Virchows Arch (2001) 438:228–231
DOI 10.1007/s004280000306

ORIGINAL ARTICLE

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Tumours may be innervated

Received: 27 March 2000 / Accepted: 7 July 2000 / Published online: 4 November 2000
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Abstract It is generally assumed that tumours are not innervated. However, following an accidental observation of a nerve fibre within an adenoma of the ciliary body epithelium of the eye, we have further examined two such tumours. One pigmented and one non-pigmented adenoma of the ciliary body epithelium (APCE and ANCE, respectively) that had been surgically removed from two human eyes were processed for ultrastructural evaluation and systematically screened and analysed for the occurrence of nerve tissue under a transmission electron microscope. The adenomas were composed of epithelial tumour cell strands and interposed vascularised connective tissue. Both tumours contained a small number of fine unmyelinated nerve fibres containing clear and dense core vesicles. In both adenomas, the nerve fibres were located in the tumour periphery close to blood vessels and tumour cells. In the APCE, they were also seen in more central areas. Since nerves always have a function, this finding, if confirmed in other neoplasms, may influence our understanding of such innervated tumours.

Keywords Adenoma · Ciliary body epithelium · Innervation · Tumour · Ultrastructure

Introduction

The epithelium of the ciliary body of the eye is made up of two superimposed monolayers of cells that correspond to the outer and inner leaflet of the optic cup. The inner leaflet forms the non-pigmented and the outer leaflet the pigmented ciliary epithelium. Both epithelia can develop rare adenomas with a polymorphous appearance [14]. We studied one such adenoma of each epithelial layer; the results will be published elsewhere. Continuing our examination at the level of fine structure, we found nerve fibres within these tumours. As far as we know, nerve fibres had never previously been observed within non-neuronal neoplasms, with the exception of nervous tissue in recently described neoplasms of the adrenal gland [6]. However, this gland is abundantly innervated for purposes of its normal function. That is, these nerve fibres were already present prior to the lesion [6]. Because previous immunohistological investigations had rejected the possibility that tumours are innervated [9], we want to report our findings.

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Methods

Two intraocular tumours were removed surgically from the eyes of two female patients at the University Eye Hospital of the University of Bonn. Pathologic evaluation identified one tumour as an adenoma of pigmented and the other tumour as an adenoma of the non-pigmented ciliary epithelium (APCE and ANCE, respectively). Portions of both tumours were excised for ultrastructural study. These specimens were kept overnight in a fixative containing 2% glutaraldehyde, 2% paraformaldehyde and phosphate buffer (pH 7.3). They were rinsed in 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide for 2 h, rinsed in phosphate buffer, dehydrated in ethanol, and embedded in Durcupan ACM. Ultrathin sections (50 nm) were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 109 transmission electron microscope.

Results

Adenoma of the pigmented ciliary epithelium

The APCE measured 6×4.5×3.5 mm. It was composed of compact strands of epithelial cells embedded in a rather sparse connective tissue stroma (Fig. 1). The epithelial cells of the tumour were rich in organelles and frequently contained melanosomes and mature melanin granules. The connective tissue was vascularised. In the connec-

