designed to further characterize these experimentally induced hemangiomas immunohistolologically and to in

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3 The abbreviations used are: vWF, von Willebrand factor; bFGF, basic fibroblast growth factor; MDD, mean day of death; MLS, mean lesion score; PyV, (murine) polyomavirus; PCNA, proliferating cell nuclear antigen; pfu, plaque-forming unit(s); p.i., postinfection; poly IC, polynosinic-polycytidylic acid; PymT, polyomavirus middle T antigen; TNP-1, tissue inhibitor of metalloproteinase; uPA, urokinase type plasminogen activator; VEGF, vascular endothelial growth factor; VP1, viral capsid protein.

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vestigate whether their progression can be blocked by anti-angiogenic compounds.

MATERIALS AND METHODS

Animals. Rats of the Wistar R strain were used throughout all of the experiments. The rats were obtained from the Animal Production Center of the Katholieke Universiteit Leuven and were housed under germ-free conditions during the experiments.

Virus. Murine polyoma virus (strain Marseille) was grown on murine embryo fibroblast cultures and titrated with the plaque method of Dulbecco and Freeman on mouse embryo fibroblast cells as described previously (24).

Materials. TNP-470 was provided by Takeda Chemical Industries, Ltd. (Osaka, Japan) and stored dry at −20°C. Shortly before administration, a suspension of TNP-470 in 5% ethanol and 5% arabic gum in saline was prepared. Control rats were given injections of a suspension of 5% ethanol and 5% arabic gum in saline. Poly IC was obtained from Sigma Chemical Co. (St. Louis, MO) and Roferon A was from Roche (Brussels, Belgium). Both compounds were dissolved in PBS.

Hemangioma Induction and Mortality Experiments. Four-day-old rats were infected i.p. with either $10^{9.7}$ or $10^{8.7}$ pfu of PyV. Poly IC was injected i.p. at 5 mg/kg, twice weekly. TNP-470 was administered s.c. according to a variety of treatment schedules, as described in the “Results” section. Mortality and the appearance of cutaneous hemangiomas were recorded daily for those rats that were infected with $10^{9.7}$ pfu. The animals that had been infected with $10^{8.7}$ pfu were killed at 4 weeks p.i. The brains, lungs, liver, spleen, and kidneys were dissected and fixed in Bouin’s fluid and embedded in paraffin, and sections were stained with H&E, as described below. The two-tailed unpaired Student’s $t$ test was used to calculate the differences in the MDD. Statistical significance of the number of animals developing lesions was assessed by the $\chi^2$ test with Yates’ correction.

Histological Analysis. Four-day-old rats were infected with $10^{9.7}$ pfu of PyV, s.c. treatment with 50 mg/kg TNP-470, or carrier only, was started at 4 days p.i. and was continued twice weekly until the end of the experiment. At different times after infection, brains of untreated and TNP-470-treated animals were dissected and fixed in 10% buffered formaldehyde. Brains were sliced in a transversal plane. The distribution of hemangiomas throughout the brain turned out to be relatively homogenous. Therefore, two random slices

Fig. 1. The incidence of cutaneous and cerebral hemangiomas in PyV-infected rats. Animals were killed at different times p.i., and the appearance of cerebral hemangiomas was recorded macroscopically after necropsy. The presence of cutaneous lesions was recorded daily on feet and tail (n = 5). A parallel group was evaluated for mortality (n = 5).

Fig. 2. The histological appearance (H&E) of cerebral and i.m. hemangiomas. Early phase hemangiomas (4 days p.i.) consist of aggregates of endothelial cells (A, ×400). At 11 days p.i. (B, ×400), the tumors are composed of a layer of endothelial cells, surrounding a blood-filled lumen. At 18 days p.i. hemangiomas contain many cysts (not shown), which, after the disappearance of the septae, may coalesce into a huge blood-filled sac (C, ×200). Histological appearance of an i.m. hemangioma at 18 days p.i. (D, ×400).
were embedded in paraffin and sectioned at 5 μm. Specimens were stained with H&E and examined by light microscopy.

