average blue geometric albedos of Pluto and Charon at superior conjunction (rotation phase 0.75, near maximum light). Pluto's bolometric Bond albedo was corrected for the observed light curve amplitude between phases 0.75 and 0.0, Charon's albedo was assumed to remain constant. Definitions for Pluto's rotational phases and light curve amplitude were taken from R. Binzel and J. Mulholland [*Astron. J.* **89**, 1759 (1984)].
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A Transgenic Mouse Model for Human Neurofibromatosis

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Human T-lymphotropic virus type 1 (HTLV-1) has been associated with the neurologic disorder tropical spastic paraparesis and possibly with multiple sclerosis. The *tat* gene of HTLV-1 under control of its own long terminal repeat is capable of inducing tumors in transgenic mice. The morphologic and biologic properties of these tumors indicate their close resemblance to human neurofibromatosis (von Recklinghausen's disease), the most common single gene disorder to affect the nervous system. The high spontaneous incidence of this disease, together with the diverse clinical and pathologic features associated with it, suggests that environmental factors may account for some of the observed cases. Multiple tumors developed simultaneously in the transgenic *tat* mice at approximately 3 months of age, and the phenotype was successfully passed through three generations. The tumors arise from the nerve sheaths of peripheral nerves and are composed of perineural cells and fibroblasts. Tumor cells from these mice adapt easily to propagation in culture and continue to express the tat protein in significant amounts. When transplanted into nude mice, these cultured cells efficiently induce tumors. Evidence of HTLV-1 infection in patients with neural and other soft tissue tumors is needed in order to establish a link between infection by this human retrovirus and von Recklinghausen's disease and other nonlymphoid tumors.

WHILE HUMAN T-LYMPHOTROPIC virus type 1 (HTLV-1) has been associated with the development of lymphoma and leukemia in humans, less than 0.1 percent of individuals who have antibodies to the virus develop lymphoid malignancies and do so only after a long latency period (*1*). The clinical and morphologic features of the disease are varied and may involve the skin and bone as well as the lymphoid system (*2*). Recent studies have suggested that HTLV-1–induced disease may be accompanied by neurologic symptoms (*3–5*).

A previously obscure syndrome termed tropical spastic paraparesis (TSP) affects the nervous system and has been epidemiologically linked to infection with HTLV-1 through detection of antibodies in serum and cerebral spinal fluid (*3*). The characteristic symptoms in patients with TSP from the Caribbean appear to be related to the central nervous system with gradual development of spastic paralysis as well as facial nerve paralysis and selective sensory deficits (*3*). A similar disorder termed HTLV-1–associated myelopathy (HAM) has been described in patients from Japan (*4*). Serologic and low stringency hybridization data have provided evidence of HTLV-1–related antigens and sequences in patients with multiple sclerosis (MS) (*5*). Thus clinical evidence would suggest that HTLV-1 may be neurotropic as well as lymphotropic.

The *tat* gene of HTLV-1 under the control of its own long terminal repeat (LTR) is capable of inducing two phenotypes in transgenic mice in the absence of other viral genes (*6*). Tumors arose in all three founder mice (designated 6-2, 6-7, and 8-4) which survived longer than 3 months, an observation that is consistent with tumor development being unrelated to the site of integration of the transgene. On the basis of their spindle cell morphology, the tumors were thought to be of mesenchymal derivation. Most of the tumors were benign, although in some cases malignant features were seen.

We analyzed the offspring from two of the original three founder mice (6-2 and 8-4), as well as those from the more recently derived 12-2 founder. The tumor phenotype was faithfully transmitted to 30 of 30 F_1 transgenic progeny mice and was successfully carried into the F_2 and F_3 generations. Tumors first appeared between 90 and 130 days of age. They were usually located on the ears, nose, legs, or tail (Fig. 1A). Most tumors began as small discrete nodules, which became confluent or multinodular as they enlarged. The largest tumor that we have observed was on the tail, and it measured approximately 1 cm in its greatest dimension; it had a high mitotic index and extended to the surrounding connective tissue, features we interpreted as consistent with a malignant transformation. Granulocytic infiltration occurred in all large and small tumors, despite little or no evidence of tumor necrosis.