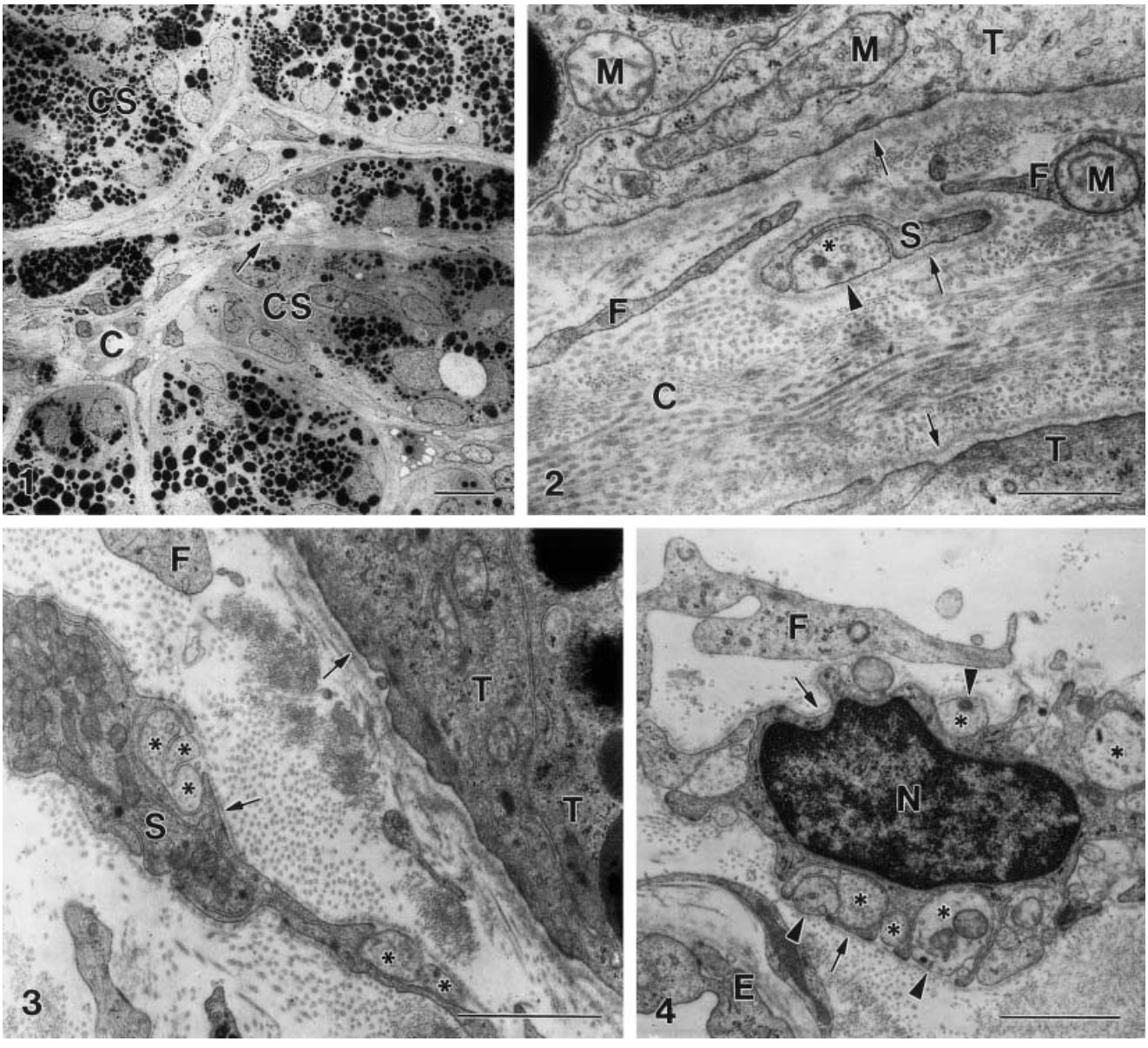

Fig. 1 The pigmented tumour cells of the adenoma of the pigmented ciliary epithelium are arranged in compact epithelial cell strands (*CS*). The *arrow* points to the location of the nerve fibre shown in detail in Fig. 2. *C* connective tissue. *Bar* 10 µm

Fig. 2 Fine nerve fibre consisting of a Schwann cell (*S*) and a single axon between tumour cells (*T*) of adenoma of the pigmented ciliary epithelium (note: nerve fibres and tumour cells have a basal lamina, whereas fibroblasts do not). *Arrowhead* bare area of axon; *arrow* basal lamina; *asterisk* axon with clear and dense core vesicles; *C* connective tissue with collagen fibrils; *F* fibroblast; *M* mitochondrion. *Bar* 0.5 µm

Fig. 3 Profile of a nerve fibre bundle with five axons (*asterisks*) in adenoma of the pigmented ciliary epithelium. *Arrow* basal lamina, *F* fibroblast, *S* Schwann cell, *T* tumour cell. *Bar* 1 µm

Fig. 4 Nerve strand in the connective tissue at the edge of the adenoma of the non-pigmented ciliary epithelium approximately 0.1 mm away from the tumour cells, with numerous axons (*asterisk*) in a Schwann cell. *Arrow* basal lamina, *arrowhead* bare area of axon, *E* endothelial cell, *F* fibroblast, *N* nucleus of Schwann cell. *Bar* 1 µm

tive tissue septa between the strands of epithelial cells, we were able to identify a few single axons and fine bundles of unmyelinated nerve fibres (Fig. 2 and Fig. 3). Their maximum density was three profiles per 0.9 mm^2 of specimen. In the vicinity of these nerve fibres, the sections did not contain any identifiable portions of blood vessels. Nerve fibres in the vicinity of blood vessels were seen only in the loose connective tissue at the margin of the tumour towards the normal ciliary body. Deeper in the tumour, the blood vessels were never associated with any visible nervous elements. All nerve fibres identified were embedded in Schwann cells either singly or in groups of a few axons which exhibited bare areas (Fig. 2 and Fig. 3). They contained clear vesicles and dense core vesicles.