**Morphometry.** Because the distribution of the hemangiomas throughout the brain turned out to be relatively homogenous, their surface proportion may be considered representative for their volume proportion. The number of hemangiomas and their surface proportion were determined in H&E-stained sections of the brains of untreated and TNP-470-treated animals. The surface proportion occupied by hemangiomas was determined by means of a conventional point-counting method using an ocular grid containing 121 equally spaced points. Scoring was done by counting the number of hit points at 10 stratified random positions of the grid, which resulted in a total of 1210 points per section. Next, the percentage of hit points as compared with the total number of points was calculated. Sections were read at ×100.

**Immunohistochemistry.** Deparaffinized sections of brains, dissected at different time points after infection, muscle, or skin tissue (dissected at 18 days p.i.) were incubated for 1 h with 1.5% blocking serum in PBS. After the removal of the blocking serum, the slides were incubated with primary mouse monoclonal antibodies directed against human PCNA (clone PC10, Calbiochem-Novabiochem, Nottingham, United Kingdom), vWF (Dako Corp., Glostrup, Denmark), VEGF (Santa Cruz Biotechnology, Santa Cruz, CA), or bFGF (clone FB-8, Sigma Chemical Co.) for 30 min at room temperature. The slides were subsequently washed in PBS for 5 min and then incubated with biotinylated secondary antimouse antibody (Dako Corp.) for 30 min at room temperature. After a 5 min wash, all of the slides were incubated with horseradish peroxidase-labeled streptavidine (Dako Corp.) for 30 min at room temperature. The immunoreactions were visualized as brown precipitates by incubation with the substrate diaminobenzidine (Dako Corp.) for 5 min. The reaction was stopped by water, and the slides were counterstained with hematoxylin. Goat polyclonal antibodies to rat uPA, TIMP-1, and human bFGF were from Santa Cruz Biotechnology (Santa Cruz, CA). Staining was performed with the goat Immunocruz Staining System (Santa Cruz) according to the manufacturer’s instructions.

**RESULTS**

**Induction of Hemangiomas.** i.p. infection of 4-day-old rats with 10^9.7 pfu of PyV resulted in the development of cerebral hemangiomas that appeared macroscopically at 7 days p.i., followed by the appearance of cutaneous hemangiomas at 14 days p.i. (Fig. 1). Rapid growth of the cerebral hemangiomas, associated with severe hemorrhage and anemia, resulted in the death of the rats within 3 weeks p.i.

**Histological Appearance of Cerebral Hemangiomas.** Brains of infected rats were dissected at 4, 7, 9, 11, 14, 18, or 21 days p.i (Fig. 2). At 4 days p.i., endothelial cells started to accumulate into an immature lesion (Fig. 2A). From day 4 to 11 after infection, endothelial cells evolved into lumenlike structures containing erythrocytes (Fig. 2B). These cells also showed numerous mitotic figures. Multiple hemangiomas of various size were present at day 11. After 14 days, some tumors were composed of multiple cystic cavities with septation, others had converged into a single, huge blood-filled cyst (Fig. 2C).

**Immunohistochemical Analysis.** Proliferating human hemangiomas are vWF-positive and express high levels of PCNA (a marker for cells in the S phase), urokinase, and the angiogenic factors bFGF and VEGF (10). We carried out an immunohistochemical analysis (using antibodies to vWF, PCNA, uPA, bFGF, VEGF, and TIMP-1) of cerebral hemangiomas (Fig. 3), dissected at different time points p.i., and of cutaneous and i.m. hemangiomas at day 18 p.i. (not shown). The immature lesions visible at 4 days p.i. were characterized by the expression of PCNA, whereas these lesions were negative for the other markers. High levels of PCNA expression were observed in all of the examined hemangiomas at any time point (Fig. 3A). The endothelial marker vWF (Fig. 3B) was weakly expressed in a subset of hemangiomas at 11 days p.i., whereas a clear positive staining for
vWF was noticed in the majority of late-stage hemangiomas (day 18 or 21). The endothelial cells lining the lacunae of the hemangiomas showed an intense reactivity for the endothelial-specific angiogenic factor VEGF at days 14, 18, and 21 (Fig. 3C). Surprisingly, these cells were negative for bFGF. Therefore, either bFGF was not present, or the antibodies that are directed against human bFGF are not cross-reactive with rat bFGF. The balance between proteolytic enzymes was also studied. The hemangiomas uniformly expressed the proteolytic enzyme uPA (Fig. 3D), whereas only a few lesions stained positive for the metalloproteinase inhibitor TIMP-1 (Fig. 3E).

