The radiobiology of human acoustic schwannoma xenografts after stereotactic radiosurgery evaluated in the subrenal capsule of athymic mice

MARK E. LINSKEY, M.D., A. JULIO MARTINEZ, M.D., DOUGLAS KONDZIOLKA, M.D., JOHN C. Flickinger, M.D., ANN H. MAITZ, M.SC., THERESA WHITESIDE, PH.D., and L. DADE LUNSFORD, M.D.

Departments of Neurological Surgery, Pathology, Radiation Oncology, and Radiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

A published experimental model with xenograft transplantation into the subrenal capsule of athymic (nude) mice was used to evaluate the early response of human acoustic schwannomas to stereotactic radiosurgery. After xenograft placement, 45 mice underwent stereotactic radiosurgery with single doses of 10, 20, or 40 Gy using a 201-source 60Co gamma unit (4-mm collimator, single isocenter, 80% isodose line). The 45 radiosurgery-treated xenografts were compared with 15 untreated xenografts and 15 xenografts in mice that underwent "sham" radiosurgery. All five study groups were matched for the following pretreatment variables: patient of origin, animal weight, average xenograft diameter, and percentage of xenograft surface vascularity. Immediately prior to sacrifice of the mice all xenografts were evaluated in situ to determine the average tumor diameter, tumor volume, and percentage of surface vascularity. Mice were sacrificed 2 weeks, 1 month, or 3 months after radiosurgery. Blinded histological review was performed by an independent neuropathologist.

Tumor volume was reduced 33.6% after 2 weeks (p = 0.023) and 45% after 3 months (p = 0.018) in the 40-Gy radiosurgery group. Tumor volume was reduced by 46.2% after 1 month (p = 0.0002) and 35.2% after 3 months (p = 0.032) in the 20-Gy radiosurgery group. An average volume reduction of 16.4% was observed after 3 months (p = 0.17) in the 10-Gy radiosurgery group. At 3 months after surgery, tumor surface vascularity was reduced by an average of 19.7% (p = 0.043) in the 40-Gy radiosurgery group and 5.8% (p = 0.12) in the 20-Gy radiosurgery group and was unchanged in the 10-Gy radiosurgery group and both control groups. Histological examination demonstrated a higher incidence of hemosiderin deposits (p = 0.026) and vascular mural hyalinization (p = 0.032) in radiosurgery xenografts versus control.

The subrenal capsule xenograft in nude mice was an excellent model for studying the in vivo radiobiology of acoustic schwannomas after radiosurgery. Both cellular and vascular effects could be assessed serially in situ and the model was stable even 4 months after transplantation. Additional studies investigating radiobiology over periods better approximating the time course of clinical neomaging changes (6 to 12 months) are warranted.

Key Words • acoustic neurinoma • radiobiology • stereotaxis • xenograft • vestibular schwannoma • nude mouse

Despite extensive clinical experience,\textsuperscript{20,22-25,29,30} the radiobiological mechanisms leading to tumor control after stereotactic radiosurgery of acoustic (vestibular) schwannomas remain largely unknown. Current dose selection criteria goals are empirically based on clinical studies suggesting that single radiation doses to the tumor margin of between 12 and 20 Gy are sufficient to control tumor growth in 86% to 95% of patients and are associated with minimal risk to adjacent cranial nerves.\textsuperscript{22-25,29,30} There are two main reasons for the lack of knowledge about acoustic tumor radiobiology: 1) few patients treated by radiosurgery have yet come to autopsy;\textsuperscript{3,29,37} and 2) a reliable animal model to study human acoustic schwannomas has been unavailable.