Primary tumors expressed concentrations of the tat protein that were several times higher than any normal tissue, as revealed by Western blot analysis. Isolated tumor cells placed in tissue culture expressed tat in the nucleus, as demonstrated by indirect immunofluorescence (Fig. 2). Tissue culture cells that produced the tat protein have been passaged at least ten times and have grown tumors in five of five nude mice.

Three of four females from the F_1 and F_2 generations of the 6-2 lines were noticeably different from their male counterparts. They expressed the *tat* gene as shown by immunoblot analysis of tail extracts, but did not show external signs of tumor development. Nevertheless, they succumbed to progressive wasting and death by 3 months of age. At autopsy, all three animals showed tumors of the cranial nerves (two animals) or nerve ganglion (one animal). In one of these animals, the intracranial tumor had its origin in the left fifth cranial nerve ganglion (Fig. 1B). In that particular case, the right fifth cranial nerve showed no evidence of tumor. Histologically, the tumor was composed of elongated, spindle-shaped cells admixed with ganglion cells. No evidence of extension into the brain was seen. A second tumor was found in the cranial foramen, but its site of origin could not be determined. The remaining two females had diffuse involvement of a plexiform type of the extracranial nerves V and VII. Coronal sections from one of these mice demonstrated extensive tumor involvement of the nerve as it passed to the right of oral cavity and tongue, while the nerves on the left side appeared normal (Fig. 1C).

Since the tumors from these three animals suggested a close association with nerve, we

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again evaluated the tumors arising on the tail, nose, feet, and ears from other animals. Gross examination revealed that all large tumors were in continuity with nerve trunks, but smaller tumors required histologic examination to identify the associated nerve. A transverse section through the tail demonstrated the progression of tumors in three of the four quadrants of the tail (Fig. 1D). As compared with the normal nerve in the left upper quadrant, the others, in a clockwise manner, were replaced by tumors at progressive stages of growth. Further examination revealed an apparent expansion of the endoneural component with proliferation of the perineural cells surrounding the nerve. Central necrosis was present in the tumor at the lower left with diffuse involvement of nerve branches extending to the dermis on the extreme left.

Since the clinical, gross, and microscopic features of the tumor suggested a neurofibroma-like lesion, we further studied the ultrastructural appearance of these tumors and the presence of cell-specific markers by immunoblot analysis. Electron microscopy of the large tumors demonstrated a proliferation of predominately fibroblast-like cells. In a few areas, however, spindle-shaped cells with elongate cytoplasm were surrounded by discontinuous basal lamina suggesting an origin from nerve sheath. We then studied early lesions measuring between 1 and 4 mm removed from the ears and tail. The earliest lesion identified (Fig. 3A) contained enlarged perineural cells with densely clumped chromatin surrounding myelinated and unmyelinated nerve axons. A distinct basal lamina was present next to the perineural cells in the upper portion of the section. The basal lamina became interrupted and then totally absent at the bottom of the section, demonstrating that perineural cells might lose differentiating features as they proliferated. A separate small tumor from the ear was found where cells were proliferating from the perineural sheath as a distinct mass surrounding a myelinated nerve (Fig. 3B). The Schwann cell nuclei in this nerve had denser chromatin than in normal nerve; granulocytes were present even in this early lesion. These studies demonstrated that tumors in the transgenic mice were of nerve sheath origin.

As a confirmation of our morphologic assignment, we searched for biochemical markers using specific antibodies. Vimentin is a useful marker for identifying mesenchymal tissues (*7*). An antibody to vimentin was used to identify a 58-kD protein in tumors

