Published in final edited form as:

Laryngoscope. 2014 June ; 124(6): E241–E244.

Extracellular Melanin in Inflammatory Middle Ear Disease

Mark Fritz, MD¹, Pamela C. Roehm, MD, PhD¹, Michael A. Bannan, MD², and Anil K. Lalwani, MD³

¹Department of Otolaryngology New York University School of Medicine, New York, NY

²Department of Pathology, New York University School of Medicine, New York, NY

³Department of Otolaryngology, Columbia University College of Physicians and Surgeons, New York, NY

Abstract

Objective—Melanin is a pigmented polymer with a known role in dermal solar protection. In vertebrates, melanogenesis has been reported in leukocyte population suggesting a potential role in innate immunity. In this study, we report the novel finding of melanin associated with chronic inflammation and speculate on its potential role in the middle ear and mastoid.

Study Design—Retrospective review of case series

Methods—Medical records of 6 patients who demonstrated melanin in the ear were reviewed.

Results—Six patients, ages from 1 to 63 were identified with extracellular melanin within middle ear and/or mastoid air cells at time of surgery. Intraoperative findings included cholesteatoma (n=3), chronic suppurative OM (n=2), and coalescent mastoiditis (n=1). Histologically, extracellular melanin was identified by Fontana-Masson stain; absence of melanocytes was confirmed by the absence of Melan-A and Prussian Blue stain. One patient had a positive stain for CD163 (a marker for macrophages).

Conclusion—This case series is the first demonstration of extracellular melanin within middle ear mucosa not associated with melanocytes or metastatic melanocytic lesions. The presence of melanin is either a variant of normal anatomy, a pathway of cholesteatoma formation, or a marker of the inflammatory immune response. Melanin's presence in the setting of inflammation suggests that there may be a heretofore unreported link between the pigmentary and immune systems in the ear.

Keywords

melanin; melanocyte; inflammation; cholesteatoma; otitis media

Correspondence and Reprint Request to: Anil K. Lalwani, MD Professor and Vice Chair Division of Otolaryngology, Neurotology, and Skull Base Surgery Department of Otolaryngology-Head & Neck Surgery Columbia University College of Physicians and Surgeons 180 Fort Washington Avenue, Harkness Pavilion HP818 New York, NY 10032 Phone: 212-305-5820 Fax: 212-305-2249
anil.lalwani@columbia.edu.

Financial Conflict: No relevant financial conflict

Introduction

Melanocytes are derived from the neural crest, the outer wall of the optic cup, and the cranial neural tube[1]. Melanocytes have been known to be present within the inner ear since the time of Corti and are found in the wall of the scala vestibuli, Reissner's membrane, interstices of modiolus, maculae utriculi and sacculi, and the interstices of inner ear bone [2]. They have also been described within the attachments of the round window membrane and submucosal and subcutaneous tissue of the niche [3].

The middle ear mucosa, like the nasopharyngeal and oral mucosa, is derived embryologically from pharyngeal pouches. Given that both oral and nasopharyngeal mucosa contain melanocytes, it would be expected that the middle ear would also harbor melanocytes. However, the literature regarding melanocytes in the middle ear is conflicting. While Lin showed melanocytes scattered in the lamina propria of the middle ear mucosa in 10/50 cadaveric specimens [2] Navarrete, only found melanin and melanocytes in the inner ear of 18 specimens [4]. Reports of primary malignant melanoma of the middle ear mucosa supports the hypothesis that melanocytes are present in the middle ear [5–11]. Of note, extracellular melanin has not been reported in the middle ear. In this report, we report on a series of 6 patients undergoing ear surgery in whom extracellular melanin was identified and discuss its potential significance.

Materials and Methods

Six patients with intraoperative findings of dark pigmentation while undergoing ear surgery were identified. Their medical, radiological, surgical and histological records were retrospectively reviewed. Histological samples were studied with Fontana-Masson stain, Prussian blue stain, and Melan-A immunohistochemical stain. The Fontana-Masson stain identifies melanin, by demonstrating an accumulation of black material wherever melanin granules have reduced silver nitrate to metallic silver. The Prussian blue stain identifies iron by a chemical reaction that takes place in the tissue producing a blue colored iron salt *in situ*. Melan-A is a melanocyte differentiation antigen, recognized by autologous cytotoxic T lymphocytes. Melan-A is also called MART-1 (melanoma antigen recognized by T cells). Melan-A is expressed in all normal melanocytes and melanocyte cell lines. Antibodies directed against Melan-A are used to identify melanocytes, as well as melanocytic lesion such as melanoma. Additional immunohistochemical staining was performed with CD163 antibody to identify macrophages.

Results

The six patients who were found to have dark pigment in the ear ranged in age from 1 to 63 years old. The four adults in the study had surgery for cholesteatoma (n=3), and coalescent mastoiditis (n=1). The two pediatric patients were undergoing cochlear implantation and were noted to have chronic otitis media with significant mucosal disease in the middle ear and mastoid cavity. With the exception of the presence of dark pigmentation, the surgical course was typical.