Recently, the athymic mouse subrenal capsule xenograft model (originally designed as a short-term chemotherapy assay for malignant tumors\textsuperscript{5}) was modified to study the growth of human acoustic tumors.\textsuperscript{14,15} We hypothesized that this in vivo xenograft model could be used to study the radiobiology of human acoustic schwannomas after stereotactic radiosurgery. The purposes of this study were: 1) to evaluate the early neoplastic cellular and vascular response of vestibular
whether viability; transplantation; both drawings are drawn to scale with each division on the measuring lines representing 1 mm. By directing treatment to the 80% isodose line, the vast majority of xenograft is guaranteed to receive between 80% and 100% of the maximum tumor dose. C: Schema for calculating the percentage of the xenograft surface covered by blood vessels. The xenograft surface was divided into four quarters, each accounting for 25% of the tumor surface area. If only a portion of any particular quadrant was covered by blood vessels, that quadrant was further divided into a square representing 16% and two “triangles,” each representing 4.5% of the tumor surface area. If the square within a given quadrant was not completely covered by blood vessels, it was further subdivided into four smaller squares, each representing 4% of the tumor surface area.

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schwannoma xenografts to radiosurgery using clinically relevant single doses (10, 20, or 40 Gy); 2) to establish whether the subrenal capsule xenograft model remained stable up to 4 months after transplantation; 3) to determine whether removing athymic mice from their immunologically protected colony for short periods to perform radiosurgery excessively increased animal mortality or decreased xenograft viability; and 4) to establish whether stereotactic radiosurgery resulted in increased animal morbidity or mortality compared with animals receiving “sham radiosurgery.”

Materials and Methods

Tumor Acquisition and Preparation

All human acoustic schwannomas were obtained intraoperatively using sterile techniques. All specimens were obtained from the center of the neoplasm in order to eliminate the possibility of sampling the tumor capsule. Electrocautery, laser, or Cavitron ultrasonic aspirator were not used during specimen retrieval. After confirmation of the diagnosis of schwannoma by frozen section, the tumor fragments were transferred to a sterile transport jar containing nutrient medium (F10), supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution of penicillin, amphotericin B (Fungizone), and streptomycin. The remainder of the tumor was submitted for routine pathological studies. The tumor specimen in nutrient medium was transported immediately to the nude mouse colony; transplantation was initiated within 30 minutes after surgical removal.

Transplantation Procedure

The animal use portion of the research protocol was approved by the Institutional Animal Care and Use Committee of the Eye and Ear Institute of Pittsburgh (Protocol No. 0096), and the Institutional Animal Care and Use Committee of the University of Pittsburgh (Assurance No. A3187-01). Congenitally athymic female nude mice,* each weighing 18 to 28 gm (mean 21.7 gm), were housed in groups of five in sterile cages, and given autoclaved chow and water ad libitum. The cages were kept in a laminar flow isolator† in a dedicated nude mouse colony with strictly controlled temperature, humidity, and 12-hour light cycles.

Transplantation was performed in the nude mouse facility under sterile conditions. The surgical technique was modified in three ways from the technique described by Lee, et al.14 Instead of chloral hydrate, each mouse was anesthetized with an intraperitoneal injection of 0.3 ml Avertine (2,2,2-tribromoethanol and tert-amyl alcohol). Under the microscope, the two perpendicular tumor implant diameters were measured using sterile electrocardiographic calipers which were then read against a fine-gradation ruler. Measurements were accurate within 0.1 mm. A 0.5-mm length of sterile coiled titanium wire was placed adjacent to the tumor implant beneath the renal capsule for x-ray targeting purposes (Fig. 1). The average tumor diameter was determined by the equation: \( D = \text{length} \times \text{width} \times \frac{1}{2} \), and the tumor volume from the equation: \( V = \text{length} \times \text{width} \times \text{width} \times \frac{1}{2} \).3

* Athymic nu/nu mice obtained from Harlan-Sprague-Dawley, Inc., Indianapolis, Indiana.
† Laminar flow isolator manufactured by Forma Scientific, Inc., Marietta, Ohio.
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Surgical Re-Exploration

Four weeks after transplantation, all mice were weighed and re-explored using identical surgical and anesthetic techniques. Measurements of the two perpendicular xenograft diameters were repeated. The percentage of the xenograft surface covered by tumor vessels was estimated under the microscope according to the schema outlined in Fig. 1C. Using this schema, the surface area vascularity of any xenograft could be estimated with an error of 4.5% (the area of the largest subdivision). A xenograft was accepted for entry into the study only if the tumor had at least maintained its initial average tumor diameter (within 5%) and had achieved at least 75% tumor surface vascularity (Fig. 2).

