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ORIGINAL ARTICLE

Melanin in human vestibular organs: what do we know now? An ultrastructural study and review of the literature

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ABSTRACT

Objective: Melanin is a pigment widely found in animals and plants. It has long been observed in the inner ear, but its function there is not even now entirely clear. The protective role of melanin against damage caused by ageing, ototoxicity and noise is still a matter of debate.

Methods: The current study discusses ultrastructural findings in the vestibular organs removed via the translabyrinthine approach for the resection of a schwannoma of the 8th cranial nerve. Our aim was to shed more light on the role of melanocytes in the inner ear, analyzing their features in human subjects.

Results: Melanin granules were present in all of the cases studied: in some samples they appeared scattered in the cytoplasm, whereas in others they accumulated in the reserve area. There was no correlation between the presence of pigment and schwannoma dimensions other than a greater frequency of accumulation in the reserve areas in the melanocytes of patients with small tumours.

Conclusions: Our findings and literature data confirm that melanocytes are an integral part of the endolymph production system and appear essential for balance and hearing functions, having a protective effect. Thanks to its semiconducting properties, melanin could be involved in absorbing the mechanical energy arising from sensory stimulation.

KEYWORDS
Melanocytes; electron microscopy; inner ear; human vestibular labyrinth

Introduction

Melanin is a pigment widely found in animals and plants. It has long been observed in the inner ear, but its function there is not even now entirely clear. Melanin in the inner ear was first mentioned by Corti [1] in 1851, but it was not until 1931 that Wolff [2] demonstrated that the pigment detected in the human labyrinth was melanin, while in 1956, Bonaccorsi [3] postulated a correlation between eye colour and cochlear melanin content.

Melanocytes derive from the neural crest and they are present in the cochlea, vestibular organs and the endolymph sac. The vestibular system is quite primitive: approximately 500 million years ago, aquatic vertebrates had ears limited to the labyrinth component and composed of two semicircular canals, a utricle and a saccule. Later, when vertebrates began to live on dry ground, they developed a 'vestibular' ear with hearing ability that became more sensitive and was perfected in the evolutionary passage from reptiles to mammals [4].

Melanin is synthesized in the cytoplasm of melanocytes whose precursors migrate and reach the inner ear after neural tube closure, with a distribution that varies from species to species [5,6]. Vestibular melanocytes are found in the subepithelial region in the highly vascularized dark cell area of the utricle and ampulla, which is mainly responsible for maintaining the ionic composition of endolymph [6–10]. They represent a biological reserve of Mg^{2+} and Ca^{2+}, essential for the enzymatic activation of many systems. In pathological conditions such as Waardenburg or Vogt–Koyanagi–Harada syndromes, melanocyte activity appears critical for inner ear function [11–16].

Depending on its biochemical properties and solubility, melanin can assume an ellipsoidal shape and dark colouring (eumelanin) or a spherical shape and red/yellow colouring without a matrix (pheomelanin), whose percentages are determined by the level of

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tyrosinase activity and genetic factors [14,17–19]. L-DOPA-quinone, the precursor of both pheomelanin and eumelanin, is formed by a multistep process starting with tyrosine activity through tyrosinase reactions in the melanosomes [20,21]. Since quinones are potentially cytotoxic, melanin pigments are restricted to specific subcellular compartments [21].

Melanin is able to reduce free radicals, bind many organic molecules and act as a semiconductor material. As a semiconductor, melanin responds to electrical, acoustic and photic stimuli and acts as a photon/electron receptor and converter of rotational energy and molecular vibration [14].

While observations of animals are abundant, human studies are limited in number because of the nature of the human vestibular system. On the one hand, the vestibular organs are small and hard to access except through rather destructive surgical intervention; on the other, the epithelium is so delicate that it needs to be immediately treated with fixing techniques on removal. This paper describes the ultrastructural findings of melanin and melanocytes in human vestibular organs harvested during the course of translabyrinthine schwannoma resections.

