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Cellular schwannoma

A clinicopathologic study of 29 cases

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Summary. A series of 29 cellular schwannomas is described in terms of their clinical presentation and course, light and electron-microscopic appearance, immunohistochemical properties and cytogenetics. The study indicates that cellular schwannoma can be defined as a subtype of classical schwannoma, characterized by spindle cells forming a compact fascicular, sometimes fibrosarcoma-like growth pattern, a low mitotic activity, a generally moderate nuclear and cellular polymorphism and a high degree of Schwann cell differentiation as seen by electron microscopy and immunohistochemistry. The tumour is characteristically located close to the vertebral column, in the mediastinum or retroperitoneum and has a benign course. Occasionally bone destruction and neurological symptoms develop. The clinical appearance together with the high cellularity, fascicular pattern and mitotic activity had led to the erroneous diagnosis of a soft tissue sarcoma in a few cases, and cellular schwannoma may thus be considered to be a pseudosarcoma. Immunohistochemically, cellular schwannomas appear to deviate from classical schwannomas and malignant peripheral nerve sheath tumours by their expression of glial fibrillary acidic protein. The chromosome analysis revealed a normal diploid stemline karyotype, with a variety of abnormal clones, including one with monosomy 22.

Key words: Neurilemoma – Pseudosarcoma – Electron microscopy – Immunohistochemistry – Cytogenetics

Introduction

The term cellular schwannoma has been used to designate a tumour that shares some gross and microscopic features of classical benign schwannoma*, but is distinguished histologically by the dominance of a cellular and fascicular spindle-cell component, with a certain mitotic activity (Woodruff et al. 1981; Fletcher et al. 1987). These features, together with a moderate cellular and nuclear polymorphism and the capacity of the often mediastinal or retroperitoneal paravertebral tumours sometimes to erode adjacent bone, have led in some instances to the erroneous diagnosis of a soft tissue sarcoma. However, the clinical outcome of the cases hitherto studied has indicated the benignity of the tumour (Woodruff et al. 1981; Fletcher et al. 1987). It has been suggested that cellular schwannoma should be included in the group of so called pseudosarcomas (Angervall et al. 1986; Fletcher et al. 1987). The ultrastructural features described by Woodruff et al. supported a Schwann cell differentiation of the tumours, as did the immunohistochemical finding of positivity for S-100 protein reported by Fletcher et al. in their series of 18 cases (1987). Two cellular schwannomas were also mentioned in an immunohistochemical and electron microscopic study of the expression of intermediate filaments in nerve sheath tumours (Gould et al. 1986).

Cytogenetic analysis has previously been performed on a few classical schwannomas (Ray et al. 1987), but, to the best of our knowledge, no reports describing the cytogenetics of cellular schwannomas are available.

The present study reports the clinical, light and electron-microscopic and immunohistochemical findings in a series of 29 patients with cellular schwannoma, with a long-term follow-up. In one case, a light and electron-microscopic and chromosomal analysis was also carried out on tumour cells cultured in vitro.

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* The term schwannoma is used as a synonym for neurilemoma in this paper

Materials and methods

At a review of mediastinal tumours operated upon at the Department of Thoracic Surgery, Sahlgren Hospital, Gothenburg, Sweden during the period 1967–1986, 20 cases fulfilled the criteria for cellular schwannoma. Nine additional cases, 7 of which had a paravertebral location in the retroperitoneum or pelvis were collected from the files of the Department of Pathology at the same hospital, making the total number of cases 29. For comparison, 8 classical schwannomas and 8 malignant peripheral nerve sheath tumours (PNST) arising from a medium-sized or large peripheral nerve, from neurofibromas or in patients with von Recklinghausen's neurofibromatosis were selected.

For light microscopy new sections were prepared from paraffin blocks and stained with haematoxylin-eosin and the trichrome staining according to van Gieson (the haematoxylin-van Gieson method).

To estimate the mitotic activity in the cellular areas, the number of mitoses was counted in 20 high power fields (objective × 25 and ocular × 10). By measuring the visual field it was possible to estimate the mitotic frequency per mm^2.

The antibodies used in immunohistochemistry, their respective working dilutions and control tissues are listed in Table 1. The avidin-biotin complex (ABC) method (Hsu et al. 1981) was performed using the Vectastain ABC-kits (Vector Labs, Burlingame, CA, USA) according of the instructions, except for a prolonged incubation time for the primary antibody (16 h at 4° C). The end products were visualized by treating the sections with a freshly made solution of 0.05% diaminobenzidine tetrahydrochloride with 0.02% H_2O_2. The antibodies and the chromogenic substrate were diluted in Tris buffer saline (TBS) (0.05 M, pH 7.4). For the study of collagen IV, the ABC-technique was applied with prior digestion with trypsin (Sigma Chemical Company, Mo, St. Louis, USA) in 0.05 M Tris buffer saline containing 0.1% $CaCl_2$ (pH 7.8) for 20 min at 37° C. In all instances the controls were positively stained. The classical schwannomas and the malignant PNST were only stained for glial fibrillary acidic protein (GFAP) and S-100 protein.

Table 1. Antibodies used in the study, their working dilutions and control tissues

Antibody	Working dilution	Control tissue
Polyclonal anti-S-100 protein[a]	1:2000	Schwann cells of nerves
Monoclonal anti-vimentin[b]	1:100	Endothelium, fibroblasts
Polyclonal anti-glial fibrillary acidic protein (GFAP)[b]	1:1000	Brain
Polyclonal anti-collagen IV[c]	1:300	External laminae of vessels
Monoclonal anti-chromogranin[d]	1:2500	Carcinoids
Monoclonal anti-neurofilament[e]	1:4	Axons of peripheral nerves
Monoclonal anti-epithelial membrane antigen (EMA)[b]	1:100	Breast carcinoma

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Electron microscopy was performed in 13 cases. In three cases, small pieces of fresh tumour tissue were placed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, fixed for 1 h in 1% OsO_4 in cacodylate buffer, dehydrated in ethanol, embedded in Agar resin and cut in a LKB Ultrotome. In 8 cases, only formaldehyde-fixed tissue was available. Small pieces from these specimens were fixed for 1 h in 1% OsO_4 in cacodylate buffer and subsequently processed as described above. From two tumours, small pieces were selected and cut out from the paraffin blocks, carefully deparaffinized in xylene, rehydrated in decreasing concentrations of alcohol, washed in cacodylate buffer, fixed in 1% OsO_4 and dehydrated in ethanol. They were then embedded as described above. 1 μm thick sections were stained with toluidine blue, and ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 electron microscope.

