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Electron microscopic examination of oligocilia in naevus of Ota

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Abstract The structure of the cilia present in dermal melanocytes of 14 patients with naevus of Ota was examined by electron microscopy. Cilia and basal bodies were found in 10 and 9 lesions, and in 39 and 18 dermal melanocytes, respectively. In each case, 1–12 cells with a single cilium or multiple cilia were observed. In a total of 3 dermal melanocytes from 2 cases, two cilia per cell were observed. The cilia contained 7, 6, 5 and 4 pairs of doublet microtubules in the periphery and no central microtubule. Another pattern with several pairs of doublet microtubules in the periphery and one or two centrally located doublet microtubules were also observed. The latter were not bona fide central microtubules but one and two doublets, which seemed to be displaced to the centre from the periphery of the cilium.

Key words Cilia · Melanocytes · Microtubules · Basal body · Cell structure · Naevus of Ota

Introduction

Cells with numerous cilia or flagella have been examined by light microscopy for many years, but electron microscopy allows the examination of a single cilium or of a group of cilia in a variety of cells. These cilia are designated as single, primary, rudimentary, or solitary cilia or oligocilia. In human skin, the first ultrastructural study of a single cilium was made in keratinocytes by Wilson and McWhorter [14] in 1963. They found cilia in some basal keratinocytes in normal epidermis and in basal cell carcinomas. Elofsson et al. [2] demonstrated that cilia are common in the melanocytes of normal epidermis. In naevus tissues, a single cilium is usually present in cells of melanocytic naevus [11]. In dermal melanocytes, the presence of a single cilium was noted by Kjaerheim et al. [9] in naevus cells of blue naevus. Other studies revealed the presence of two cilia per cell in the blue naevus [1]. However, the presence of cilia has not been reported in dermal melanocytes in the naevus of Ota.

The naevus of Ota is a congenital or acquired unilateral pigmented lesion on the face, characterized by the presence of melanocytes in the dermis. We have recently reported chronological changes in the histology of dermal melanocytes of patients with naevus of Ota during laser irradiation [7]. During the study, we frequently observed oligocilia in cells of tissue obtained from these patients prior to irradiation. Our report is the first that describes the presence of oligocilia in dermal melanocytes in patients with naevus of Ota.

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Materials and methods

Skin tissues were obtained at biopsy from 14 Japanese patients with naevus of Ota (4 males and 10 females). No patients had received previous treatment. Biopsy material was obtained from the temporal lesion under local anaesthesia (1% lidocaine with adrenaline at 1/100000) using a disposable trepan (diameter, 4 mm; Despopunch, SFM, Germany; Table 1). Biopsies were performed with the written consent of each patient.

Specimens were bisected, and one piece was fixed in 10% neutral buffered formalin solution for light microscopy while the other was fixed in 2.5% glutaraldehyde solution for electron microscopy. The fixed tissues were postfixed in osmium tetroxide, dehydrated and embedded in epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined under an electron microscope (model H-7100, Hitachi, Tokyo).

Results

The cytoplasm of dermal melanocytes present in the naevus tissue contained numerous melanosomes. The golgi