Materials and methods

The study was performed on preparations obtained from the semicircular canals of patients undergoing surgery via translabyrinthine access for the resection of a schwannoma of the 8th cranial nerve. The series included 16 men and 15 women, all Caucasian, with a mean age of 51 years (range: 27–77). Ten subjects underwent surgery at the Department of Otolaryngology-Head and Neck Surgery at Bologna University Hospital and 21 at Legnano City Hospital. The tumours varied in size from 5 to 40 mm, and measured 10 mm or less in eight out of the 31 cases (Tables 1 and 2). The schwannomas were assessed using magnetic resonance imaging with gadolinium-based contrast agents. Cases undergoing resurgery were not included. Vestibular function, as measured by electronystagmography, was found to be impaired to a greater or lesser extent in all patients studied. Except for two patients whose first symptom was an episode of vertigo, clinical presentation coincided with the onset of tinnitus associated with hearing loss. The patient with a 5 mm neoplasm opted for surgery due to severe vertigo symptoms refractory to medical therapy.

After removal, the vestibular organs (semicircular canals) were immediately prepared for examination following standard procedures. Specimens were fixed in 2.5% glutaraldehyde in a phosphate buffer, dehydrated in ethanol and embedded in araldite. Semi-fine (0.5–1.0 micron) sections were then prepared, stained with toluidine blue and analyzed under an optical microscope. Thin sections stained with uranyl acetate and lead citrate were examined under a Philips 400 T transmission electron microscope (TEM) and a Hitachi H-800 TEM at the Electron Microscopy Centre of Ferrara and the Electron Microscopy Centre of the University of Bologna respectively. The study started in Bologna and continued at the University of Ferrara when the first author moved. Our research was carried out in accordance with the principles of the Declaration of Helsinki and informed consent was obtained from all patients.

Table 1. Generall information and tumour size in patients who underwent schwannoma resection at Bologna University Hospital.

	Sex	Age	Size
1	F	62	12 mm
2	M	71	15 mm
3	M	56	28 mm
4	F	39	18 mm
5	F	61	20 mm
6	F	47	12 mm
7	F	64	9 mm
8	F	53	18 mm
9	F	56	14 mm
10	M	44	25 mm

Table 2. General information and tumour size in patients who underwent schwannoma resection at Legnano City Hospital.

	Sex	Age	Size
11	M	58	15 mm
12	M	44	18 mm
13	M	27	12 mm
14	M	44	30 mm
15	M	46	40 mm
16	F	31	20 mm
17	M	77	40 mm
18	M	55	15 mm
19	F	58	30 mm
20	M	37	30 mm
21	F	41	25 mm
22	F	50	10 mm
23	M	52	15 mm
24	M	40	12 mm
25	M	51	10 mm
26	M	55	8 mm
27	F	45	35 mm
28	F	45	10 mm
29	M	65	10 mm
30	F	47	5 mm
31	F	65	10 mm

Results

The samples examined were sections of the semicircular canals of the dark cell area. The images show the

basal membrane and epithelium made up of dark cells, light cells and transitional cells (Figure 1). The dark cells, assembled in one or two layers close to the sensory epithelium and in a single layer close to the canal wall, had a cube-like or slightly flattened shape with numerous microvilli. The cytoplasm contained the endoplasmic reticulum and was mitochondria-rich with basolateral invaginations that appeared piled on top of one another or in a sagittal section depending on the direction of the cut (Figure 2). Light cells also contained high quantities of mitochondria, while numerous cytoplasmic processes were found next to the dark cells. Tight junctions were evident in the dark cells, whereas gap junctions were not noted.

The melanocytes almost always displayed a light cytoplasm and were located near the dark cells in the subepithelial region. They contained spherical granules, mitochondria, a well-developed Golgi apparatus and frequent pinocytotic invaginations.