Fresh tissue from one tumour was minced into small pieces of 1–2 mm in diameter and subsequently digested in a collagenase solution (1000 U collagenase/ml, 146 mM NaCl, 4 mM KCl, 5 mM $CaCl_2$, 20 mM Hepes pH 7.3 and 0.1% glucose) for 18 h at 37° C. The tumour cell suspension was washed twice in fresh culture medium after which the pellet was resuspended in Eagle's alfa MEM supplemented with 10% fetal calf serum and 200 mM L-glutamine, and explanted in 75 cm^2 tissue culture flasks. Cells were also seeded in small petri dishes for ultrastructural analysis. The cells were grown in humidified 5% CO_2/95% air at 37° C. Cells and tissue fragments were allowed to adhere to the bottom of the culture flasks overnight. The following day, the culture medium was changed to remove non-attached cells and tissue fragments. The culture medium was then changed twice a week until harvest.

The growth medium was removed from the petri dishes and the monolayer of cells was fixed in 3% glutaraldehyde for 30 min at 20° C, post osmificated (1% OsO_4 for 30 min) and thereafter routinely processed for resin embedding in the dishes. The monolayer of tumour cells embedded in the resin was loosened mechanically from the bottom of the dishes. Thereafter small blocks were sawn out. 1 μm toluidine blue stained sections were used to study the in situ morphology under the light microscope. Ultrathin sections were prepared and routinely contrast stained with uranyl acetate and lead citrate.

Chromosome preparations from short-term cultures were performed as soon as a sufficient number of mitoses were available. Sixteen hours before harvest colchicine was added to a final concentration of 0.02 μg/ml. The methods used for chromosome preparation and subsequent G-banding were those described by Stenman and Mark (1983). Magnified photographs were used for the karyotype analysis. The nomenclature follows that of ISCN (1985).

Results

Clinical data

There was a strong female predominance, 21 (72%) women and 8 (28%) men. The median age of the patients at the discovery of the tumours was 55 years (15–79 years). In 21 cases (72%), the tumours were discovered accidently at chest x-ray, abdominal- or gynecological examination carried

out either at routine health examinations or follow up of other diseases. In these cases, the patients had not experienced any symptoms whatsoever. In three cases the tumours were discovered when the patients sought medical attention because of symptoms such as chest pain and fatigue, which in retrospect seem unlikely to have been caused by the tumours. Three patients had neurological symptoms; two with tumours arising in spinal nerves (lumbago, rhizopathy) and one with a tumour in the tibial nerve (paresthesia). One patient with a large, dorsally located mediastinal tumour experienced dysphagia. Two patients had superficially located tumours which were discovered as palpable lumps. In twenty-seven patients, the tumours were located posteriorly in the trunk, in close association with the vertebral column. These tumours had a median size of 6 cm (2–12 cm). Twenty of these tumours were located in the mediastinum and 7 in the retroperitoneum or pelvis. The two superficially located tumours (ankle and neck) measured 1.5 cm and 2 cm respectively. All the tumours were removed surgically. Usually, the tumours, including those with nerve association, could be enucleated by blunt dissection. At surgery, 9 were considered to arise from an identifiable nerve structure; 5 from a spinal nerve, two of which extended into the dura and formed so called dumbbell tumours, two from the thoracic part of the sympathetic trunk and one from the 2nd intercostal nerve and the tibial nerve respectively. In one case, extensive tumour destruction of bone could be demonstrated radiologically and at surgery (Fig. 1). In two cases, two closely located but separate lesions were detected. The follow-up data is summarized in Table 2. No deaths were caused by the tumours and no local recurrence or metastasis occurred in any of the patients. One patient in our series had von Recklinghausen's neurofibromatosis. She had had numerous cutaneous and deep neurofibromas and schwannomas, some of which were of the cellular type, removed over a 17-year period.

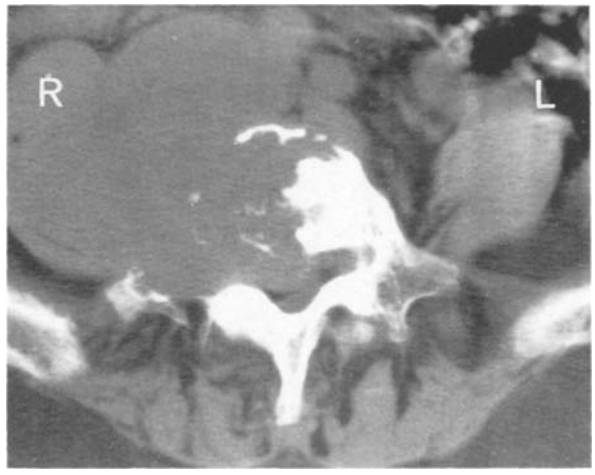

Fig. 1. Computed tomography showing a large retroperitoneal cellular schwannoma with advanced destruction of the body of the 5th lumbar vertebra, in a 71-year old woman. The tumour, which had initially been asymptomatic, had been discovered at a routine gynecologic check-up three years earlier. The initial computed tomography led to the suspicion of a schwannoma, and the patient underwent repeated computed tomographies, which showed a slow tumour growth and slow yet progressive vertebral destruction. Due to the development of neurologic symptoms and the subsequent advanced vertebral destruction, the patient underwent surgery and the multilobulated tumour, 8 cm in diameter, apparently originating from an adjacent spinal nerve, was enucleated. Apart from a slight decrease in sensation in the right leg, the patient is currently in good health, 18 months postoperatively

Table 2. Follow-up data ($n=28$)[a]

Median duration	7 ys (3 months–19.5 years)
Outcome	*n*
Alive, no evidence of disease	25
Alive, von Recklinghausen's neurofibromatosis	1
Dead from tumour	0
Dead from unrelated disease	2
Local recurrence	0
Metastasis	0

[a] one patient was lost to follow-up

Gross appearance

The gross appearance of the tumours was uniform, all being well demarcated and seemingly encapsulated. They were rather firm and usually round or ovoid in shape. The cut surfaces were greyish-white in colour, often showing irregular brown or yellow patches and sometimes had a whorled appearance. Cyst formation and/or areas of haemorrhage were sometimes seen, but necrosis was not found.