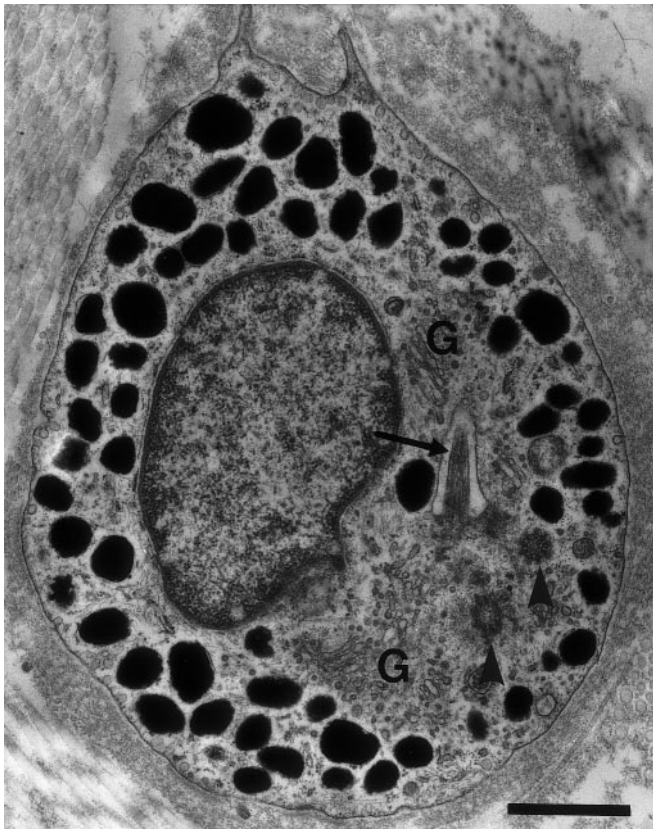


Fig. 1 Electron microscopic features of a dermal melanocyte in a patient with naevus of Ota. The dermal melanocyte is filled with numerous melanosomes. Note the presence of a single cilium (*arrow*) and two centrioles or basal bodies (*arrowheads*) in the midst of abundant Golgi apparatuses (*G*). *Calibration bar* 1 µm

apparatus was well developed but other organelles, such as mitochondria, endoplasmic reticulum and ribosomes, were rarely detected. Cilia, in addition to basal bodies or centrioles, were observed among the large number of Golgi apparatus (Fig. 1). Cilia and basal bodies were found in 10 and 9 lesions and in 39 and 18 dermal melanocytes, respectively. Between 1 and 12 cells with a single cilium or cilia were observed in each case. In most cases, only a single cilium was observed per cell, although in a total of 3 dermal melanocytes from 2 cases, two cilia were detected in each cell (Table 1). Examination under a high magnification showed that the cilium originated from the distal end of the basal body and appeared to lie in a tunnel formed by an invagination of the plasma membrane, thus communicating with the extracellular space (Fig. 2). Most cilia measured up to 0.5 µm in length, while one giant basal body, measuring 0.22 µm in diameter and 1.3 µm in length was observed (Fig. 3). In cross sections of the centrioles or basal bodies the wall of them was composed of nine evenly spaced triplet microtubules (Fig. 4). The cilium appeared to lie in a hollow extracellular space and contained several pairs of doublet microtubules. The cilia contained 7, 6, 5 and 4 pairs of doublet microtubules in the periphery and no central microtubule (7+0, 6+0, 5+0 and 4+0 pattern). We also found that the microtubule pair pattern was 8+1, 7+1, 6+2 and 6+1, although they were not genuine central microtubules as seen in the bronchial mucosa (which comprise two separated microtubules), but rather, one and two doublets, which seemed to be displaced to the centre from the periphery of the cilium (Fig. 5).

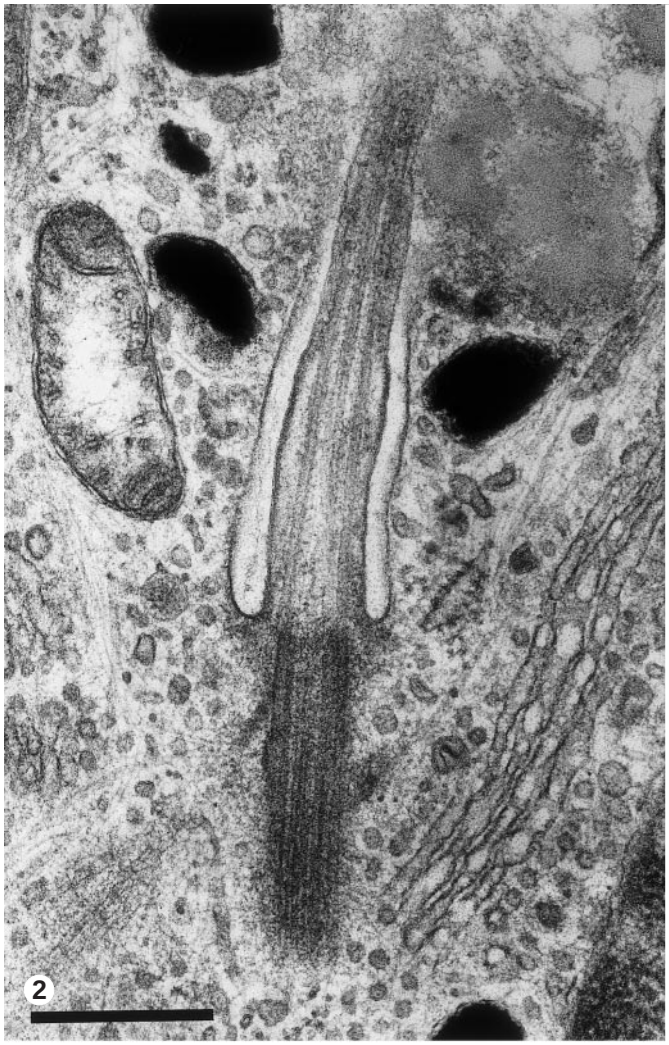
Table 1 Patient characteristics and number of melanocytes containing cilia and centrioles or basal bodies in each patient (– no cilium, centriole or basal body were detected)