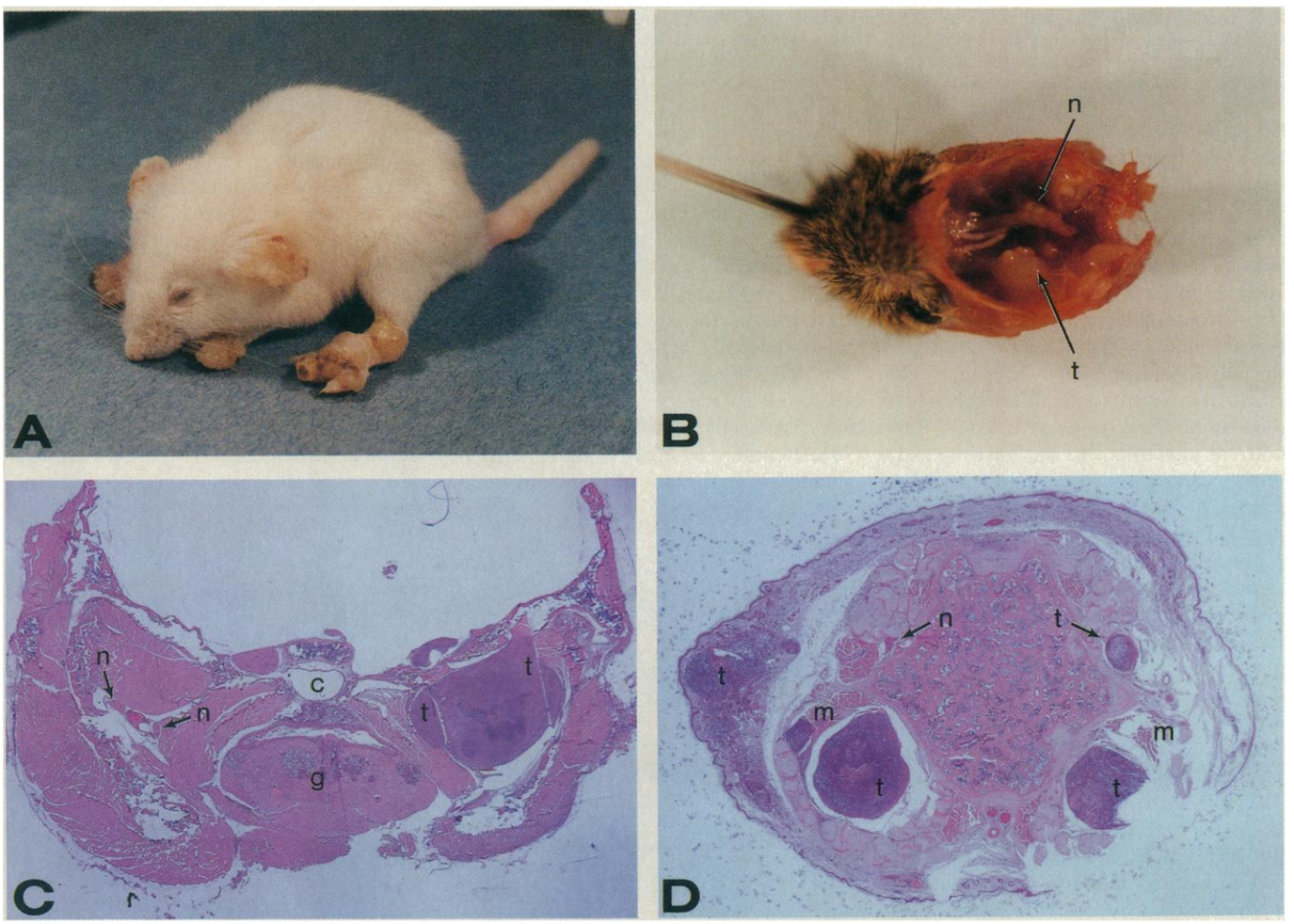

Fig. 1. Gross and histologic features of the transgenic tat mice. (**A**) Male mouse (140 days old) from the 6-2 line with multiple tumors of front and hind legs, ears, and tail. Tumors are subject to trauma and frequently develop crusted areas overlying necrosis. (**B**) Cranial cavity dissection of female mouse from the 6-2 line. Anterior is to the left. Tumor (t) of trigeminal nerve ganglion with extension along ophthalmic and mandibular branches is visible. Unaffected right trigeminal nerve (n) and ganglion are at the top. A second, smaller tumor mass can be seen in the foramen magnum. (**C**) Coronal section of head from a female mouse from the 6-2 line. Two unaffected nerve (n) trunks are present at the left. Tumors (t) have entirely replaced the nerve trunks on the right with compression of surrounding muscle tissue. Nasal cavity (c), tongue (g). Tissues were prepared as described (*20*). Hematoxylin and eosin stain. Original magnification, ×10. (**D**) Tail of male mouse from the 6-2 line. Dorsal surface of tail is at top. Nerve (n), muscle (m), and connective tissue elements of tail are duplicated in each of four quadrants. Bone and bone marrow are in center of tail. Tumors (t) of various sizes are seen replacing the nerve trunks. Tissue preparation as described in (*20*). Original magnification, ×25.