Light microscopic appearance

All the tumours were predominantly cellular and the cells were mostly spindle-shaped. The cells were usually arranged in distinct fascicular bundles, sometimes forming a "herring-bone"-like pattern, giving the tumours a fibrosarcoma-like appearance (Fig. 2A, B). Sometimes a whorled or storiform pattern was seen. Distinct areas of Antoni-A and Antoni-B type tissue were not evident. However, many tumours showed a tendency towards a biphasic pattern including areas of a loose, myxoid

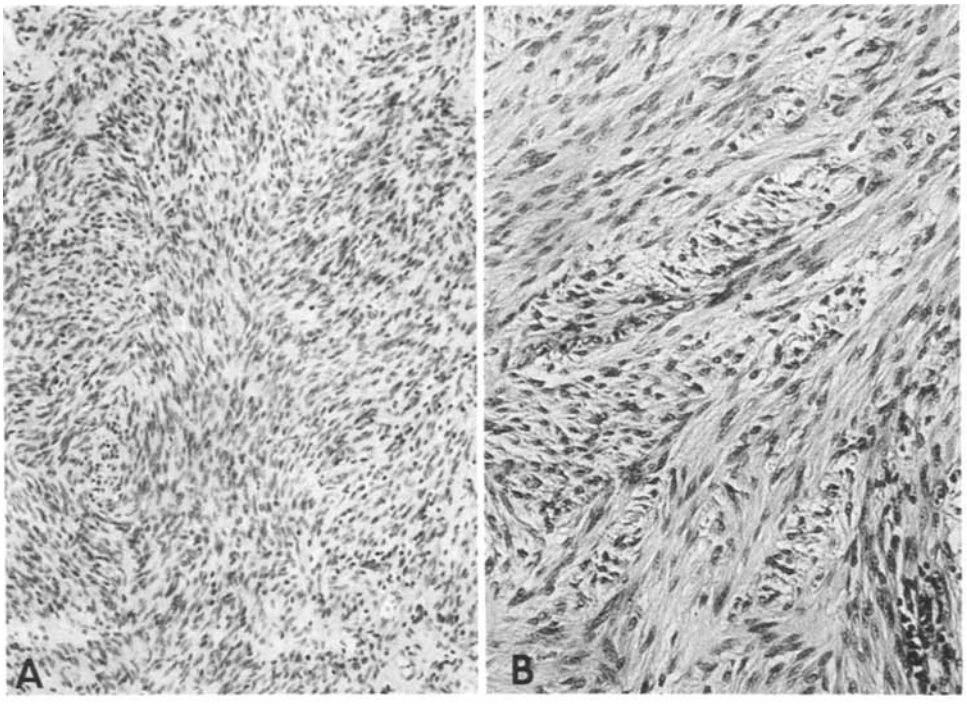

Fig. 2 A, B. Characteristic cellular area with spindle-shaped tumour cells arranged in a cellular, dense fascicular pattern; in areas there is a tendency towards a herring bone-like arrangement of the tumour cells, creating a resemblance to fibrosarcoma. H & E, ×240

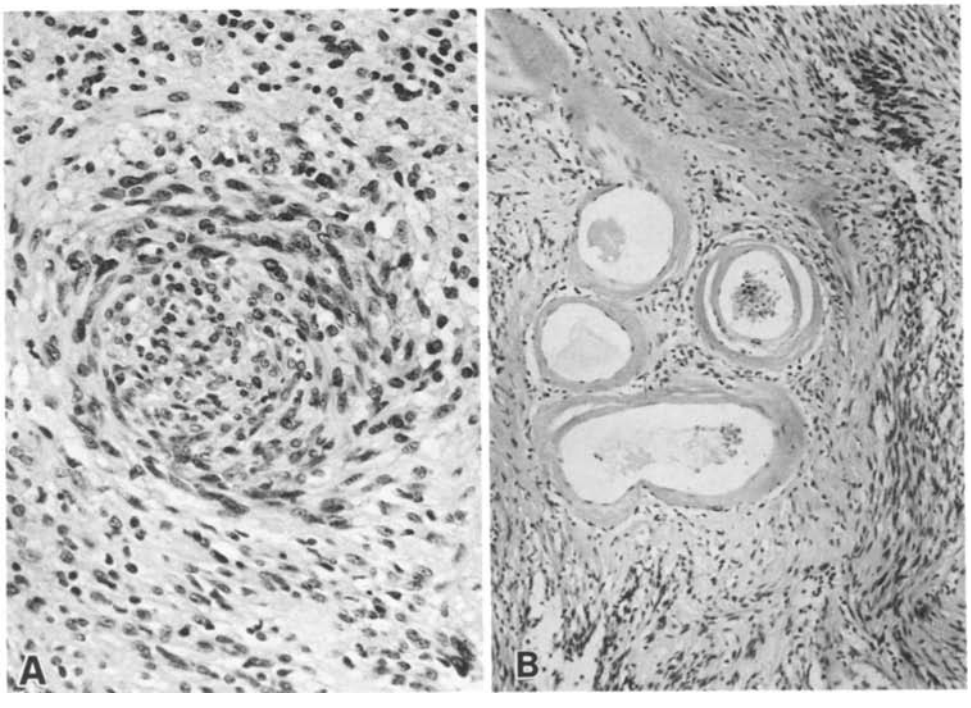

Fig. 3. Tumour cells arranged in a whorled pattern, so-called neural whorl (**A**). Tumour vessels with hyaline thickening of the walls within a less cellular area, similar to the picture seen in classical schwannoma (**B**). H & E. **A** ×240, **B** ×100

tissue indistinguishable from the Antoni-B tissue of classical schwannomas within the dominating compact cellular fascicular areas. True Verocay bodies were not seen, but in many tumours there was a certain rhythmical arrangement of the nuclei in areas and so called "neural" whorl structures (Fig. 3A). The lesions displayed vessels of varying sizes, most of which showed hyaline thickening of the wall, as in classical schwannoma (Fig. 3B). The tumour cells were spindle shaped, sometimes extremely elongated and slender, with wavy contours. The nuclei were oval, rather dense and sometimes cigar-shaped. Some of the tumours showed varying degrees of degenerative changes, mainly cyst formation, minor foci of haemorrhage and an abundance of foam cells. In these tumours, and