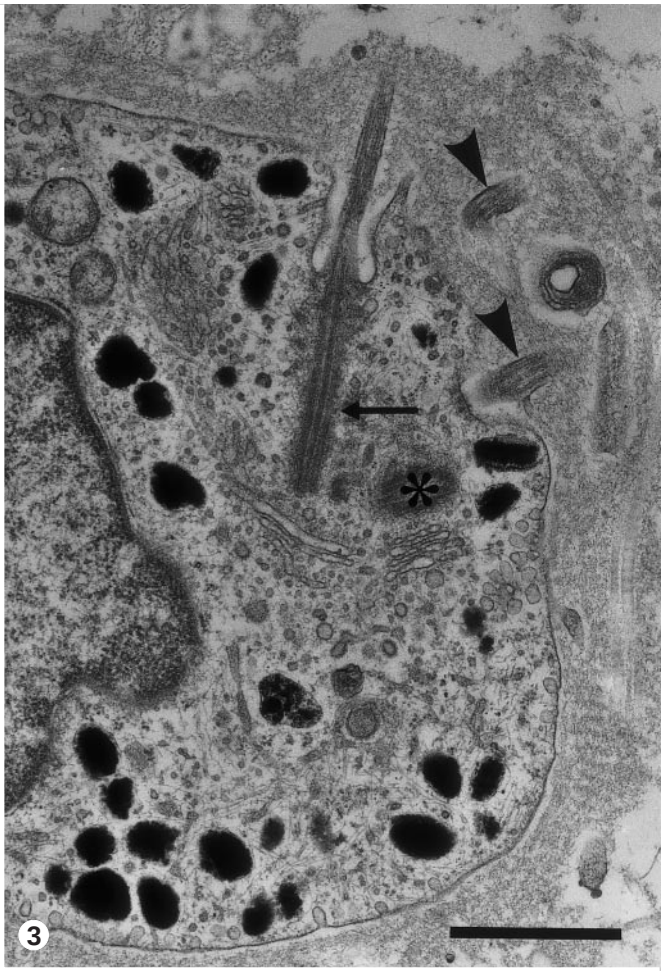
Patient	Age (years)	Sex	Cilia		Centriole or basal body
			Single	Two	
1	37	M	10	2	1
2	55	M	7	–	2
3	19	F	5	1	4
4	29	F	4	–	2
5	27	F	3	–	2
6	31	F	2	–	1
7	15	F	2	–	1
8	46	M	1	–	1
9	22	F	1	–	–
10	21	M	1	–	–
11	5	F	–	–	4
12	44	F	–	–	–
13	34	F	–	–	–
14	17	F	–	–	–

Discussion

Ultrastructural studies have shown that the basal body is a hollow cylinder with the same structure as a centriole and that the basal body is essentially a centriole with modified activity [5]. Centrioles and basal bodies in human cells are reported to be about 0.25 µm in external diameter and 0.4 µm long and fairly constant in size [6]. We found a giant basal body in a dermal melanocyte measuring 0.22 µm in diameter and 1.3 µm in length, or about three times the length of the usual centriole. A giant basal body was previously reported in the tissues of a kidney affected by lupus [10]. At present, neither the mechanisms leading to the development of the giant centrioles nor their function are understood.