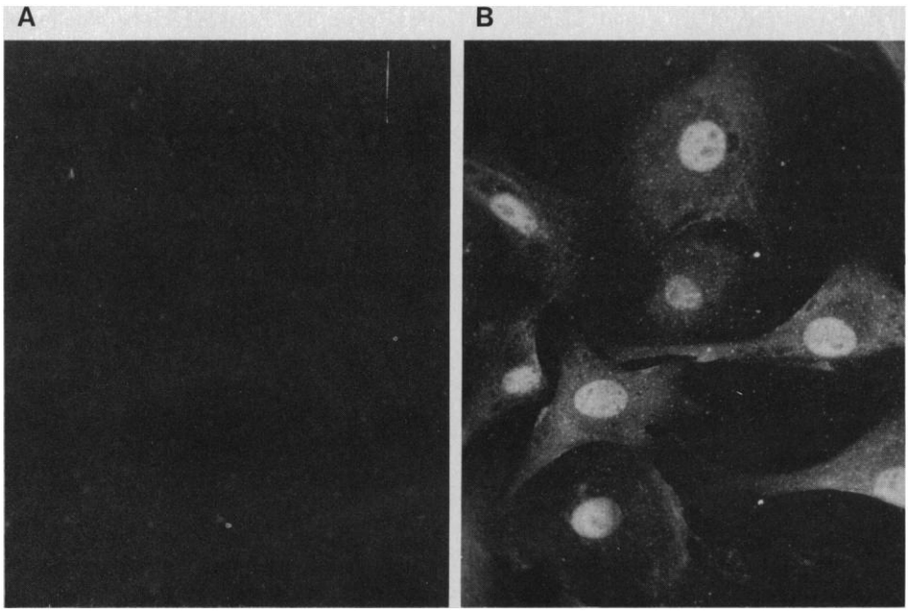

Fig. 2. Indirect immunofluorescence of tissue culture tumor cells. (**A**) Preimmune rabbit serum. (**B**) Rabbit antiserum to a tat fusion protein (*22*). Rhodamine-conjugated, goat antibody to rabbit immunoglobulin G was used as second antibody. The tissue preparation has been described (*20*), as well as the immunofluorescence procedure (*21*).

from the 6-2 and 8-4 mouse lines (Fig. 4A, lanes 1 and 2) and in L cells (lane 3). Since muscle tissue from the transgenic animals expresses high levels of the tat protein (*6*), it was important to determine whether desmin would be found in the tumor. The absence of desmin in tumor tissues from lines 6-2 and 8-4 confirms that the tumors did not arise from muscle (Fig. 4B, lanes 1 and 2). The polyclonal sera used in our study showed cross-reactivity to other intermediate filaments. The cross-reactive band was of a different molecular size and intensity and, therefore, distinguishable from the authentic 53-kD protein band. The selective expression of vimentin but not desmin in the transgenic tumors is consistent with a mesenchymal derivation.

Blood coagulation factor XIIIA has been successfully used to distinguish fibroblastic characteristics of perineural cell neurofibromas in humans (*8*). Extracts of tumors from both the 6-2 and 8-4 transgenic lines revealed an 80-kD protein by immunoblot analysis with an antibody to human factor XIIIA (Fig. 4C, lanes 1 and 2). This component is not detected in cultured mouse L cells (lane 3), muscle (lane 4), or nerve (lane 5). Reactivity to this antibody confirmed the fibroblastic nature of the proliferative cell in the transgenic mouse tumors. Our evaluation was restricted by the lack of specificity of our rabbit antibody to the mouse S-100 protein on Western blot. However, subsequent immunohistochemical analysis has failed to detect S-100 in these tumors, suggesting a paucity of Schwann cells.

Neurofibromatosis (NF) in humans occurs in two main forms (*9*). Von Recklinghausen neurofibromatosis (VRNF) refers to the typical form of the disease with cafe au lait spots and dermal neurofibromas. Central or bilateral acoustic NF refers to a less frequent syndrome with bilateral tumor involvement of the vestibulocochlear nerve and occasionally brain or spinal cord tumors. The diverse clinical manifestations of NF have also suggested a larger classification scheme with as many as eight categories (*10*). Neurofibromatosis is thought to be inherited as an autosomal dominant, single gene disorder. As such, it is one of the most common genetic syndromes affecting the nervous system, with an estimated incidence of 1 in 3000 live births (*11*).