Experimental Protocol and Treatment

Radiosurgery was performed within 1 week of initial re-exploration. Day 0 of the study protocol was considered to be the day of radiosurgery. Mice were transported from the nude mouse colony to the gamma knife suite in a special pathogen-filtered transport cage. After intraperitoneal anesthesia with 0.3 ml Avertine, the mouse was attached with tape to a sterile Leksell stereotactic frame which was modified for work with small mammals. Anteroposterior and lateral orthogonal targeting radiographs were obtained. The titanium-wire fragment, clearly visible on x-ray films, was used as the irradiation target. Radiosurgery was performed using a 4-mm collimator of the 201-source $^{60}$Co Leksell gamma unit. The gamma unit radiation delivery rate during the period of study declined from 239.0 to 229.6 cGy/min. Radiation delivery was prescribed to the 80% isodose line in order to include the majority of each xenograft in the treatment volume (axial radius 1.7 mm, coronal radius 1.875 mm, sagittal radius 1.875 mm (Fig. 1)). Radiographs repeated after radiosurgery in selected animals revealed no movement of the target marker in the anesthetized animals during the treatment process.

The research protocol was composed of five arms (two control arms and three treatment arms) containing 15 mice each. The first control arm was a nude mouse "colony control group;" these mice never left the colony and were not treated with radiosurgery. In the second control arm the mice were removed from the colony and underwent "sham radiosurgery" (0 Gy). Among the treatment groups, in the first arm the xenografts were treated with 10 Gy to the 80% isodose line (10 to 12 Gy to the xenograft); in the second arm the xenografts were treated with 20 Gy to the 80% isodose line (20 to 25 Gy to the xenograft); and in the third arm the xenografts were treated with 40 Gy to the 80% isodose line (40 to 50 Gy to the xenograft). Each protocol arm was composed of three groups of five mice, all of which were re-explored either 2 weeks, 1 month, or 3 months after treatment, respectively. An additional five mice were sacrificed after their initial re-exploration (4 weeks after transplantation) to serve as "baseline histological controls" for comparison with the xenografts in the two study control arms.

In an effort to prevent differences in individual tumor biology from confounding the interpretation of the experimental results, every attempt was made to distribute tissue from each donor patient evenly throughout all five experimental arms (Fig. 3). Mice in each experimental arm were also matched for three additional pretreatment variables: animal weight (a general measure of animal health), average xenograft diameter, and percentage of xenograft surface vascularity (Table 1).

Gross Evaluation and Sacrifice

At the appropriate time after entrance into the research protocol, each mouse was weighed and surgically re-explored. Measurements of the two perpendicular xenograft diameters and the percentage of surface vascularity were repeated. The kidney containing the xenograft was then surgically removed and immediately

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Fig. 2. A: Intraoperative photograph of a xenograft immediately after implantation. B: The same xenograft 4 weeks later at re-exploration. The xenograft had increased 0.5 mm in average tumor diameter and had achieved 81% surface vascularity during the 4-week interval.

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2 Leksell stereotactic frame and gamma unit manufactured by Elekta Instruments, Tucker, Georgia.
Fig. 3. Scattergram depicting the distribution of xenografts from all 18 donor patients among the five experimental arms of the study.

placed in 10% buffered formalin, after which the mouse was killed humanely with an anesthetic overdose.