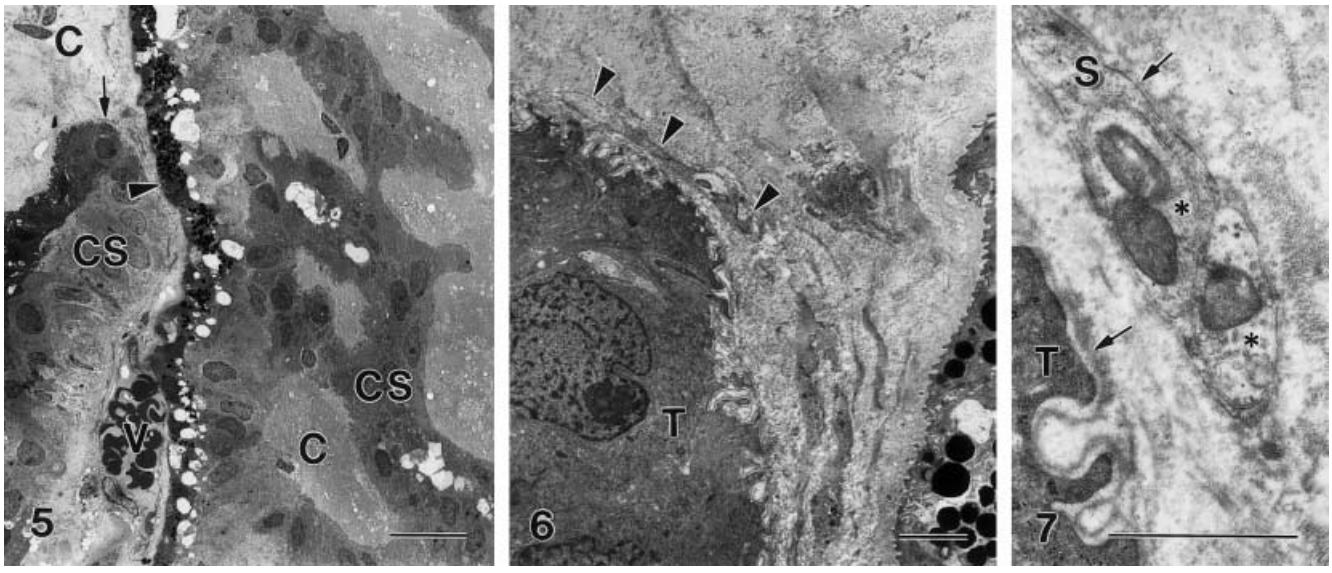

Fig. 5 The adenoma of the non-pigmented ciliary epithelium contains abundant connective tissue (*C*) between the epithelial cell strands (*CS*). The *arrow* points to the location of the nerve fibre bundle shown in detail in Fig. 6. *Arrowhead* pigmented ciliary body epithelium, *V* blood vessel. *Bar* 20 µm

Fig. 6 Higher magnification of area marked by *arrow* in Fig. 5, showing a nerve fibre bundle (*arrowheads*) in the vicinity of tumour cells (*T*) within the adenoma of the non-pigmented ciliary epithelium. *Bar* 2 µm

Fig. 7 Higher magnification of a nerve fibre bundle marked by *arrowheads* in Fig. 6 (the next section). *Arrow* basal lamina, *asterisk* axon, *S* Schwann cell, *T* tumour cell. *Bar* 1 µm

Adenoma of the non-pigmented ciliary epithelium

The ANCE measured 3.5×2.5 mm. The tumour was composed of epithelial sheets and strands, forming a spongy structure with interposed abundant connective tissue (Fig. 4). The epithelial cells of the tumour contained many organelles, but only exceptionally melanosomes and melanin granules. The connective tissue was loose, poor in fibres, sparsely vascularised, and accounted for about half of the tumour mass. Only at the base of the adenoma did the connective tissue contain more blood vessels that invaded the tumour. This border zone contained many axons embedded in Schwann cells exhibiting bare areas, clear vesicles and dense core vesicles. Other axons were located between blood vessels and tumour strands in the periphery of the adenoma, while very rarely finer nerve fibre bundles were detected in very close proximity to tumour cells (Fig. 5, Fig. 6 and Fig. 7). No neural elements were identified in the more central areas of the tumour.

Discussion

The neural tissue in the tumours we examined represents a relatively small proportion of the total tissue, and the number of fibres identified in our study was small; their mere presence, however, proves that they must reach active cellular elements of the adenomas. Their formation and persistence necessarily result from the action of neurogenic growth factors [3] produced by cells seeking innervation.