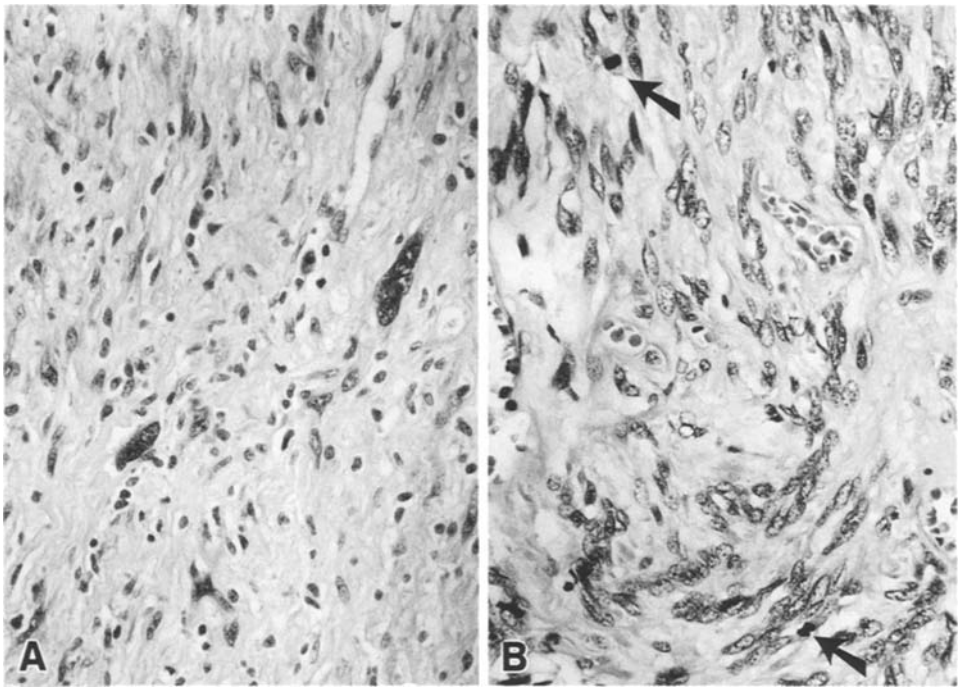

Fig. 4. Tumour cells with large hyperchromatic and polymorphous nuclei, considered to be a degenerative phenomenon (**A**). Two mitotic figures are indicated by arrows (**B**). H & E, ×240

to a lesser degree in others, there was some cellular and nuclear polymorphism, similar to that seen in the so called ancient neurilemoma (Fig. 4A). Mitotic activity ranged from 0–0.2 mitoses/mm^2 (0–4/20 examined fields) (Fig. 4B). No atypical mitoses were detected. In many of the tumours there were clusters of inflammatory cells, mainly lymphocytes, within the capsule and along the tumour margin. Similar clusters of often perivascularly arranged lymphocytes were also observed occasionally centrally within the tumours. Areas of necrosis were not seen. All lesions were well demarcated and the vast majority were encapsulated by a thin fibrous membrane. Even in tumours which appeared to have arisen from major nerves at surgery, few normal nerve structures were associated with the tumours microscopically. The lesion which had caused bone erosion also revealed a fairly sharp demarcation.

Electron microscopic appearance

Ultrastructurally, the tumour cells of the 13 studied cases were fusiform, sometimes with extremely elongated bipolar cytoplasmic extensions. The single nucleus was usually oval or cigar-shaped and had fairly even contours. In a minority of the tumours the nuclei were highly clefted and irregular, with a polymorphous appearance. The nuclei usually contained one centrally located nucleolus. The heterochromatin was usually arranged in irregular condensed clumps along the periphery of the nucleus. Occasional tumour cells had very prominent large heterochromatin lumps distributed throughout the entire nucleus (Fig. 5A). The cytoplasm was fairly poor in organelles, of which polysomes were the most prominent, while rough endoplasmic reticulum (RER) as well as mitochondria were relatively sparse. An abundance of cytoplasmic intermediate filaments was seen in all the tumours. These were mostly arranged in a reticular network, but sometimes formed condensed distinct bundles. Microtubules were occasionally observed. Degenerative changes, including an abundance of lysosomes and lipid droplets, were frequent findings. Multiple centrioles were seen in 5 cases and in 4 cases cilial structures were observed (Fig. 5D). The most striking ultrastructural feature of the tumour cells was the richness of fine interdigitating cytoplasmic projections. The cytoplasmic membrane was lined by a sometimes multilayered external lamina (Fig. 5B). In areas of closely apposed cytoplasmic projections, the external lamina was discontinuous and usually single-layered, or completely absent. There were frequent cell junctions, mainly of desmosome-like type. The tumour cells were tightly packed in the cellular areas, leaving little room for collagen and intercellular substance. In looser areas there were moderate amounts of intercellular collagen, which in three instances was of the long-spacing type (so-called Luse bodies). In one tumour axon-like elongated structures, poor in organelles, were surrounded by cytoplasmic pro-

