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CLINICAL GROWTH RATE OF ACOUSTIC SCHWANNOMAS

CORRELATION WITH THE GROWTH FRACTION AS DEFINED BY THE MONOCLONAL ANTIBODY Ki-67

Abstract—The growth rate of acoustic tumors, although slow, varies widely. There may be a continuous spectrum or distinct groups of tumor growth rates. Clinical, audiologic, and conventional histologic tests have failed to shed any light on this problem. Modern immunohistochemical methods may stand a better chance. The Ki-67 monoclonal antibody stains proliferating cells and is used in this study to investigate the growth fraction of 13 skull base schwannomas. The acoustic tumors can be divided into two different growth groups, one with a rate five times the other. The literature is reviewed to see if this differentiation is borne out by the radiologic studies. Distinct growth rates have been reported: one very slow, taking 50 years to reach 1 cm in diameter; a second rate with a diameter increase of 0.2 cm/year, and a third rate five times the second, with a 1.0 cm increase in diameter per year. A fourth group growing at 2.5 cm/year is postulated, but these tumors cannot be followed for long radiologically, since symptoms demand surgical intervention. The clinical implications of these separate growth rates are discussed.

The rate of growth of acoustic tumors remains something of an enigma. The idea perpetrated by Cushing of a homogeneous group of slowly growing tumors with gradually progressive symptomatology was soon realized to be inaccurate.[1] Some tumors appear to grow relatively rapidly, others so slowly as to be almost static, and some may even decrease in size.[2] This variation is growth rate is also apparent following partial removal, some return with a vengeance, others cause no symptoms for the rest of the patient's life,[3] and some may even disappear.[4]

Conventional histologic examinations have been unable to shed any light on this variation in clinical growth patterns.[1] Any laboratory technique used for this task is hampered by a number of problems. One is that the change in size of a tumor, even if it were constant, does not give a constant progression of symptoms. This is well-illustrated

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by the patient whose disabling dizziness improves by central compensation as the tumor starts to enlarge into the cerebellopontine angle and no further symptoms occur for some time despite continuing enlargement. Another problem is that the change in size of a tumor may be due to edema, hemorrhage, or cyst formation or other degenerative processes rather than a cellular change.[5] A third problem is that the cellular growth may not be constant throughout the life of a patient and any cytologic test can only be used to evaluate the cells at the time of removal. A tumor may grow quickly for a while, then stop or grow slowly for years, and then have a burst of growth. Hormonal changes may play a part in this variation.

In considering growth rates of acoustic tumors, it must be remembered that many, if not the majority, of the total number are incidental postmortem findings on temporal bone sections and often have been asymptomatic in life.[6] With the advent of sophisticated screening and less invasive methods of diagnosis, some of these tumors may be entering clinical series. It is known that the incidence of tumors found in different areas of the same country seems to vary with the effort made to find them.[7]

Assessment of the cellular growth rate may help in determining the correct strategy for managing these tumors at different stages of life. It could also allow us to determine whether there is a continuous spectrum of growth rates or distinct groups of tumors. Modern cytologic techniques may provide the answers. Rasmussen et al.[8] have used flow cytoflurometric DNA analysis to quantify the proportion of cells in the "S" phase of the cell cycle but did not find any correlation with duration of symptoms and age or size of the tumor, although the highest "S" phase percentage was in the only recurrence tested. Wennerberg and Mercke[9] also used flow cytometry to determine the "S" phase percentage. The variation was great (range, 1.4 to 19.9%) but did not correlate with tumor volume as measured on the preoperative scans.[9] Our study uses the Ki-67 monoclonal antibody to label proliferating cells. It is designed to see if this technique can be applied to acoustic neuroma and other benign skull base tumors and determine if proliferation is related to clinical course or operative findings.

METHODS

The monoclonal antibody Ki-67 used has been characterized as reacting with a human nuclear antigen expressed in the "G1," "G2," "S," and "M" phases of the cell cycle.[10] The staining pattern is similar to that observed for DNA polymerase alpha.

We used an immunohistochemical method that has been previously described.[11,12] To determine the percentage of stained nuclei, ten high-power fields (magnification ×250) per case were randomly chosen and all nuclei counted. The number of nuclei stained by the Ki-67 antibody was expressed as a percentage of the total count. An example high-power field is shown in Figure 1.