Neurofibromas in humans are tumors of nerve sheath origin and may be formed by a proliferation of Schwann cells, fibroblasts, and perineural, mast, and histiocytic cells. In approximately 60 percent of cases, the tumors are S-100–positive, indicating a predominance of Schwann cells. In the other cases, there is a mixture of cell types (*12*). The Schwann cell is thought to be derived from the neural crest as are the melanocytes found in the dermal cafe au lait spots. The origin of perineural cells is in dispute, with recent evidence suggesting a mesenchymal derivation (*12*). In the transgenic mice, the tumors are composed of fibroblastic cells, which appear to have their origin from the perineurium. It is not surprising that Schwann cell or perineural cell characteristics could not be found by electron microscopy in the large or malignant mouse tumors because in malignant human neurofibrosarcomas differentiating features are frequently lost (*12*).

Several other observations of characteristics of the transgenic *tat* mice have interesting correlates with human neurofibromatosis. Puberty and pregnancy may result in accelerated growth of human neurofibromas suggesting a hormonal effect (*13*). Tumors in pregnant transgenic *tat* mice rapidly increased in size and number, frequently requiring transfer of offspring to a foster female.

In one founder line (6-2), three females developed intracranial or extensive extracranial tumors of cranial nerves. In humans,

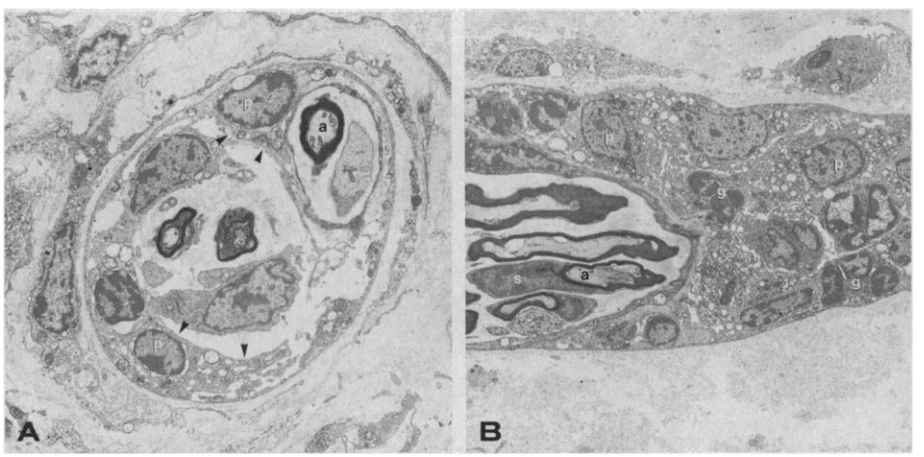

Fig. 3. Electron micrograph of tat mouse nerve and ear tumor. (**A**) Earliest lesion identified in a mouse peripheral nerve from the 6-2 line. Perineural cells (p) surround myelinated and unmyelinated axons (a). Arrowheads at top identify basal lamina which is missing over perineural cells at bottom. Original magnification, ×7500. (**B**) Proliferation of fibroblastic (p) cells from perineurium of peripheral nerve from an ear tumor. Schwann cell (s) surrounds myelinated axon. Multiple granulocytes (g) have migrated between neoplastic cells. Original magnification, ×4500. Tissues for electron microscopy were prepared as described (*20*).

cranial nerve V is a frequent site of plexiform neurofibromas (*10*).

Infiltration by mast cells is a characteristic feature of human neurofibromas, the significance of which is unknown (*10*). An inflammatory cell infiltrate composed primarily of neutrophils has been seen in all transgenic *tat* tumors. Their presence may be related to production of a chemotactic factor rather than trauma since granulocytes are seen infiltrating even early lesions identifiable only by electron microscopy. We have seen a florid myeloid hyperplasia with peripheral granulocytosis in two of the oldest surviving mice. In humans, an increased incidence of nonlymphoid leukemias has been found in patients with NF (*14*). The circulating white cells from the tat mice with granulocytosis lacked sufficient blast forms to warrant a diagnosis of leukemia.