**Histological Analysis**

Tumor specimens fixed in 10% buffered formalin were embedded in paraffin. All specimens were cut at 5 to 6 μm thickness and stained with hematoxylin and eosin. Selected sections were also prepared with Masson trichrome stain. The Schwann cell composition of the xenografts was confirmed by immunocytochemical staining for S100 protein using standard immunoperoxidase methods. Histological sections were evaluated by a neuropathologist (A.J.M.) who was blinded to the experimental history of the xenograft under examination. Xenograft histology was compared with the original donor tumor histology.

**Statistical Analysis**

The adequacy of interarm matching for continuous variables such as change in mouse weight, average tumor diameter, and percentage of xenograft surface vascularity was evaluated by analysis of variance of the mean. The change in average tumor volume and surface vascularity for each xenograft (between the time of entrance into the protocol and mouse sacrifice) was expressed as a percentage value based on the original value for each parameter determined at the time of initial surgical re-exploration. Mean relative values were compared between experimental arms at each scheduled sacrifice period by analysis of variance. The presence or absence of histological features on microscopic analysis as well as unexpected mortality rates were compared between experimental arms by chi-squared analysis.

**Results**

**Xenograft Survival Rate**

The overall “take rate” for tumor implants (no reduction in size plus 75% or greater surface vascularity) as assessed at initial surgical re-exploration was 78.3%. The mean overall “take rate” per donor tumor specimen was 80.9 ± 10.6% (± standard deviation) (range 60% to 100%).

**Adequacy of Matching**

Figure 3 is a scattergram depicting the distribution of tumor material from the 18 donor patients throughout the 15 groups in the five study arms. The xenografts in all five arms were well matched for patient of origin. The results for matching of the five study arms for mouse weight, average tumor diameter, and percentage of surface area vascularity of the xenograft are presented in Table 1. There was no statistically significant difference between the five study arms in any of the three parameters.

**Mortality in Experimental Groups**

Twenty-five mice died prior to their scheduled sacrifice date after they were entered into study groups; they were replaced with 25 additional xenografted mice. Radiosurgery itself did not appear to be the major cause for early mortality: the sham-radiosurgery arm had a higher unexpected death rate than any of the three radiosurgery arms (33.3% vs. 18.6%). Taking the mice out of the nude mouse colony and/or anesthetizing them for a third time tended to increase the incidence of early mortality (22.9% for all mice leaving the colony vs. 6.3% for colony control mice); however, this difference did not achieve statistical significance (p = 0.19). After 1 month the effect of removing the mice from the colony was no longer apparent and the mortality rate was fairly uniform across all study arms (16.7% for all experimental arms over the last 2 months of the protocol).

**Xenograft Size and Vascularity**

The relative change in average tumor volume and tumor surface vascularity for each experimental arm as a function of time is depicted in Fig. 4. There was no significant difference between the colony and the sham-radiosurgery control groups in terms of change in tumor volume or change in tumor surface vascularity. The environmental immunological challenge from removing mice from the colony had no effect on xenograft viability.

Tumor volume was reduced by 33.6% after only 2 weeks in the 40-Gy radiosurgery group (p = 0.023), and

<table>
<thead>
<tr>
<th>Study Arm</th>
<th>Mean Mouse Weight (gm)</th>
<th>Mean Xenograft Diameter (mm)</th>
<th>Mean Xenograft Surface Vascularity (%)</th>
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</thead>
<tbody>
<tr>
<td>colony control</td>
<td>22.3 ± 2.47</td>
<td>2.52 ± 0.56</td>
<td>94 ± 6.94</td>
</tr>
<tr>
<td>sham radiosurgery control</td>
<td>24.7 ± 2.22</td>
<td>2.25 ± 0.46</td>
<td>89 ± 12.30</td>
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<td>10 Gy radiosurgery</td>
<td>24.0 ± 1.79</td>
<td>2.33 ± 0.35</td>
<td>90 ± 10.40</td>
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<tr>
<td>20 Gy radiosurgery</td>
<td>23.9 ± 2.12</td>
<td>2.13 ± 0.46</td>
<td>89 ± 12.70</td>
</tr>
<tr>
<td>40 Gy radiosurgery</td>
<td>24.0 ± 2.99</td>
<td>2.15 ± 0.30</td>
<td>92 ± 10.80</td>
</tr>
<tr>
<td>overall</td>
<td>24.0 ± 2.47</td>
<td>2.25 ± 0.46</td>
<td>91 ± 11.0</td>
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</table>