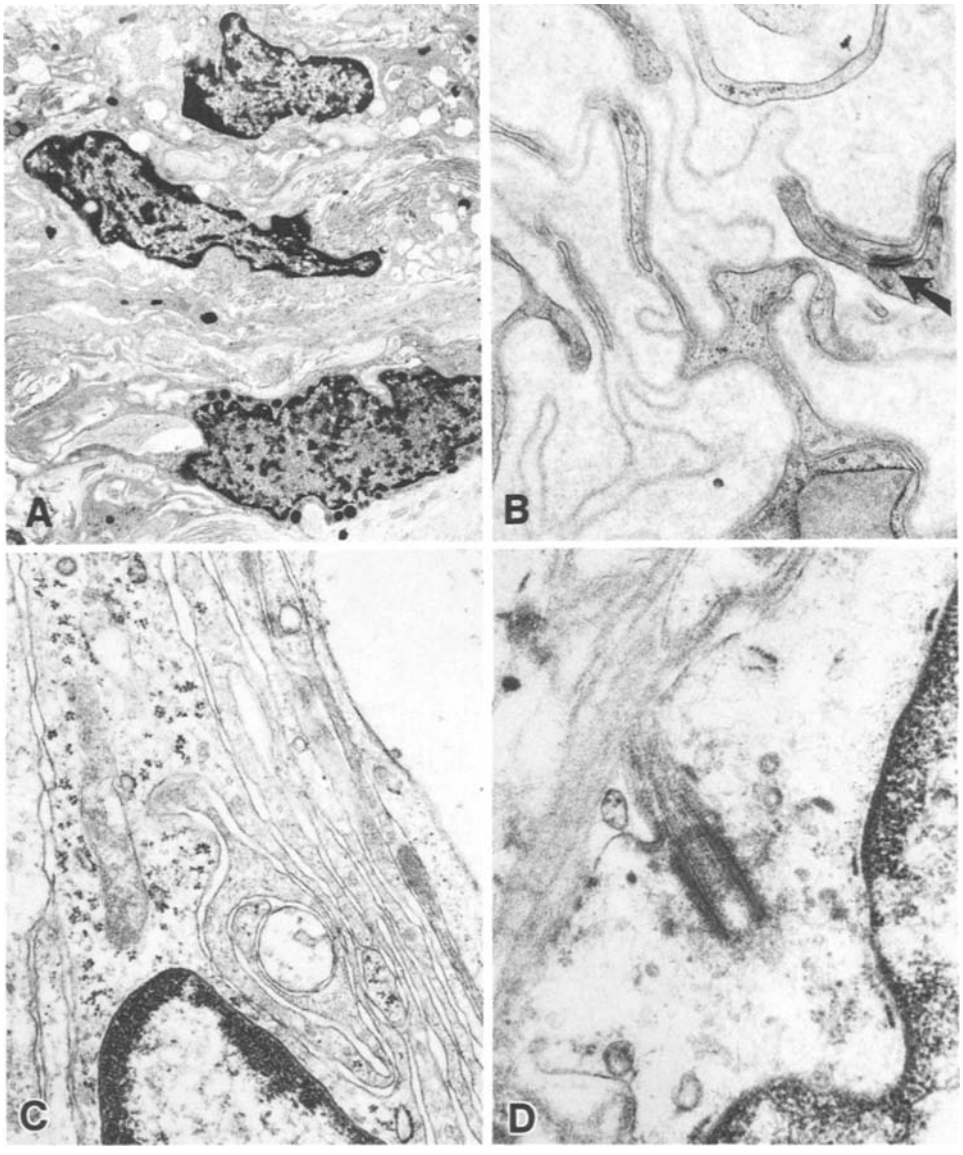

Fig. 5. Elongated tumour cells with somewhat polymorphous, clefted and irregular nuclei and long, slender, interdigitating cytoplasmic projections. The heterochromatin is mainly condensed along the nuclear membrane and forms some clumps within the centre of the nuclei (**A** × 5500). The interdigitating cytoplasmic projections are outlined by distinct external lamina material. A desmosome-like junction is seen (*arrow*) (**B** × 21 500). Cytoplasmic projections of tumour cells giving rise to a so-called pseudomesaxon formation (**C** × 25 000). A cilium-basal body at the cytoplasmic membrane, which is covered by multiple layers of external lamina (**D** × 58 000)

jections, resembling so-called pseudomesaxon formations (Fig. 5C). Myelin-like bodies were observed in one tumour. Melanosomes or premelanosomes were not observed.

Immunohistochemistry

The results of the immunohistochemical analysis are summarized in Table 3. In all the tumours but two, the vast majority of the tumour cells revealed a strong cytoplasmic positivity for S-100 protein (Fig. 6A) and in all the tumours but one there was an evenly distributed positivity for vimentin (Fig. 6B). The tumour which was negative for vimentin was also negative for S-100 protein. Intrinsic vimentin controls, such as endothelium and fibroblasts were also negative in this case, indicating that the tissue had been damaged in some way during processing. All the tumours showed some degree of positivity for GFAP. Unlike the staining for S-100 protein and vimentin, the staining for GFAP was much more irregular, varying considerably from tumour to tumour, and from area to area within one and the same tumour (Fig. 7A). Most contained areas where the vast majority of the cells revealed a strong cytoplasmic staining. The GFAP staining enhanced the sometimes extremely elongated shape of the cells, as well as their wavy contours. In 10 of the tumours perivascular enhancement of GFAP staining was noted. In no case were fibroblasts or endothelium positively stained for GFAP. In 19 tumours there was a positive reaction for collagen IV. This staining gave prominence to external lamina material in the tu-

Table 3. Immunohistochemical results

Antigen	Numbers positive	Staining pattern
S-100 protein	27/29	even[a]
Vimentin	28/29	even[a]
GFAP	29/29	uneven[b]
Collagen IV	19/29	uneven[b]
Chromogranin	0/29	
Neurofilament	0/29	
EMA	0/29	

[a] Even intensity and distribution in all positive tumours
[b] Irregular distribution and uneven intensity within positive tumours

mour vessels and to the external lamina material surrounding individual cells and groups of tumour cells (Fig. 7B). The positivity was unevenly distributed. Sometimes a pattern with strong staining in the central parts and weaker staining in the periphery of the tumours was observed and there was generally less staining in the limited areas with loose, Antoni-B-like tissue. In the tumours which were negative for collagen IV external lamina material in the vessels provided positive intrinsic controls. All tumours were negative for chromogranin, neurofilament and epithelial membrane antigen (EMA).

Of the 8 classical schwannomas, two showed a rather extensive positivity for GFAP, while three showed staining of very few scattered cells. The remaining three were negative. All the classical schwannomas were strongly positive for S-100 protein. Of the 8 studied malignant peripheral nerve sheath tumours, none showed any positivity for GFAP, two showed extensive positivity for S-100 protein, while three showed a few scattered positive cells and three were negative.

Light and electron-microscopy of cultured cells

The cell cultures were characterized by a monolayer of spindle-shaped, stellate or polygonal cells with long slender interdigitating cytoplasmic projections. Ultrastructurally, the nuclei had evenly distributed euchromatin and few and small clumps of heterochromatin. There was a single large nucleolus in most nuclei. The cytoplasm contained well developed RER, abundant mitochondria and a network of intermediate filaments. Close to the cytoplasmic borders some condensed bundles of thin filaments of actin type were observed. Scattered cells contained large glycogen deposits and groups of lysosomes. There were no well-developed external laminae, but the external surface of the cytoplasmic membrane was sometimes covered by a poorly defined rim of condensed granular and fibrillary material. The few collagen fibres observed were often enclosed within spaces between interdigitating cytoplasmic projections.