MATERIALS

Thirteen skull base tumors were studied. Of these, eight were acoustic tumors and the other five were miscellaneous tumors, as summarized in Table 1. The acoustic tumors were all smaller than 2 cm, with the exception of one that was referred after a recurrence following previous surgery. The clinical picture of each tumor was studied for length of symptoms, progression of symptoms, and operative findings (Table 2). These tumors form a relatively homogenous group with very little to differentiate between them on a clinical level, all having short histories and being of similar sizes. The exceptions are one tumor with the unusual symptom of hemifacial spasm and one that was invading the VIIth nerve at surgery. The tumors all had some evidence of progressing symptoms although it is difficult to be certain that this represents a change in size. Only four of the tumors had significant periods of time between radiologic test studies, to give an approximate growth rate using this measure. Conventional histo-

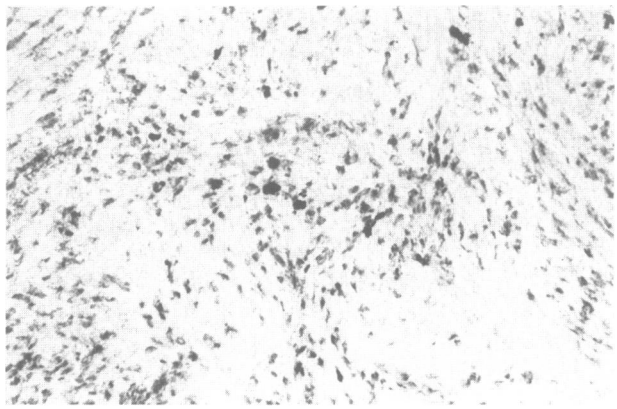

Figure 1. Photomicrograph of the Ki-67–stained proliferating cells in an acoustic tumor. Case 10. (Final magnification, ×330.)

Table 1. Miscellaneous Tumors

Case	*Sex/ Age (yr)*	*Lesion*	*Size (cm)*	*Ki-67 (%)*
1	M/48	Trigeminal schwannoma (invading bone)	5 × 4	2.59
2	F/17	Parapharyngeal neurofibroma (neurofibromatosis 1)	10 × 5	0.87
3	M/64	Facial schwannoma	1.4 × 1.4	0.63
4	M/31	Vagus schwannoma	2.5 × 1.5	0.63
5	M/40	Hemangioma (internal auditory meatus)	0.5	0.26

Table 2. Acoustic Tumors

Case	Sex/ Age (yr)	Symptoms	Duration	Size (cm)
6	M/29	Progressive deafness Mild tinnitus Slight disequilibrium	7 yr	1.6
7	F/33	Increasing deafness Severe disequilibrium	4 yr	1.0
8	F/38	Deafness Tinnitus Hemifacial spasm	1 yr 1 month 3 yr	1.3
9	M/38	Severe tinnitus Slight deafness Occasional dizziness	2 yr 1.5 yr	1.5
10	F/49	Tinnitus Slight deafness	1 yr 1 yr	1.5
11	M/51	Increasing tinnitus	3 yr	1.3
12	F/55	Recurrence of tumor after suboccipital removal	6 yr	4 × 6
13	M/63	Severe tinnitus	4 yr	0.4

logic examinations showed that all the acoustic schwannomas contained areas of Antoni type A and B tissue except patient 8, which contained only type A and was more cellular than the other tumors. They were all benign, with no mitosis or abnormal nuclei.

RESULTS

The Ki-67 assessments of proliferation rate are shown in Tables 1 and 3. Ki-67 value is expressed as a percentage of proliferating cells compared with total number of cells in all of the high-power fields counted. For the acoustic tumors, they range from 3.15 to only 0.36%. The tumors with the higher rates are those that are known to be enlarging on the limited radiologic evidence available. The tumor that is known to be growing only very slowly has the lowest Ki-67 percentage. There is a separation of the acoustic tumors into two groups by the Ki-67 value.

The miscellaneous tumors have a range in values between 2.59 and 0.26%. The very low percentage of 0.26% may represent no overall growth because the tumor was found to be a small hemangioma.

Table 3. Ki-67 Values for Acoustic Tumors

Case	Ki-67 (%)	Clinical Details	Growth
10	3.15	Invading VII nerve (3 mm in 3 mo)	1.2 cm/yr
12	2.75	Recurrence	1.0 cm/yr
8	2.33	Hemifacial spasm	1.1 cm/yr
7	1.64		NA
9	0.58		NA
11	0.49		NA
6	0.36		NA
13	0.36	Intrameatal (4 mm in 4 yr)	0.1 cm/yr

NA: not available.