The melanosomes were prevalently mature and dark coloured (eumelanin). Melanin granules were present in all of the cases studied: in some samples they appeared scattered in the cytoplasm, whereas in others they accumulated in the reserve area (Figures 3–5). Pigment accumulated in the reserve areas of the melanocytes in three of the eight patients with small tumours. In some cases, melanocytes were noted trespassing the epithelium in the midst of the dark cells, containing numerous melanosomes accumulated in the cytoplasm (Figure 6). Fusiform band structures were frequently noted in the extracellular space (Figure 7). Cells that were morphologically attributable to macrophages with characteristics similar to those of melanocytes but with a darker cytoplasm were also noted (Figure 8).

The capillaries observed consisted of non-fenestrated endothelium whose cells were connected to one another by means of a junctional apparatus. They showed well-developed microvilli with pinocytotic invaginations in the cytoplasm, and a basement membrane. The capillaries were generally surrounded by the cellular body or cytoplasmic processes of melanophages or melanocytes and by dark cells (Figure 9).

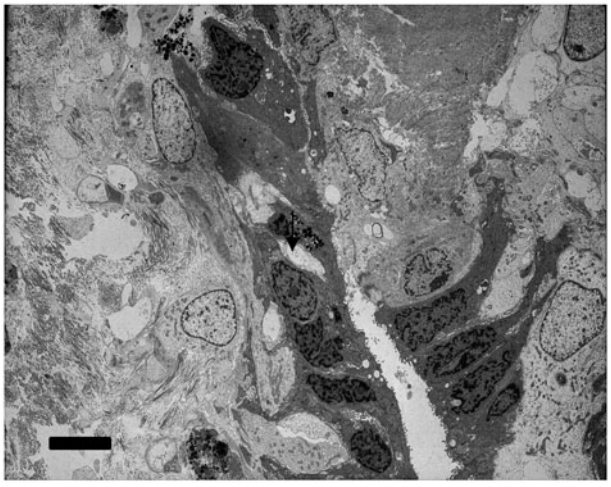

Figure 1. The epithelium, made up of dark cells and light cells; scale bar = 4.4 μm.

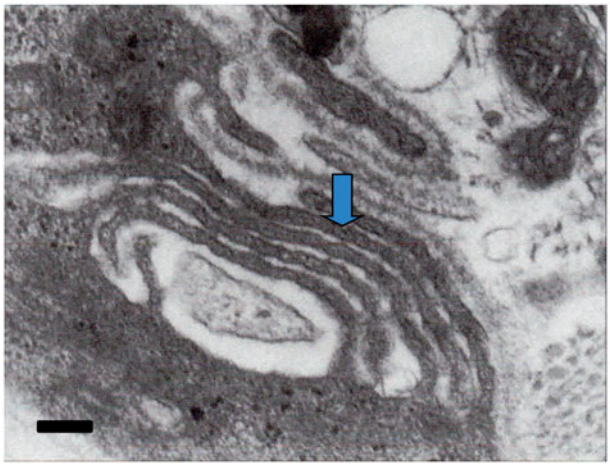

Figure 2. Higher magnification of a basocellular invagination of a well-developed system. It is likely to embrace a cytoplasmic edge of a menanocyte; scale bar = 0.3 μm.

Discussion

Due to the difficulty of accessing the human vestibular system and the few melanocytes in the vestibular labyrinth, this study on melanin in the posterior inner ear was based on morphological features [22,23]. We observed melanin in the dark cell areas of the semicircular canals of all the cases examined. For this reason, and as the pigment is present in the normal human

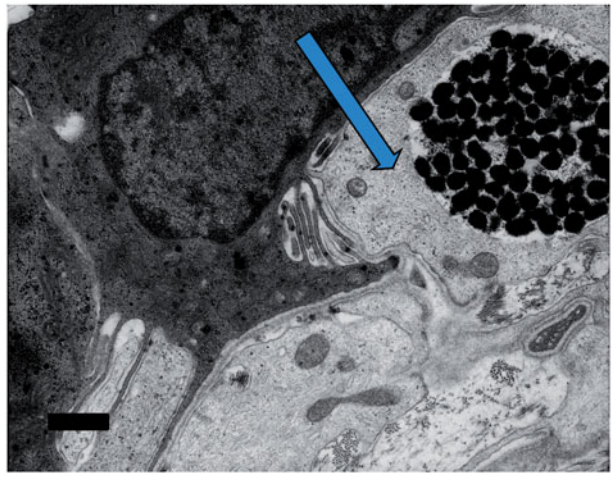

Figure 3. Pigment granules clustered in reserve areas; scale bar = 0.6 μm.