Cytogenetic analysis

A total of 42 cells were karyotyped from two consecutive preparations performed after growth peri-

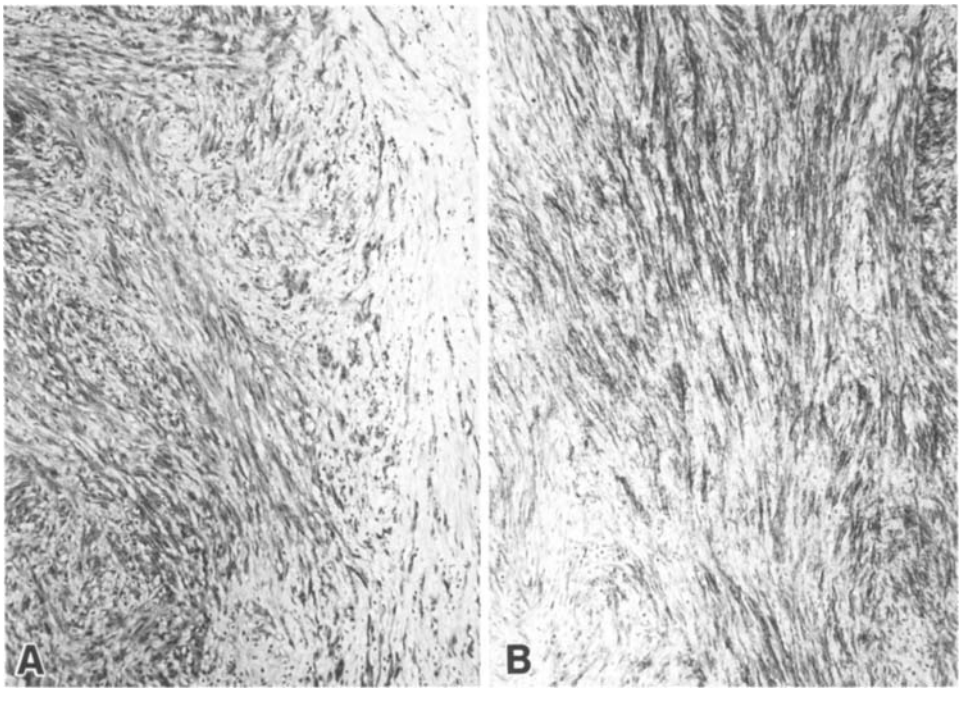

Fig. 6. Strong cytoplasmic positivity for S-100 protein (**A**) and anti-vimentin (**B**) is seen in the vast majority of the tumour cells. The less intensely stained area to the right in Fig. 6A corresponds to a less cellular part of the tumour adjacent to its encapsulation. Immunohistochemical staining, ABC-technique, ×100

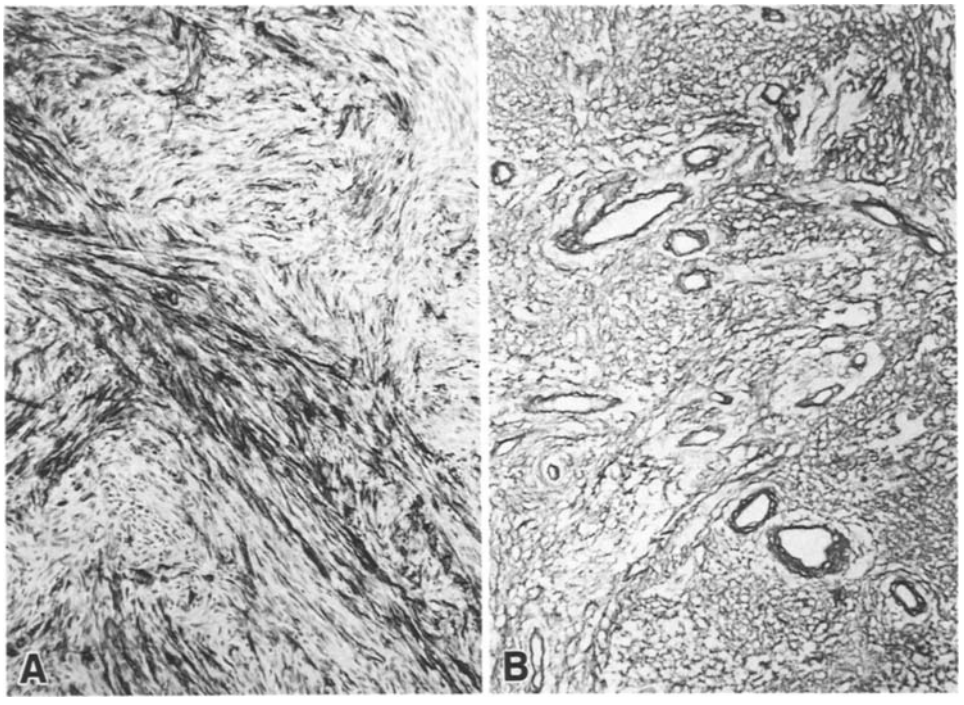

Fig. 7. The positive cytoplasmic staining for GFAP enhances the extremely elongated shape of the tumour cells (**A**). Positive staining for collagen IV, corresponding to external lamina material, is observed around individual cells and groups of tumour cells, and in the vessel walls (**B**). Immunohistochemical staining, ABC-technique, ×100

Table 4. Chromosome counts (superscript figures indicate the number and distribution of karyotyped cells)

	Chromosome numbers								Total cells
	39	41	44	45	46	47	82	91	
Preparation 1	1^1	1^1	5^5	9^6	24^{15}	2^1	1^1	1^1	44^{31}
Preparation 2				2^1	23^{10}				25^{11}

ods of 6 and 9 days, respectively (Table 4). Both preparations showed a diploid stemline without visible rearrangements. The first preparation also contained a multitude of mostly hypomodal variant cells, and clones with different abnormalities. Thus, there were 4 cells with monosomy 15, three cells with monosomy 22 (Fig. 8) and three cells with loss of the X chromosome. Furthermore, there was one hypodiploid and one hypotetraploid cell, both of which had lost two chromosomes 5, and two cells with loss of one chromosome 9. Most cells with the above-mentioned recurrent deviations also contained random losses and gains of single chromosomes. The only structural deviations recorded were two cells with deletion of the entire long arm of chromosome 3, i.e. del(3)(pter→cen:). One of these cells also had a chromosome 1 short arm deletion, del(1)(qter→p21:). One additional cell was monosomic for chromosome 3.

Discussion

The light and electron-microscopic and immunohistochemical findings of the tumours described in the present study all provide support for a Schwann cell derivation. The tumours were mainly distinguished from classical schwannoma by the light-microscopic finding of a predominant component of cellular, spindle-celled, fascicular and sometimes fibrosarcoma-like tissue and from malignant peripheral nerve sheath tumours by the high degree of Schwann cell differentiation observed immunohistochemically and ultrastructurally and the benign clinical course. Thus, the term cellular schwannoma, suggested for this type of tumour by Woodruff et al. (1981), seems well justified.