Cutaneous and i.m. hemangiomas started to appear at 11 days p.i. and evolved into blood-filled cysts (Fig. 2D) that were histologically similar to cerebral hemangiomas. Immunohistological analysis of these lesions at 18 days p.i. (not shown) revealed consistent and high expression of PCNA. Staining for VEGF was uniform but less intense, and again no immunoreactivity was observed for bFGF. The hemangiomas showed a marked reactivity for uPA, whereas most lesions were TIMP-1 negative.

Effect of TNP-470 on Hemangioma-associated Mortality. When s.c. treatment with TNP-470 (either 50 mg/kg/day twice a week or 10 mg/kg/day five times a week) was initiated at 3 days p.i., a marked delay in tumor-associated mortality was observed [MDD, 19.2 ± 1.1 for the control group as compared with 28.2 ± 3.3 (P < 0.001) and 25.6 ± 0.9 (P < 0.001) for the 50-mg/kg and 10-mg/kg treatment groups, respectively (Fig. 4)]. Even when the start of treatment was delayed until cerebral hemangiomas were already clearly visible (i.e., 9 days p.i.), TNP-470 caused a significant delay in tumor-associated mortality [MDD, 24.6 ± 2.4 (P < 0.05) and 24.6 ± 1.5 (P < 0.001) for the 50-mg/kg and 10-mg/kg treatment groups, respectively (Fig. 4)]. Furthermore, the animals that had received TNP-470 therapy showed remarkably fewer cerebral hemangiomas and almost no hemorrhages as compared with the control animals.

Effect of TNP-470 on Number and Size of Hemangiomas in the Brain. Both the number and the total volume of the developing hemangiomas were determined in brain sections of untreated or TNP-470-treated rats dissected at the different time points described above.
Immature hemangiomas were observed as soon as 4 days p.i. (see above). From 4 until 18 days p.i., the number of cerebral hemangiomas increased exponentially (Fig. 5A). Although at day 11, the number of hemangiomas was already 50% of the number reached at day 18, these hemangiomas were small as indicated by the low volume% of the brain that was occupied by these lesions (Fig. 5B). The increase in the size of the hemangiomas occurred predominantly between day 11 and day 18. At day 18, the tumor volume represented about 20% of the total brain volume. The plateau reached at day 18 represents the maximum attainable number of hemangiomas and hemangioma size, inasmuch as further tumor growth will lead to death of the animals.

s.c. treatment with TNP-470 (50 mg/kg, twice a week, starting at 3 days p.i.) resulted in a markedly lower number of hemangiomas in brain sections, as compared with the control group at 11 days after infection. In fact, an increase in the number of lesions as observed in control rats after day 9 was not detectable in TNP-470-treated animals. Also, these hemangiomas remained very small in size until 18 days p.i., as reflected by a volume% of about 3 as compared to 20 for the control animals. However, after day 18, the volume % of the cerebral hemangiomas in the treated animals started to increase significantly. This was the result of a small number of hemangiomas that continued to grow, ultimately leading to the death of the animals (see above).

Macroscopically, few hemangiomas were visible at day 7. The increase in size and number of hemangiomas as well as the effect of TNP-470 on these parameters is presented in Fig. 6.