DISCUSSION

The value of the Ki-67 monoclonal antibody test has been established in surgical neuropathology as a method for evaluating the proportion of proliferating cells.[11,13] The method does have its limitations, with errors arising from sampling only the small fragment of tumor available for frozen section. The advantages over other labeling methods, such as tritiated thymidine and bromodeoxyuridine, are that it does not need to be injected preoperatively and labels all of the proliferating cells rather than those just in the "S" phase.[14] In slow-growing tumors such as those studied here the proliferation rate is a good indicator of overall tumor growth, the proportion of cells dying being minimal. As can be seen in Table 3, the acoustic tumors do appear to have been divided into two groups by the Ki-67 percentage, one with a mean value of 0.45% and the other with a mean value of 2.47%, a proliferation rate on average five and a half times higher in one group than the other. Statistical analysis using Fisher's test confirms that they are different groups ($p > 0.05$). Three of the four tumors that fall into the higher rate group have some unusual features that could represent aggressive growth. One was invading the facial nerve and had radiologically increased by 3 mm in 3 months (equivalent to 1.2 cm/yr); another caused the unusual symptom of hemifacial spasm and had an otherwise short history. This latter tumor's histologic appearance was unusually cellular. The growth rate in diameter was 1.1 cm/yr but is possibly inaccurate because two different imaging modalities were used at different hospitals. The third tumor is a recurrent tumor 6 years after total removal by the suboccipital approach. The fourth patient had no distinctive features except marked disequilibrium. The other tumors, also being of short duration, did not cause any aggressive signs or symptoms. Table shows how the Ki-67 test has also ranked the miscellaneous tumors in order of aggressiveness. The trigeminal schwannoma of the infratemporal fossa, which although cytologically benign behaved very aggressively in invading the bone of the skull base, was at the top, and at the bottom was the nongrowing hemangioma of the internal auditory canal. It therefore appears that this test may be able to differentiate the more aggressive skull base and acoustic tumors from those with a less aggressive growth pattern. This has previously been shown for pituitary adenomas, which have two groups distinguishable by conventional histologic features and clinical growth patterns. The noninvasive adenomas have a mean Ki-67 value of 0.60%, whereas invasive adenomas have a mean value of 1.15%.[12] The Ki-67 values in the present study point to there also being two different growth patterns in the acoustic tumors, with one having

five times as many proliferating cells. Since there is no other histologic evidence for comparison to confirm this difference, we must look to the in vivo evidence from cases studied clinically.

There is some support for the hypothesis of distinctly different growth rates in the literature, both from the long-term results of incomplete surgery and also from series followed by radiologic means. The results of partial removal were well documented in the 1940s and 1950s.[3,15,16] For example, 25 of Olivecrona's 83 partial removals survived with no evidence of disease (average follow-up, 12 years), whereas others had rapid recurrences.[3] A more recent retrospective study from the Mayo Clinic confirms this variation, with 14 of 26 partial removals surviving with no evidence of disease (follow-up, 14 to 21 years).[17] This points to the existence of two different groups of tumors. Olivecrona was the first to comment that the nature of the tumor rather than the amount left behind dictated the subsequent fate of the patient. The hypothesis has been that surgery in some way alters the growth rate of the tumor. Interference of the blood supply has been considered a possible cause.[17] There is no direct evidence for this, and it is hard to believe that a tumor that has a propensity for producing new blood vessels in its surroundings can be stripped of its supply by removing its center. An alternative explanation for these tumors not recurring is that they had stopped growing anyway, or at least the part left behind had. The surgery need not have altered the growth pattern that was destined for that subgroup that did not recur.

Studies of clinical courses do not really help in determining growth rates. There is a vast variation in length of history, and this does not correlate at all with size of tumor.[18,19] Audiologic progression is similarly of little use in assessing rates of growth. Clinicopathologic study has isolated one group of tumors with a different course.[18,20] Some of the intracanalicular tumors are thought to form a separate group that does not enlarge to cause symptoms beyond the VIIIth nerve. These could be the tumors that, were it not for the current active searching, would not have been seen until postmortem. Flow cytometry has shown that the greatest variation in "S" phase percentage was in these smaller tumors when taken as a group, but no relationship was mentioned with clinical course.[9] This variation may represent different growth rate groups.