In virtually all reported cases of cilia in pathological skin tissue the commonest type of cilium encountered has been the 9+0 cilium. The basic difference between classic cilia and oligocilia is seen in the cross section of the cilium. In the former, the internal structure consists of an outer ring of 9 pairs of microtubules and 2 separated central tubules (9+2 axoneme), whereas the 2 central tubules are absent in the latter (9+0 axoneme). Since it is generally believed that the central tubules are associated with motility, it follows that the oligocilia are immotile [6]. In dermal melanocytes, we found that the cilia had the 7, 6, 5 and 4 peripherally located microtubule pairs, whereas the two separated central tubules were absent. Therefore, the cilia of dermal melanocytes appeared to be immotile. In many studies, the microtubular pattern has not been re-





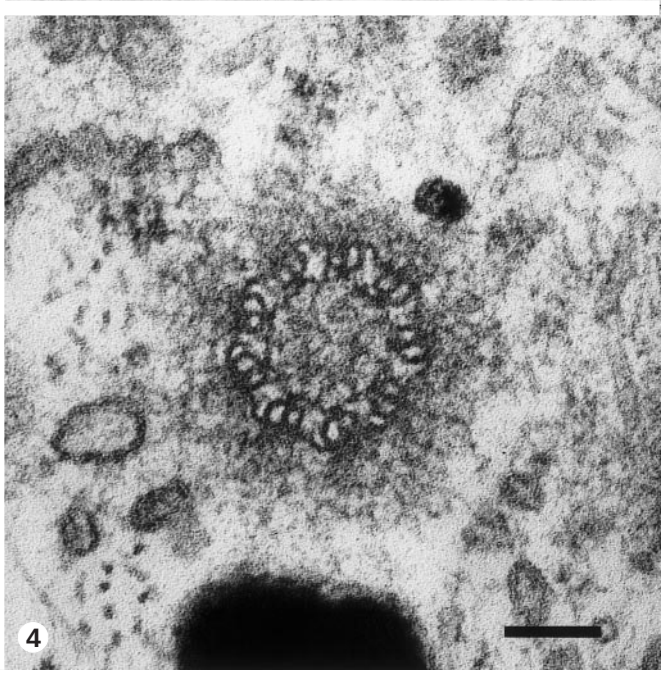


Fig. 2 A basal body and cilium. Note that the cilium originates from the distal end of the basal body. *Calibration bar* 0.5 µm

Fig. 3 A giant basal body (*arrow*), measuring 0.22 µm and 1.3 µm in length, is shown in one naevus of Ota. Another basal body (*asterisk*) and two cilia (*arrowheads*) possibly extending from the cell are also observed. *Calibration bar* 1 µm

Fig. 4 A cross section of a centriole or basal body. It contains nine pairs of triplet microtubules. *Calibration bar* 0.1 µm