Light-microscopically, many tumours showed a biphasic pattern with small areas of loose myxoid tissue, indistinguishable from Antoni-B tissue of classical schwannomas, within the dominating cellular, spindle-celled and fascicular areas. The encapsulation of the tumours, the characteristic hyaline thickening of their vessel walls and the presence of areas with a certain rhythmical arrangement of the nuclei, as well as so called "neural" whorls (Woodruff et al. 1981) also produced a resemblance to classical schwannoma. A low, but readily demonstrable, mitotic activity is a feature of this lesion which distinguishes it from classical schwannoma. The moderate nuclear polymor-

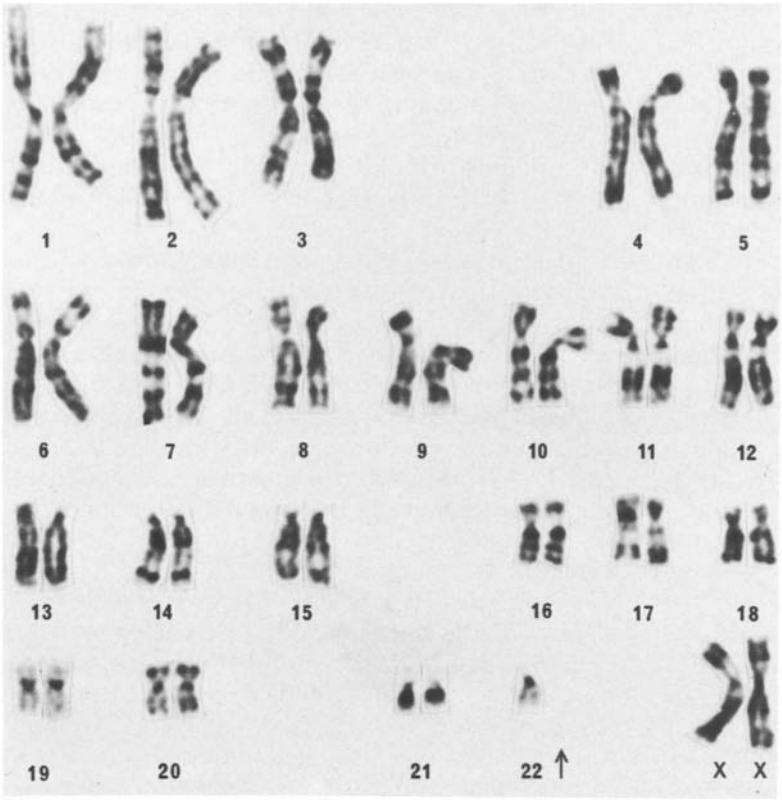

Fig. 8. G-banded karyotype of a hypodiploid cell showing loss of one chromosome No. 22 (*arrow*)

phism found in many instances was similar to that which can be found in old, degenerated schwannomas, so called ancient neurilemoma (Ackerman and Taylor 1951; Dahl 1977), as were the cysts, foci of haemorrhage and groups of foam cells which were occasionally found. However, moderate nuclear and cellular polymorphism were also a frequent finding in tumours lacking other signs of degenerative change.

Cellular schwannomas differ from malignant peripheral nerve sheath tumours (PNST) mainly by their lack of geographical areas of necrosis and by their lower mitotic rate (Woodruff et al. 1981). They also differ in terms of S-100 protein expression. The results of the present series regarding the 8 studied malignant PNST are in agreement with our previous observations and those of others, indicating that a significant proportion of malignant PNST are completely negative for S-100 protein (Nakajima et al. 1982; Weiss et al. 1983; Daimaru et al. 1985; Matsunou et al. 1985; Lodding et al. 1986; Wick et al. 1987). Furthermore, malignant PNST usually show a much less pronounced Schwann cell differentiation seen ultrastructurally than does cellular schwannoma (Erlandson and Woodruff 1982; Dickersin 1987).

Ultrastructurally, the tumour cells presented features of Schwann cells, such as the bipolar, extended shape with numerous interdigitating cytoplasmic projections, which were usually outlined by an external lamina. The ultrastructural findings are thus similar to those seen in Antoni-A areas of classical schwannoma (Erlandson and Woodruff 1982). In some respects, however, the tumours in this series deviated ultrastructurally fom classical schwannoma. For instance, long-spacing type of collagen (Luse bodies) a frequent finding in classical schwannomas (Ghadially 1980; Erlandson and Woodruff 1982; Enzinger and Weiss 1988) was rare, only seen in three of the 13 tumours studied. Furthermore, the extracellular matrix was less conspicuous and the occurence of multilayered external laminae was less frequent than in classical schwannomas. In fact, in many cellular and fascicular areas there were only scattered segments of a discontinuous single layered external lamina separating the cells, and there were closely apposed tumour cells completely lacking any enclosing external lamina. The finding of multiple centrioles in several tumour cells in 5 of the 13 tumours, and the occurence of fully developed cilia in 4 of them is of interest. We have previously encountered oligocilia and cilium basal bodies in classical schwannoma and malignant PNST, including epithelioid malignant schwannoma (Lodding et al. 1986). Melanosomes or premelanosomes were not observed

in any case, but they have previously been described in a single case of cellular schwannoma (Gould et al. 1986).