The advent of computerized tomography and magnetic resonance imaging has allowed in vivo quantification of growth rates, although the accuracy in following a tumor using the current measurements has been drawn into question.[21] The studies that have made use of these methods to determine a growth rate have been mainly directed at elderly patients and those with long histories prior to presentation and are therefore a very biased population. Nevertheless, they do provide some useful indicators of growth rates. The results of articles dealing with the radiologic assessments of growth rates are summarized in Table 4. These studies do not all use the same methods of measurement or even the same radiologic modalities and are only broadly comparable.

Table 4. Radiologic Assessment of Growth Rates

Reference	*Not Operated**	*Operated**
Wazen et al[22]	No growth (1)	No growth (11)
	0.2 cm/yr (5)	0.2 cm/yr (1)
	0.9 cm/yr (2)	1.0 cm/yr (1)
Silverstein et al[23]	No growth (3)	No growth (11)
	0.2 cm/yr (5)	0.2 cm/yr (2)
	1.0 cm/yr (1)	1.0 cm/yr (2)
		"Rapid" (1)
	(Some overlap of patients with Wazen et al)	
Shea et al[4]		No growth (4)
		0.15 cm/yr (2)
Zollner and Brockenheimer[24]	No growth (1)	
	0.3 cm/yr (2)	
Gardner et al[25]	No growth (4)	
	0.2 cm/yr (2)	
Nedzelski et al[26]	0.02 cm/yr (6)	>3 yr follow-up
	0.2 cm/yr (15)	<3 yr follow-up
	Decrease (2)	
Tos and Thompsen[19]	Growing (3)	
	No growth (18)	
Valvassori and Guzman[27]	Growing <20% increase (12)	
	<50% increase (6)	
	>50% increase (2)	
	No growth (15)	

*Figures in parentheses are number of patients (total, 136).

There appear to be three different and to a great extent separate tumor growth rates: (1) Those that do not grow, regress, or grow at a rate of 0.02 cm/year, taking 50 years to reach 1 cm; (2) those that grow at 0.2 cm/year; and (3) those that grow at around 1.0 cm/year. There may be a fourth growth rate, since some of the larger tumors appear to grow at a rate equivalent to 2.5 cm/yr. However, this may be an artifact due to the use of diameter measurements in the larger lesions. Tumors growing at this rate are not going to be followed for long because the symptoms will demand intervention.

The tumors analyzed by the Ki-67 test in this study fall into the second and third growth rates. These "rates" of increase in size are stated only as an indication of there being separate groups rather than a continuous spectrum. A more realistic measure of growth is increase in volume as calculated from sequential scans. This has also shown distinct groups, one with a doubling time of 205 to 545 days and the other from 1090 days to no growth.[28] This is an approximate fivefold difference between the average of these two rates.

It is also apparent that the size of a tumor at presentation does not seem to relate to the subsequent growth rate in a series of 21 tumors,[26] a fact that points to there being varying rates of growth in the life of a tumor. There is also a general clinical feeling that tumors do indeed change growth rates during the life of the patient, and there are some well-documented cases in the literature in which this

has occurred.[19] What stimulation causes these benign tumors to change growth rates is as yet unknown.

Of the tumors followed in the literature, 60% are in the very slow to no growth group, 30% in the 0.2 cm/yr, and 10% in the 1.0 cm/yr. This distribution is biased by the selection of cases to be followed, since the faster growing tumors will not be followed. Nevertheless, this information leads to the temptation to watch a tumor to see if it is growing or static. There are problems with this approach. Small tumors less than 1.5 cm appear candidates for watching, but they can be removed via the transotic approach with minimal morbidity (99% preservation of facial nerve function).[29] Should they be left to enlarge to 2 cm, the risk to the facial nerve is much greater (60% preservation). It seems therefore prudent to remove such tumors without delay. Tumors that present already greater than 1.5 cm have less to lose because the morbidity and indeed mortality of surgery tend to plateau until the tumor has reached 2.5 cm. It is reasonable to watch tumors presenting between 1.5 cm and 2.5 cm to see which growth rate they have.

Once a particular growth rate has been established for a patient, this must still be followed, since there is the continuing risk of a rapid increase occurring. If the change in growth rate is found to be gradual, then some greater leeway becomes available, but it can suddenly start to grow at 2.5 cm/yr, so follow-up will have to be very frequent.

CONCLUSION

The Ki-67 test has identified two of the growth rates that occur in acoustic tumors. The higher rate is some fivefold the lower rate. This adds another piece of evidence for the existence of differential growth rates. The main body of evidence for this is emerging from radiologic studies, although we must await long-term studies to show if these groups are really distinct or part of a spectrum. Such studies are also needed to determine what proportion of tumors change growth rates in the life of a patient.

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