* There was no statistically significant difference between any of the five study arms in any of the three parameters (analysis of variance). Means are expressed ± standard deviation.
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remained reduced by 35.0% after 1 month ($p = 0.026$) and by 45% after 3 months ($p = 0.018$) following treatment (Fig. 5). In the 20-Gy radiosurgery group, tumor volume reduction became significant 1 month after radiosurgery (46.2% reduction, $p = 0.0002$) and remained significant 3 months following radiosurgery (35.2% reduction, $p = 0.032$). In the 10-Gy radiosurgery group, tumor volume was reduced 3 months after radiosurgery, but the magnitude of reduction was not statistically significant compared with control xenografts (16.4% reduction, $p = 0.0166$).

A significant reduction in xenograft capsular surface vascularity was observed in the 40-Gy radiosurgery arm compared with a combined colony and sham-radiosurgery control group (Fig. 5). This reduction was apparent 2 weeks after treatment (9.8% reduction, $p = 0.012$), and remained significant 3 months after treatment (19.7% reduction, $p = 0.043$). The 5.8% reduction in xenograft surface vascularity observed in the 20-Gy radiosurgery arm was not statistically significant 3 months after treatment ($p = 0.121$). Although xenograft capsular surface vascularity initially decreased in the 10-Gy radiosurgery arm, by 3 months after treatment no apparent macroscopic vascular effect from radiosurgery was observed.

**Histological Results**

The five baseline histological control xenografts that were evaluated after the first surgical re-exploration (4 weeks after transplantation) all appeared as lenticular well-encapsulated masses in the subrenal capsule space. The mass compressed and indented the kidney surface but did not invade the renal parenchyma (Fig. 6). The renal capsule was permeated by a syncytial net of

![Graphs depicting the percent changes in tumor volume (A) and in tumor surface vascularity (B) over time for all five study arms. Each data point represents the mean value for five mice sacrificed at that time point after entering the protocol. The vertical bars on the far right represent the standard error of the mean for the 3-month value for each study arm. Symbols: closed circles = colony control; crosses = sham-radiosurgery control; open circles = 10-Gy radiosurgery; squares = 20-Gy radiosurgery; triangles = 40-Gy radiosurgery.](image)

**Histological Results**

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![A: Intraoperative photograph of a xenograft at re-exploration, just prior to receiving radiosurgery with a dose of 40 Gy. B: The same xenograft photographed just prior to sacrifice of the mouse, 1 month later. The average tumor diameter had decreased from 2.1 mm to 1.9 mm and the tumor surface vascularity had decreased from 100% to 81%, with a corresponding reduction in blood vessel density.](image)
capillaries. Tiny penetrating parenchymal capillaries were also evident. The majority of the penetrating parenchymal capillaries appeared to arise from the renal capsule capillary net, but penetration of capillaries from the renal parenchyma was rarely observed. Cellular morphological characteristics and density resembled the original neoplasm (except for the absence of areas of Antoni B cellular architecture or Verocay bodies). Masson trichrome stain revealed no increase in the collagenous connective tissue content of the xenografts compared with the original tumors. All control xenografts were strongly positive for S100 protein (confirming the Schwann cell origin).