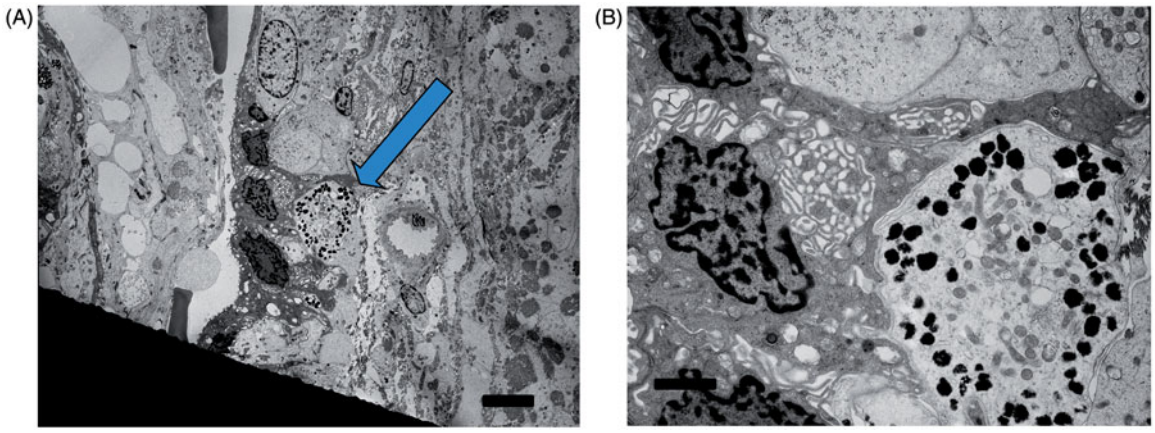

Figure 4. (a) Pigment granules scattered sparsely throughout the cytoplasm of the cell body; scale bar = 4.8μm. (b) Higher magnification (a); scale bar = 1.1 μm.

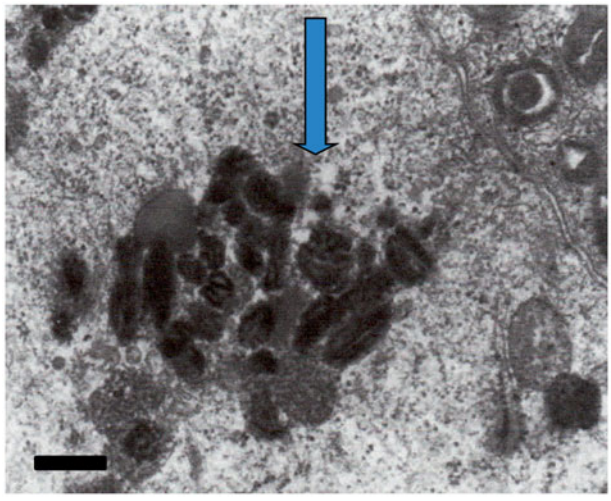

Figure 5. Eumelanin granules; scale bar 0.3 μm.