Effect of TNP-470 on Hemangioma Incidence and Growth after Infection with a Lower Virus Load. To study the effect of TNP-470 in a less aggressive model for cerebral hemangioma growth, 4-day-old rats were infected with a 10-fold lower virus load (10^6.7 pfu) than in...
The previous experiments. Under these conditions, cerebral hemangiomas developed within 14 days p.i., and cutaneous lesions were not observed. Treatment with TNP-470 was started at different time points after infection (Table 1); all of the animals were killed at 4 weeks p.i.; and the brains were examined macroscopically for the appearance of hemangiomas. In addition, brains as well as lungs, kidney, spleen, and liver were examined histologically. Macroscopically detectable cerebral hemangiomas were found in 76% of all of the control animals (86% after histological examination). No hemangiomas or other tumors were observed in any of the other organs. Interestingly, control rats that did not develop hemangiomas were all male. TNP-470 therapy (50 mg/kg, twice a week) started before the appearance of brain tumors (at days 3 and 9, as evaluated macroscopically by dissection of a parallel control group) significantly inhibited the incidence of hemangiomas; i.e., 29 and 33% of the rats developed cerebral hemangiomas as compared with 76% in the control group (Table 1). There was a significantly lower number of hemangiomas present in the brain after TNP-470 treatment, and these tumors were markedly smaller in size (for MLS, see Table 1). At a dose of 50 mg/kg twice a week, TNP-470 caused stabilization of already established hemangiomas, as can be derived from the fact that the MLS was still significantly lower in those animals in which treatment was started at 14 days p.i.

**Effect of TNP-470 on Body Weight Gain of Noninfected Rats.** Administration of TNP-470 (50 mg/kg, twice a week) led to some delay in body weight gain (body weight of the TNP-470 treated animals after 4 weeks of treatment was 83% of that of the control animals) when treatment was initiated at the age of 7 days (3 days p.i.). This delay was less pronounced (body weight of the treated animals was 88% of that of the control animals) when treatment was started at the age of 13 days (9 days p.i.). Furthermore, the delay in body weight gain occurred mainly during the first 9 days of treatment, after which body weight increased in parallel with that of the control animals.

**Effect of INFα-2a and the IFN Inducer poly IC on Hemangioma-associated Mortality.** Current treatment of hemangiomas mainly relies on INFα-2a therapy. We, therefore, wanted to investigate whether IFN would also be active against PyV-induced hemangiomas. Because rat IFN is not available, and human INFα-2a did not prove active in our model, we treated PyV-infected rats with the IFN α/β-inducer poly IC. Rats received 5 mg/kg/day poly IC, twice weekly, i.p. This resulted in a significant delay in tumor-associated mortality, i.e., MDD was 31.3 ± 4.4 for the poly IC group versus 22.0 ± 3.3 for the untreated animals (P < 0.005; Fig. 7). poly IC treatment was well tolerated and did not cause any delay in body weight gain.
with previous reports showing that immature hemangiomas do not express VWF, whereas proliferating juvenile hemangiomas stain positive for this endothelial cell marker (10). In addition, rapid clinical growth has been correlated with the high expression of PCNA and VEGF (10). We detected the endothelial cell-specific angiogenic factor VEGF in the majority of PyV-induced hemangiomas, whereas no positive staining for bFGF was observed. However, because there are no antibodies available against rat bFGF, ant-human bFGF antibodies had to be used, which may be poorly cross-reactive with rat bFGF. Finally, hemangiomas showed consistent and high expression of the proteolytic enzyme uPA and stained very weakly for the proteinase inhibitor TIMP-1. The balance between proteolytic enzymes determines the extent of extracellular matrix degradation. This is important to provide space for the migration of the proliferating endothelial cells into the surrounding tissue. We hypothesize that the net proteolytic activity, observed in our model, may cause basement membrane breakdown that then allows the activated (i.e., by VEGF) endothelium to form a new capillary network, which leads to the development of the hemangiomas.

At present, there is no model that faithfully recapitulates human hemangioma pathology. Hemangiomas have been shown to develop in mice transgenic for the early region of the polyoma virus genome (26). However, these tumors possess a low tumorigenicity and a high latency period. Other models that have been described concern allograft or xenograft models in which animals were inoculated with hemangioendothelioma cells that arose spontaneously (13) or after exposure to UV light (32) or carcinogenic agents (33), or with endothelial cells overexpressing bFGF (16) or PymT antigen (14, 15). The former have not been extensively characterized, whereas the latter were formed by endothelial cell recruitment (14–16) rather than through cell proliferation (which is assumed to be responsible for human hemangioma progression).