All 30 colony control and sham-radiosurgery control xenografts had the same histological characteristics as the five baseline histology control xenografts. Every third specimen was tested for S100 protein and all remained strongly positive even 3 months after entering the experimental protocol (4 months after transplantation). Every third specimen was stained with Masson trichrome and none demonstrated an increased content of collagenous connective tissue with time. All specimens remained free of parenchymal mononuclear inflammatory cell infiltration, confirming the absence of graft rejection up to 4 months after transplantation. Rare and isolated foci of hemosiderin, usually associated with hemosiderin-laden macrophages, were evident in seven specimens, which were sacrificed at least 2 months after transplantation. The number of penetrating parenchymal capillaries arising from the capsular capillary net increased with time after transplantation. No mural hyalinization of penetrating parenchymal capillaries or capsular capillaries was evident in any of the 35 control specimens (five baseline histology controls, 15 colony controls, and 15 sham-radiosurgery controls).

Every third specimen from the 45 mice that underwent stereotactic radiosurgery was tested for S100 protein and all were strongly positive. There were two statistically significant histological findings in radiosurgery xenografts when compared with control xenografts (Fig. 6). First, radiosurgery xenografts were more likely to have areas of hemosiderin, probably resulting from microhemorrhage (21 xenografts, p = 0.026). Foci of hemosiderin were evident in xenografts sacrificed as early as 2 weeks after radiosurgery. Second, 13% of radiosurgery xenografts demonstrated hyalinization of capillaries (p = 0.032). Blood vessel mural hyalinization was observed in the penetrating parenchymal capillaries, but also affected capsular capillaries at the lateral circumferential edge of the xenograft. Blood-vessel mural hyalinization was found in xenografts as early as 2 weeks after radiosurgery and tended to increase in frequency over time. Intraparenchymal fibrosis and edema were increased in radiosurgery xenografts compared with control xenografts, but this difference did not achieve statistical significance. No foci of coagulative necrosis, vascular endothelial proliferation, or intraparenchymal hemorrhage were observed in any of the radiosurgery or control xenografts.

**Discussion**

**Histopathological Studies of Acoustic Schwannomas After Radiosurgery in Humans**

Information about the radiobiology of human vestibular schwannomas has been limited to three autopsy studies performed on patients who underwent stereotactic radiosurgery, and a series of *in vitro* tissue culture studies performed in 1980. The three autopsy studies are summarized in Table 2. The number of patients was too small and the time courses too varied to arrive at a dose-time response relationship for any of the observed effects. Not all cases had subcellular stud-
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<table>
<thead>
<tr>
<th>Authors, Year, &amp; Patient Data</th>
<th>Findings</th>
</tr>
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<tr>
<td>Anniko, et al., 1981 (Case 2) tumor diameter: 2.0 cm tumor-margin dose: 12 Gy maximum dose: 40 Gy time to autopsy: 10 mos</td>
<td>avascular by preoperative angiography in a sphere corresponding to ~20 Gy radiation; centrally: loss of tumor cells and &quot;ground substance&quot;; occasional intracellular myelin figures; morphologically preserved tumor architecture peripherally; no necrosis noted; histology of tumor vasculature not described</td>
</tr>
<tr>
<td>Norén, et al., 1983 (Case AS) tumor diameter: 2.8 cm tumor-margin dose: 10 Gy maximum dose: 40 Gy time to autopsy: 6 mos</td>
<td>sharply defined central region of coagulative necrosis in an area estimated to have received ~20 Gy radiation; morphologically preserved tumor architecture peripherally, containing hyalinized tumor blood vessels with endothelial proliferation; lymphocytes infiltrated the border between the two regions</td>
</tr>
<tr>
<td>Thompson, et al., 1990 (Case 3) tumor diameter: 3.5 cm tumor-margin dose: 12–20 Gy maximum dose: 40 Gy time to autopsy: 11 wks</td>
<td>morphologically preserved tumor architecture with extracellular lakes of proteinaceous fluid, edema, vascular congestion, clusters of hemosiderin, and recent and remote focal hemorrhages; no necrosis noted; histology of tumor vasculature not described</td>
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</table>

ies with electron microscopy, molecular genetic studies, or in vitro functional studies to determine cell growth potential. Thus, it is impossible to determine the physiological status of the "morphologically preserved" neoplastic cells noted in these three cases.