The immunohistochemical analysis indicates that the prominent networks and bundles of cytoplasmic filaments observed ultrastructurally are composed of vimentin and/or GFAP. Vimentin, which is considered to be the predominant intermediate filament of Schwann cells (Autilio-Gambetti et al. 1982), has previously been demonstrated in classical schwannoma and in the two cases of cellular schwannoma studied by Gould et al. (1986). In contrast to the staining for vimentin, the GFAP staining in our cases was usually unevenly distributed. As the anti-GFAP antibody did not produce staining of the endothelium of vessels, of fibroblasts or of other mesenchymal tissue surrounding the tumours, and the staining distributions for GFAP and vimentin were quite different, cross reactivity of the GFAP antibody with vimentin appears unlikely. Our findings indicate that there are tumour cells in cellular schwannomas which co-express vimentin and GFAP. The presence of GFAP in normal Schwann cells and in the tumour cells of classical schwannomas has been the subject of several studies in recent years, which have produced highly conflicting results (Trojanowski et al. 1984; Memoli et al. 1984; Gould et al. 1986; Iwashita and Enjoji 1987; Stanton et al. 1987; Johnson et al. 1988; Kao et al. 1989; Winek et al. 1989). Of the 8 superficial classical schwannomas we studied for comparison, only two contained many tumour cells positive for GFAP, while three showed staining in only a very limited number of cells. Three contained no positive cells. Kawahara et al. (1988) found that most of their schwannomas which were located in association with the spinal canal, at deep locations in the trunk or close to major joints were positive for GFAP, while the subcutaneous superficial schwannomas were not. Two of their tumours, both of which were positive for GFAP, were described as "cellular", and were located in the spinal canal. GFAP has previously been demonstrated in normal Schwann cells of the sciatic nerve (Yen and Fields 1981; Jessen and Mirsky 1984), the vagus nerve and sympathetic nerves (Yen and Fields 1985). Non-myelin forming Schwann cells of spinal nerve roots and the sympathetic nerve trunk have also been shown to contain GFAP (Jessen and Mirsky 1984), and it has been suggested that Schwann cells which do not take part in myelin formation are more likely to express GFAP (Yen and Fields 1985). Our findings appear to support the theory proposed by Kawahara et al. (1988) that schwannomas which arise in deep, central locations may be more liable to express GFAP than others, since they may arise from non-myelin forming Schwann cells. GFAP has not been demonstrated immunohistochemically in malignant PNST (Memoli et al. 1984; Trojanovski et al. 1984; Gould et al. 1986) and was not demonstrated in any of the 8 malignant PNST studied in this investigation.

The presence of external lamina material, demonstrable as positive staining for collagen IV (Martínez-Hernandez and Amenta 1983) in 19 of the 29 tumours, corresponds with the ultrastructural findings. Also those collagen IV-negative tumours which were studied by electron microscopy were found to produce external laminae ultrastructurally. Neurofilaments were not demonstrated immunohistochemically in any of the tumours. This finding agrees well with most reports in the literature on this intermediate filament in classical schwannoma (Trojanovski et al. 1984; Gould et al. 1986). All the tumours in the present study were negative for EMA. In a study of acoustic schwannomas, Winek et al. (1989) found focal positivity for EMA in 6 of 7 cases. Most studies of both acoustic and other schwannomas regarding EMA, however, have indicated a lack of EMA expression (Schnitt and Vogel 1986; Perentes and Rubinstein 1987; Ariza et al. 1988). Since most meningiomas have been found to express EMA, it has been suggested that this property may be of use in the differential diagnosis between meningiomas and central schwannomas (Schnitt and Vogel 1986). Furthermore, the finding that the cellular schwannomas of this series do not express EMA helps to distinguish them from the so-called perineurioma – a benign PNST with light-microscopic and ultrastructural similarities to cellular schwannoma, which, however, is S-100 protein negative and EMA positive (Pinkus and Kurtin 1985; Weidenheim and Campbell 1986; Perentez and Rubinstein 1987; Theaker et al. 1987; Ariza et al. 1988).

The large size of the tumours of this series may be a reflection of the asymptomatic clinical course, the deep, central location of the tumours, and the subsequent late detection. The strong predilection for central, paravertebral locations, in the mediastinum, retroperitoneum or pelvis of the tumours in this series is in agreement with the studies by Woodruff et al. (1981) and Fletcher et al. (1987). The large number of tumours with a mediastinal location in the present series is probably partly due to selection of the material. The strong female predominance in this series deviates from the sex distribution observed in other series of both classical and cellular schwannoma (Harkin and Reed

1969; Woodruff et al. 1981; Fletcher et al. 1987; Enzinger and Weiss 1988). This is hard to explain, as there was no obvious selection of women in the gathering of our cases. Of the 63 cases of cellular schwannoma described in some detail in the literature, including this series, only three have been associated with von Recklingshausen's neurofibromatosis (Woodruff et al. 1981; Gould et al. 1986; Fletcher et al. 1987).

All the tumours in the present study had a benign clinical course, as had all the cases reported by Woodruff et al. (1981) and Fletcher et al. (1987). However, in both this series and in that of Woodruff et al. (1981) there were tumours with extensive bone destruction. This is not a unique feature of cellular schwannomas, as it has also been described in cases of giant classical schwannoma in the lumbosacral region (Abernathey et al. 1986). There is no evidence that cellular schwannoma should in fact represent a low-grade de novo malignant schwannoma, or a schwannoma undergoing malignant change, as has been suggested by Ducatman et al. (1986). It can be noted that two tumours of this series were originally believed to be neurofibrosarcoma and leiomyosarcoma respectively and two were considered to be atypical leiomyomatous tumours of borderline malignancy. Similar misinterpretations, as well as the diagnosis of malignant fibrous histiocytoma, have been reported in two previous series (Woodruff et al. 1981; Fletcher et al. 1987). Localized fibrous mesothelioma is another tumour which may be considered in the differential diagnosis when dealing with mediastinal lesions. However, this lesion does not express S-100 protein immunohistochemically and lacks the typical Schwann cell features observed ultrastructurally in cellular schwannoma (Enzinger and Weiss 1988).

The chromosome analysis performed on one of the cellular schwannomas revealed a normal diploid stemline karyotype. However, a variety of abnormal clones and variant cells were also detected, most of which had chromosome counts in the hypodiploid region. Thus, there were three different clones with numerical deviations showing monosomy 15, monosomy 22 or loss of the X chromosome, and one structurally rearranged clone showing loss of the entire long arm of one chromosome No. 3. These findings are in agreement with the available chromosome data in the literature from a few classical schwannomas, where clonal loss of one chromosome 22 has been observed (Ray et al. 1987). In an unpublished cytogenetic study of classical schwannomas, we have also frequently found a loss of chromosome 22. It is interesting that one of these cases also showed loss of one chromosome 15. Although we only have results from one cellular schwannoma, the available data indicate that these tumours do not differ cytogenetically from classical schwannomas. Using molecular methods, Seizinger et al. (1986) recently reported the loss of heterozygosity for genes on chromosome 22 in acoustic schwannomas. Their findings indicated the loss of the entire chromosome 22 rather than a partial deletion. Loss of chromosome 22 has previously been described in other tumours originating from the nervous system, viz. meningiomas and malignant gliomas (Mark et al. 1977; Zang 1982; Bigner et al. 1984). Recently, a tentative meningioma locus was identified on the basis of deletion mapping (Dumanski et al. 1987). The segment of chromosome 22 which was consistently lost was located between bands q12.3 – qter. Whether the same locus on chromosome 22 is involved in the pathogenesis of meningiomas, gliomas, acoustic and other schwannomas, or whether there is a cluster of discrete genes on 22q, awaits further examination.

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