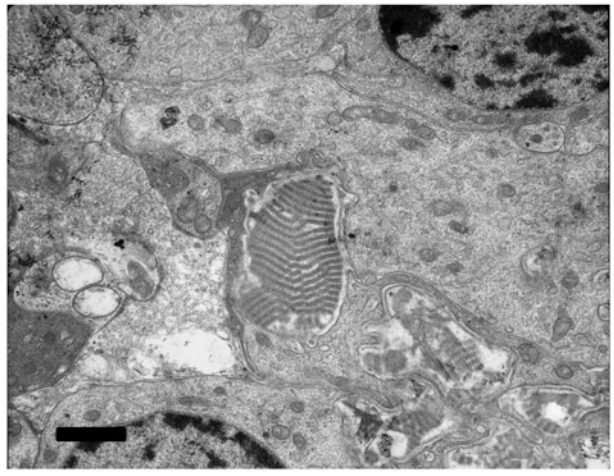

Figure 7. Structured fusiform bands similar to Luse bodies; scale bar = 1.1 μm.

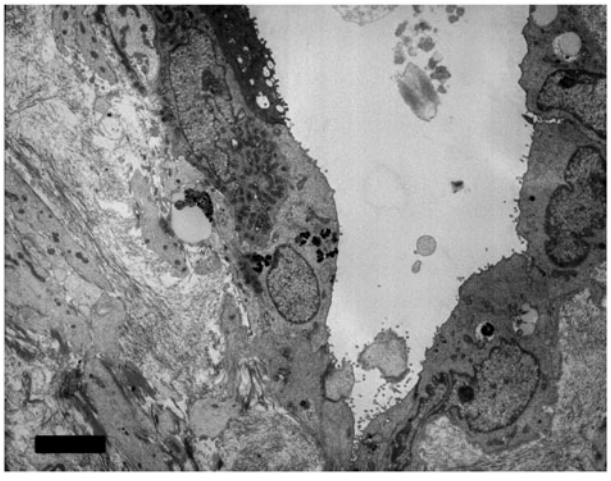

Figure 6. A melanocyte that has passed beyond the basal membrane; scale bar = 4.8 μm.

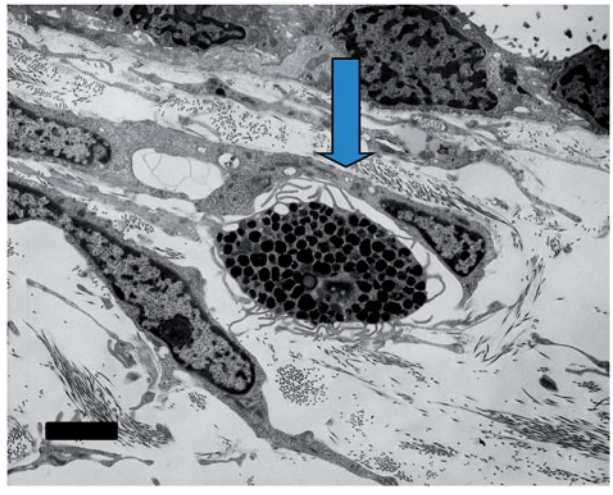

Figure 8. A macrophage in the centre of the field observation; scale bar = 2.5 μm.

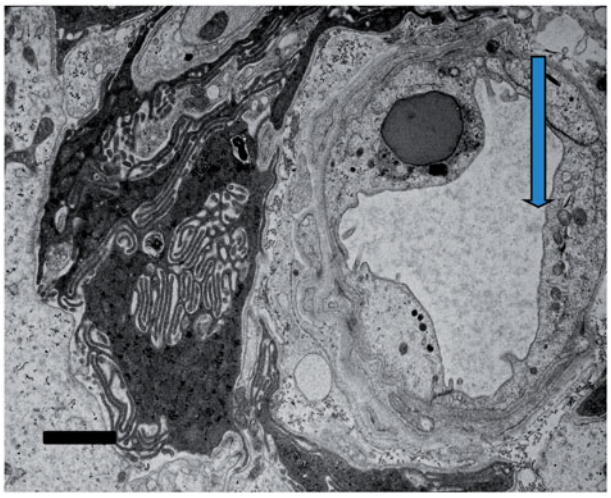

Figure 9. A capillary in a dark cell area of non-fenestrated endothelium; scale bar =1.1 µm.