In vitro organ culture studies of human vestibular schwannomas permit assessment of single-dose radiation effects on neoplastic cells and intervening extracellular matrix but do not allow determination of the effect of single-dose radiation on tumor vasculature. In the study by Anniko, et al.,3 cells received single radiation doses of 30 to 150 Gy and were assessed by electron microscopy. Irreversible subcellular changes were evident at all doses tested, and began with vesiculation of cellular cytoplasm within 3 to 5 hours in up to 40% of specimens. By 24 hours intracellular myelin figure formation was observed followed by basal lamina disintegration and loss of intervening ground substance. Individual cell disintegration became apparent after 1 week with 30 to 50 Gy radiation and earlier with higher doses. The authors could not establish a dose-dependent response for intracytoplasmic subcellular changes, but did note a dose-dependent response to generalized architectural fragmentation. Doses below 30 Gy were not examined.

In Vivo Models for Vestibular Schwannomas

Spontaneous or induced Schwann-cell tumors are reported in several animals,12 16,27,33 but none of the spontaneous tumors occurred readily or in a high incidence.33 In addition, nitrosourea-induced tumors were often peripheral rather than central in origin, often involved cranial nerves other than the vestibular nerve, and were malignant rather than benign in over 50% of cases.12 16,23 Both of these types of tumors also suffer from the drawback of not being human in origin.

Xenografts from various human neoplasms have been studied in normal animals secondarily immunosuppressed by either whole-body irradiation in mice4 or pharmacological agents in immunoprivileged anatomical sites of animals such as the cheek pouch of the golden hamster9,40 and in congenitally immunocom-petent nude mice.4,15,18,19,21,26,34–36 Human Schwann-cell neoplasms can be successfully xenografted into both the sciatic nerve4,15 and the subrenal capsule of nude mice.8,18,19 While xenograft growth is slightly better in the sciatic nerve than in the subrenal capsule,19 gross evaluation of xenograft vascularity in situ is difficult in the sciatic nerve because of the presence of perineural fibrosis and the absence of a transparent covering over the entire xenograft surface (RL Martuza, personal communication, 1990). Furthermore, distinguishing neoplastic human Schwann cells from mouse sciatic nerve Schwann cells requires an immunoperoxidase stain for a uniquely human antigen, along with special cryostat sectioning.19 The nude mouse subrenal capsule xenograft model is ideally suited for the study of acoustic schwannomas because: it permits the accurate quantitation of even very small changes in tumor size; it allows for repeated accurate quantification of xenograft surface vascularity in situ; and it utilizes a naturally rich vascularized bed to provide nutrients for the tumor fragment until it acquires its own neovascularity.5,7,18,19,32

Clinical Applicability of Nude Mouse Xenograft Data

In general, human xenografts faithfully preserve their original cellular, architectural, and genetic characteristics after transplantation into nude mice.9,31,34,39 The subrenal capsule xenograft model has been used for many years as a screening method to select potentially clinically effective chemotherapy regimens,6,7 and has been included as an assay in the National Cancer Institute drug development program.14

We have established that the nude mouse subrenal capsule xenograft model for human vestibular schwannomas is stable and histologically faithful to the donor tumor up to 4 months after transplantation (3 months after entrance into the experimental protocol). Previous studies determined the stability of this model for a mean of 60.5 ± 21.5 days after transplantation (range 32 to 97 days).19 Ideally, we would like to assess the radiobiology of this tumor 6 to 12 months after radio-

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surgery, since the median onset of loss of intratumoral contrast enhancement and tumor shrinkage in vestibular schwannoma patients occurs at a median of 6 and 12 months after radiosurgery, respectively.12,22-25,29,30 Since the median life expectancy of nude mice ranges from 18 to 24 months,11,13 these longer-term studies may be possible.