The model described here produces in a reproducible fashion, and with a short latency period, proliferating, cavernous hemangiomas that express PCNA, VWF, VEGF, and uPA and are associated with the infiltration of macrophages (24), hemorrhage, and severe anemia—characteristics of aggressive life-threatening hemangiomas. Furthermore, the use of newborn rats makes this model interesting for the preclinical evaluation of angiostatic compounds because only compounds with sufficient selectivity, i.e., potent anti-angiogenic activity and low toxicity, would result in protective activity.

We, therefore, evaluated the effect of the angiogenesis inhibitor TNP-470, which is in clinical trial for the treatment of a variety of solid tumors, including Kaposi’s sarcoma (22, 23), on hemangioma growth and associated mortality. We found that TNP-470 is highly effective against hemangioma development and progression in our model. Infection of 4-day-old rats with a high virus inoculum (108.7 pfu) resulted in the development of cutaneous hemangiomas at 14 days p.i. and cerebral hemangiomas within 7 days p.i. From day 7 to day 18 there was an exponential increase in both the number and size of the cerebral hemangiomas in untreated animals. No such increase in hemangioma number was observed after s.c. treatment with TNP-470. Moreover, the volume % of the hemangiomas in the brain of TNP-470-treated animals was only 15% of that observed in the untreated animals at 18 days p.i. After 18 days, an increase in volume % was observed, caused by a small number of hemangiomas that had continued to increase in size. As a consequence, TNP-470 caused a marked delay in tumor-associated mortality even when treatment was delayed until 9 days p.i., i.e., a time at which brain lesions were already macroscopically detectable.

When a 10-fold lower virus inoculum was used, only cerebral hemangiomas developed, and no cutaneous or i.m. hemangiomas could be observed. TNP-470 treatment started at 3 or 9 days p.i. afforded complete protection against hemangioma formation in 71 and 67% of the animals, respectively (as compared with 24% for the untreated control animals); and the hemangiomas that did appear were much smaller than those in the control animals. Even when therapy was started at a time when brain tumors were already macroscopically visible (by day 14), stabilization of the growth of cerebral hemangiomas was observed in 40% of the treated animals. Curiously, 24% of the untreated animals, infected with the lower virus inoculum (108.7 pfu) did not develop hemangiomas: all of these animals appeared to be male, although the male:female ratio was 50:50 in all of the experiments. This may be relevant to the situation in humans, in which the incidence of hemangiomas is 3-fold higher in females than in males (2).

Current hemangioma therapy is mainly based on the use of IFNα-2a (Roferon). However, because rat IFN is not available (and human IFNα-2a did not prove effective in our model), we decided to treat rats with the IFN α/β inducer poly IC. This therapy, when initiated at 3 days p.i., proved as effective as TNP-470 in delaying hemangioma-associated mortality. Thus, as is the case with human hemangiomas, PyV-induced hemangiomas in rats respond to IFN therapy.

The hemangiomas that were induced in newborn rats after infection with PyV developed after a short latency period and had the histological and immunohistochemical features reminiscent of human cavernous hemangiomas. The angiostatic compound TNP-470 as well as the IFN inducer poly IC caused a protective effect in this model. Taking these factors together, our model may serve as a good functional model for human hemangiomas. However, in contrast to the PyV-induced hemangiomas, the trigger leading to endothelial proliferation in humans is not yet known. It is unlikely that transformation of endothelial cells takes place in juvenile hemangiomas as spontaneous involution occurs. An increased expression of the angiogenic factors bFGF and VEGF has been reported in proliferating hemangiomas (10), which implies an important role for these growth factors in hemangioma progression. Moreover, some deficiency in the proliferating endothelial cells such as overexpression of tyrosine kinase receptors could make them more susceptible to different angiogenic factors or other external signals. Because (a) VEGF is also expressed in PyV-induced hemangiomas and (b) both the activity of angiogenic factors and PymT are regulated by tyrosine kinase receptors, it is tempting to hypothesize that PyV-induced and human hemangiomas may share a common pathway resulting in the activation of the angiogenic process and, subsequently, hemangioma progression.

In conclusion, the model presented here may be useful to unravel the angiogenic mechanisms (e.g., the involvement of angiogenic factors, cell adhesion molecules, and matrix-degrading enzymes) underlying hemangioma development and progression. Furthermore, our model may be suitable to assess the effects of specific inhibitors of angiogenesis on vascular tumor growth.

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