foetus, the hypothesis that under normal conditions melanocytes in the inner ear are scarcely active no longer seems plausible [23]. Eumelanin granules were prevalently observed. Therefore, using the data collected from Caucasian human specimens by Erbele et al. [24] as a case-control, it can be assumed that vestibular schwannoma does not significantly alter melanocyte function in terms of melanin production. In this regard, Navarrete et al. [25] demonstrated that the quantity of melanin in the inner ear is directly correlated to skin pigmentation, while studies by Erbele et al. [24] and Sun et al. [26] uncovered racial differences in melanin pigmentation, showing that there is a greater pigmentation in the inner ear of Afro-Americans. In this study, all the subjects were Caucasian and this represents a limitation.

The relationship we found between melanocytes and dark cells confirms the existence of real functional units maintaining the chemical composition of the inner ear fluids, as well as the active role of melanin in metabolic functions [27–29]. Animal studies have demonstrated that following gentamycin treatment or after experimentally induced osmotic alterations, the melanocytes pass beyond the basal membrane and their cytoplasmic processes extend into the intercellular space of the dark cells, with a statistically significant dose-dependent increase in the number of melanosomes [12,30]. Some melanocytes found in an intraepithelial location in a few of our patients with small schwannomas could be an expression of greater metabolic requirements during the developmental stage of the neoplasm. This 'activation' could be related to initial neurotoxic damage caused by the vestibular schwannoma as the chemical difference between perilymph and endolymph systems is known to be necessary for vestibular function [31]. In this regard, hyperpigmentation and movement of the melanosomes in the endolymphatic sac have been described in patients with Ménière disease [32].

In many of our cases, we found intercellular spindle-shaped dark band material that could also be the expression of a higher metabolic activity in this area, as could our observation of a particular cell type found in the dark area. This cell was very similar to a hybrid phenotype described by Zhang et al. [33] who demonstrated that a hybrid phenotype found in the stria vascularis, with characteristics similar to those of both macrophages and melanocytes, is essential for hearing since it determines the integrity of the interstitial fluid-blood barrier modulating the protein expression of the tight junctions. Although we examined numerous cases, no gap junctions were noted, probably due to epithelial fragility and the difficulty of orienting and fixing specimens collected from the vestibular labyrinth during surgery. The gap junctions represent the intracellular electrical coupling site allowing the passage of ions that link the cells electrically and chemically, for example in the case of calcium [12,13,21,34,35]. To date, only Masuda et al. have demonstrated frequent gap junctions in the melanocytes of the human inner ear [36,37]. As potassium cycling seems to be preserved when the sensory cells are damaged, cell communications in the dark area appear basic for hearing and balance functions [38–41]. We know that impaired potassium transport results in deafness and the mechanisms underlying cell junction-associated disorders still require further research [42].

The close proximity of melanocytes to capillaries seems related to the complex hormonal regulation of inner ear fluids and suggests that a 'holistic' approach to the pathologies of this organ is most appropriate: dark cells express adrenergic, purinergic and vasopressin receptors, while inner ear melanocytes have receptors that respond to the alpha-melanocyte-stimulating hormone, a neuromodulator in many physiological functions [16,43–47].

Based on the data reported in the literature, if we compare the function of skin melanocytes to those of the inner ear, we can hypothesize that melanin's semiconducting properties could be involved in absorbing the mechanical energy that arises from sensory stimulation and thus have a protective effect. Unlike what takes place in the skin, the melanosomes of the inner ear are not transferred to other cells, so the presence in cells of potentially cytotoxic molecules like quinones must be related to an activity necessary to

organ function [18]. The peculiarity of inner ear physiology lies in receiving sound pressure waves or sensing movements of the head. While it has been demonstrated that melanin has a protective effect against damage due to noise, it is more difficult to prove its effect on vestibular organ stimulation. The membranous labyrinth was originally located on the outside of aquatic vertebrates and the melanocytes in the human inner ear could represent the vestiges of the primitive function of monitoring water currents against the organism's body surface.

Disclosure statement

No potential conflict of interest was reported by the authors.

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