Previous reports using this model (and only considering xenograft size) had reported a xenograft "take rate" ranging from 57.1% to 77.3%.18,19 Our higher "take rate," considering both tumor size and tumor surface vascularity, probably reflects optimum tumor acquisition and the very short time interval in our study between surgical removal and xenograft transplantation.

Interpretation of Preliminary Results

Our results with the subrenal capsule xenograft model demonstrate a statistically significant reduction in tumor volume after stereotactic radiosurgery using either 20 or 40 Gy. Since we did not observe areas of intratumoral coagulative necrosis, ischemic infarction, or mononuclear inflammatory cell infiltration (to suggest rejection), we believe that tumor size reduction occurred via individual neoplastic cell death, which would be consistent with the electron microscopy findings in tissue culture noted by Anniko, et al.2,3 The fact that the xenograft size reduction after treatment with 10 Gy was not statistically significant may reflect the relatively short experimental follow-up time compared with the median onset of tumor shrinkage of 12 months in clinical series.22-25,29,30 While coagulative necrosis has been observed in autopsy studies25 as well as in surgical pathology specimens after microsurgical resection of irradiated tumors,22 it was not observed in any of the xenografts after radiosurgery. This observation may also reflect the relatively short follow-up period in this experimental setting compared with the 6-month median follow-up time in the three autopsy studies.

The ability of irradiated neoplastic cells to continue to proliferate even if they appear "morphologically intact" by light microscopy is unknown. The tissue culture studies by Anniko, et al.,3 suggest that, while these cells may be "morphologically intact," they still may be significantly damaged as evidenced by the frequent finding of cytoplasmic inclusion bodies on electron micrographs. If this is the case, these "morphologically intact" cells may be incapable of reproduction; however, this possibility requires further investigation using molecular genetic and in vitro cell viability studies.

Our preliminary results also clearly show a significant effect of radiosurgery on vestibular schwannoma tumor vasculature,1 which is consistent with the clinical findings of loss of intratumoral contrast enhancement12,22-25,29,30 as well as angiographic tumor blush after stereotactic radiosurgery.7 The fact that this effect was observed in nude mice only after 40 Gy radiation may reflect the relatively short experimental follow-up interval.

Our schema for estimating xenograft surface vascularity (outlined in Fig. 1) is an improvement in sensitivity over the previously used 0 to 4+ grading scale.10,19 However, this modification still fails to take into account surface vasculature density. In some cases the total surface area covered with tumor vessels did not change, but the density of these vessels was reduced after radiosurgery. Currently available grading systems remain insensitive to this effect.

Future Radiobiology Studies

Future studies using the nude mouse subrenal capsule model for human vestibular schwannomas include an ongoing study to assess the stability and histological fidelity of this model up to 9 months after transplantation. Should the model remain stable over this time period, further radiobiology studies will be designed to assess the effects of radiosurgery across longer time periods that more realistically reflect the time course of neuroimaging changes in our patient experience. Future histological studies will also include electron microscopy evaluation of subcellular radiobiological effects. Additional studies will include submission of xenograft samples for cell culture and molecular biology assays to assess the effects of radiosurgery on subsequent in vitro cell viability and molecular genetic alterations.

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This work received the Preuss Award for Brain Tumor Research from the Joint Section on Tumors of the American Association of Neurological Surgeons/Congress of Neurological Surgeons at the Annual Meeting of the Congress of Neurological Surgeons in Washington, D.C., October 31–November 3, with support. Address reprint requests to: Mark E. Linskey, M.D., Department of Neurological Surgery, Room F-948, Presbyterian-University Hospital, 230 Lothrop Street, Pittsburgh, Pennsylvania 15213.