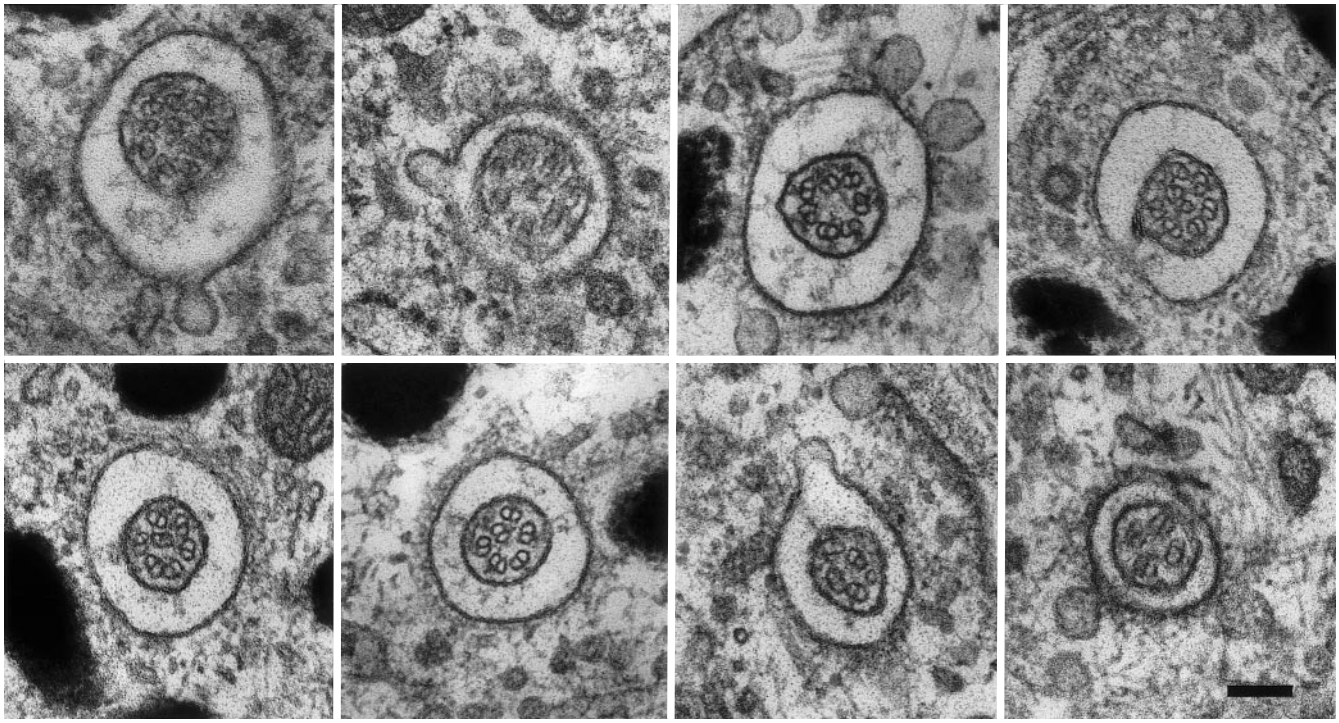


Fig. 5 Several types of cilia consisting of 8+1, 7+1, 7+0, 6+2 (*top*, from *left* to *right*), 6+1, 6+0, 5+0 and 4+0 patterns (*bottom*, from *left* to *right*) of microtubule pairs. Each centrally located tubules is composed of a pair of two microtubules (a single doublet). Note: there are no tubules composed of two separated microtubules in the center of the cilia. *Calibration bar:* 0.1 μm

ported, simply because the number of cilia seen was too small and transversely sectioned cilia were rarely encountered. In mouse kidney, all the cilia had the 9+0 pattern near their base, but the pattern gradually changed peripherally to 8+1, 7+2, 7+1, 6+2, 6+1, 6+0, 5+1, 5+0, 4+1, 4+0 and 3+0. One or two centrally located microtubules seemed to be displaced to the centre from the periphery of the cilium [4]. In human keratinocytes, the axoneme of the cilia has 9 tubular doublets and no central tubules but loses its regular arrangement toward the tip of the cilium [3]. We found the 8+1, 7+1, 7+0, 6+2, 6+1, 6+0, 5+0 and 4+0 patterns of microtubule pairs and X+(1 or 2) patterns also seemed to be displaced to the centre from the periphery of the cilium. We presume that the cilia of dermal melanocytes in naevus of Ota had the 9+0 pattern in their base, although we failed to observe this pattern. Flood and Totland [4] have previously reported that the lowest frequency in the mouse kidney was that of the 9+0.

The significance and function of the cilia in skin tissue are still obscure. Several different theories have postulated that they may have chemoreceptor or pressure sensory functions, or that they are evolutionary remnants. However, none of these theories explains all the features of cilia, and all have been refuted [8]. Recently, the potential importance of the mechano-sensory function of cilia has gained more attention [12, 13].

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