Both tumours were composed of a mixture of epithelial tumour cells and vascularised connective tissue, so different target tissues for the nerves can be considered. Further clarification with morphological methods is not possible since the "synapses à distance" that are typical of autonomic fibres do not exhibit special membrane structures towards the target cells. Furthermore, the synaptically active varicosities of the axons may be situated several hundred nanometers away from the target cells. Thus, theoretically, the epithelial tumour tissue, the blood vessels of the tumour, fixed and wandering cells of the tumour stroma could be innervated. There are, however, some topological and fine structural features that may help in associating nerve fibres with target cells [8, 12]. In both tumours that were examined, axons exhibiting bare areas and containing various populations of neurovesicles were situated in the immediate vicinity of the blood vessels and tumour cell strands. Such synaptically active, transmitter-secreting axonal regions in close proximity to tissues clearly indicate that these tissues are innervated.

Nerve fibres without a function do not exist. Thus, possible consequences of neuronal activity in a tumour must be considered. Besides the innervation of the blood vessels, further influences of the nervous system on a tumour can only be speculated upon. For example, the neurological system can act as an intermediary in healing processes [2], and the possible actions of neuropeptides secreted by axons are manifold. For example, axons influence the cells of the immune defence system by producing substance P (SP). This can act as a signal for mast cells [10], which respond by secreting tumour necrosis factor alpha (TNFα) [1]. In contrast, some neuro-

peptides, such as SP, calcitonin gene related protein (CGRP) and neurokinin A, support the mitotic activity of several types of cells in vitro [7], and neuronal input can lead to a translation of subcellularly segregated mRNA [13]. Finally, the present observation of nerve fibres provides a structural basis for communication between the autonomic nervous system and tumours. This could explain the effects of stress or other psychological factors on tumour behaviour, which has been demonstrated in many studies [4, 5, 11].

Our findings that delicate nerve fibres were electronmicroscopically detectable in two adenomas cannot be generalised to all tumours. However, we are curious as to whether the broad spectrum of neoplasms does include other tumours containing neuronal elements.

Acknowledgements The authors wish to thank Parand Pour Nouroz and Claudine Strack for excellent technical assistance. The work was supported by "Aktion Kampf der Erblindung".

References

1. Ansel J, Brown J, Payan D, Brown M (1993) Substance P selectively activates TNF-α gene expression in murine mast cells. J Immunol 150:1–8
2. Ansel JC, Armstrong CA, Song I, Quinlan KL, Olerud JE, Whrite Caughman S, Bunnett NW (1997) Interactions of the skin and nervous system. J Invest Dermatol Symp Proc 2: 23–26
3. Campenot RB (1977) Local control of neurite development by nerve growth factor. Proc Natl Acad Sci U S A 74:4516–4519
4. Holden JR, Pakula IS (1999) Tumor necrosis factor-alpha: is there a continuum of liability between stress, anxiety states and anorexia nervosa? Med Hypotheses 52:155–162
5. LeShan L (1966) An emotional life-history pattern associated with neoplastic disease. Ann N Y Acad Sci 125:780–793
6. Li Q, Johansson H, Grimelius L (1999) Innervation of human adrenal gland and adrenal cortical lesions. Virchows Arch 435:580–589
7. Liu M, Warn JD, Fan Q, Smith PG (1999) Relationship between nerves and myofibroblasts during cutaneous wound healing in the developing rat. Cell Tissue Res 297:423–433
8. Luff SE, Young SB, Anderson WP (1999) Extended angiotensin converting enzyme inhibition changes the innervation of renal glomerular afferent arterioles. J Auton Nerv Syst 77: 114–124
9. Mitchell BS, Schumacher U, Kaiserling E (1994) Are tumours innervated? Immunohistological investigations using antibodies against the neuronal marker protein gene product 9.5 (PGP 9.5) in benign, malignant and experimental tumours. Tumor Biol 15:269–274
10. Naukkarinen A, Harvima I, Paukkonen K, Aalto ML, Horsmanheimo M (1993) Immunohistochemical analysis of sensory nerves and neuropeptides, and their contacts with mast cells in developing and mature psoriatic lesion. Arch Dermatol Res 285:341–346
11. Ramirez AJ, Craig TKJ, Watson JP, Fentiman IS, North WRS, Rubens R (1989) Stress and relapse of breast cancer. Br Med J 298:291–293
12. Seifert P, Spitznas M (1999) Vasoactive intestinal polypeptide (VIP) innervation of the human eyelid glands. Exp Eye Res 68:685–692
13. Wanner I, Baader S L, Oberdick J, Schilling K (2000) Changing subcellular distribution and activity-dependent utilization of a dendritically localized mRNA in developing Purkinje cells. Mol Cell Neurosci 15:275–287
14. Zimmerman LE (1970) The remarkable polymorphism of tumours of the ciliary epithelium. Trans Aust Col Ophthalmol 2:114–125