The genetics of human NF has been partially elucidated. VRNF has been mapped to chromosome 17 (*15*), although variant forms may not conform to this. Studies with probes localized to chromosome 22 have suggested that deletions are present on chromosome 22 in the bilateral acoustic neuroma variant of NF (*16*). Most large studies have reported a spontaneous case rate of approximately 50 percent (*11*). If this were a single gene disorder, it would represent the highest gene mutation rate for any genetic disease. Multiple genetic loci may more reasonably account for the high spontaneous mutation rate and the clinical diversity observed in NF patients. Alternatively, environmental factors, such as carcinogens or viral infection, may also play a role in the development of NF (*17*).

Our data show that the *tat* gene under the control of the HTLV-1 LTR has neurotropic properties and is capable of generating, directly or indirectly, morphologically similar tumors in widely separated sites. It is possible that infection by HTLV-1 may account for a percentage of cases of NF reported as spontaneous mutations. In these cases, the epidemiology of a viral disease with a long latency period may mimic a disease thought to be a spontaneous genetic event. If proviral DNA is transmitted vertically or through transplacental infection, it could produce the appearance of a dominant inherited disease. An infectious etiology for all cases of NF is unlikely because of the apparent uniform distribution of this disorder around the world, though clustering of NF in HTLV-1 endemic areas has not been investigated.

The development of benign tumors that are induced by a defined primary event and then progress to malignancy may provide a useful system to define the molecular events in carcinogenesis. An important similarity between our transgenic animal model and human NF is the re-creation of multiple but separate simultaneous events resulting in neoplasia. This is characterized best by the many neoplastic tumors in various stages of development seen in the tails of the *tat* mice. In one large study of patients with NF, 3.6 percent of the cases progressed to malignancy (*9*). We have seen a similar low percentage of tumors showing malignant characteristics in the transgenic mice. In this system, we suggest that high levels of the *tat* protein result in cellular proliferation which is subject to regulation by angiogenic factors or limitation by the immune system (*19*). In only a limited number of tumors will secondary events occur that result in malignant transformation.

The neurotropic properties of HTLV-1 in humans have only recently been recognized (*3–5*). The time between infection and development of symptoms may be as long for the nervous system as it is for the lymphoid system. By use of the transgenic methodology, problems of latency and biologic variability may be overcome. Since restriction of infection to specific cell types has not been demonstrated, we anticipate similar disease associations to be seen in humans, and suggest a reevaluation of association of HTLV-1 with human neoplasms. This will become more important, as HTLV-1 continues to spread.

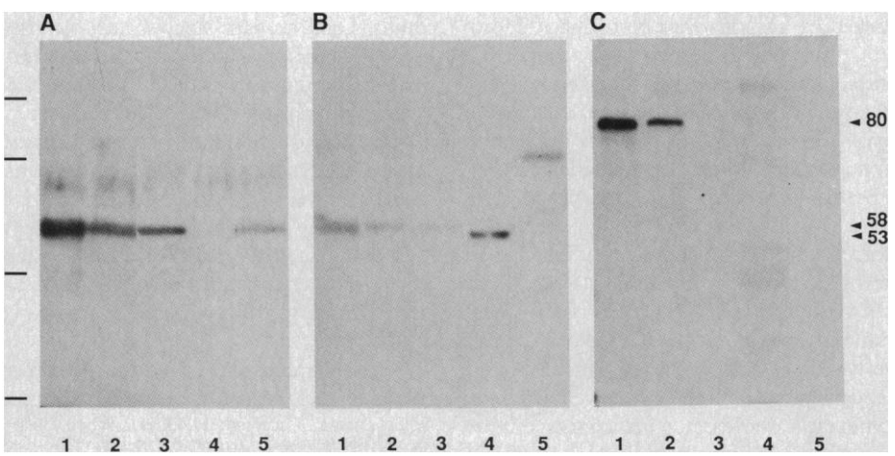

Fig. 4. Analysis of cell-type specific markers in mouse tumors. (Lane 1) Tumor from a 6-2 mouse; (lane 2) tumor from a 8-4 mouse; (lane 3) mouse L cells; (lane 4) muscle from normal mouse; (lane 5) nerve from normal mouse. (**A**) Polyclonal rabbit antibody to CHO cell vimentin. (**B**) Rabbit antibody to chicken desmin (Dako). (**C**) Polyclonal rabbit antibody to human factor XIIIA (Behring Diagnostics). All primary antibodies were diluted 1 to 200 and reacted with ^{125}I-labeled protein A from *Staphylococcus aureus*. Immunoblot procedure was followed as described (*22*).

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