In all patients, Fontana-Masson stain was positive and Prussian blue iron stain was negative, confirming the presence of melanin and absence of iron which can also have a similar appearance histologically. Melanocytes were not seen in any of our specimens as evidenced by negative Melan-A staining. Melanin was mainly found in the connective tissue within these samples.

In one patient, additional immunohistochemical analysis showed CD163 positive cells consistent with the presence of macrophages. In this patient, melanin was present within the macrophages as well as within the extracellular space similar to other patients.

Discussion

In this study, we demonstrate that the dark pigment noted in the middle ear and mastoid mucosa during surgery was positive for Fontana-Masson stain, and thus consistent with melanin. The melanin was extracellular and there were no benign or malignant cells of melanocyte lineage as demonstrated by negative Melan-A staining. In 1982, Lin showed the presence of melanocytes scattered within the lamina propria of the middle ear mucosa in 20% of cadaver specimens, but no extra-melanocytic melanin was appreciated within the mucosa [2]. Similarly, Olszewska et al. described the presence of melanocytes within the basal layer of cholesteatoma matrix [16]. Their study saw a smaller, percentage of melanocytes within the cholesteatoma matrix in comparison to normal skin samples (2–6% versus 10%). In addition, melanocytes were also observed around the glands and blood vessels, along with single cells found among the subepithelial connective tissue cells. However, these studies did not note extracellular melanin.

The significance of melanin in the setting of chronic ear disease is unclear. In the inner ear, several studies in animals have demonstrated increase melanin in the setting of inner ear stress. Gratton showed dramatically increased stria melanin content in 7/36 chinchillas 30 days after exposure to non-continuous, impulse-type noise [12]. Additional studies have shown increased pigmentation after elevated temperature and noise exposure [13], ototoxic drug administration [14], and mechanical trauma [15]. Thus, injury could be a stimulus for melanin production in the middle ear.

Alternatively, melanin may induce or be a response to inflammation. In the anterior chamber of the mouse eye, melanin has been found to augment immunologically mediated intraocular inflammation [17]. In this study, a stronger inflammatory response was noted after injecting sensitized antigen with melanin than antigen alone into the aqueous humor of the mouse eye. This proinflammatory effect was hypothesized to result from the ability of melanin to bind to antibodies and the subsequent prevention of drainage from the trabecular meshwork. Thus, presence of melanin in the middle ear may reflect a more severe inflammation or infection.

The putative role on melanin in the immune response would be more consistent with the recent shift in understanding of the function of melanocyte away from being simply a solar protector to its being an integral part of the immune system in plants and animals [18]. Fungal melanin has been shown to activate the alternative complement pathway in human sera [19]. Isolated grape melanin has been shown in rats to decrease inflammation and

suppress the activation of Th1 lymphocytes in the development of cell-mediated immunity[20]. Additionally, melanin has been found to be a part of the innate immunity of invertebrates: melanin deposits are found around microorganisms or parasites in nodules or capsules forming a mechanical barrier, and thereby restricting the growth of invading organisms[21]. Atlantic salmon produce melanin around granulomatous tissue; and this could serve as a protective mechanism against oxidation in chronic inflammatory conditions [22]. Moreover, in human studies, IFN- γ treated melanocytes can efficiently process and present antigen through MHC class II molecules to Th1-like T cells, effectively describing an integral role within the immune system [23]. Thus, the extracellular melanin observed in this study may be a byproduct of the immune response in the setting of chronic ear disease.

In summary, this paper is the first report of amelanocytic melanin in the middle ear associated with a variety of chronic middle ear pathologies. The function of this melanin remains unknown; however, there is increasing evidence that the pigmentary and immune systems are linked. Further studies are warranted to study the function and effects of melanin within the middle ear space.

References

1. AS B. Extra-Cutaneous Melanin. *Pigment Cell Research*. 1988; 1:4.
2. Lin C-S ZF. Studies on Melanocytes, VI. Melanocytes in the Middle Ear. *Arch Otolaryngol*, 1982. Aug.1982 108:2.
3. Gussen R. Round Window Niche Melanocytes and Webby Tissue. *Arch Otolaryngol*, 1977. Nov. 1978 104:7.
4. Navarrete CM RC, Paparella MM Schachern. Normal and Metastatic Melanin in the Temporal Bone. *American Journal of Otolaryngology*. 1995; 16(1):7.
5. McKenna EL, Holmes WF, Harwick R. Primary melanoma of the middle ear. *The Laryngoscope*. 1984; 94(11):1459–1460. [PubMed: 6492967]
6. Sherman IW, Swift AC, Haqqani MT. Primary mucosal malignant melanoma of the middle ear. *J Laryngol Otol*. 1991; 105(12):1061–4. [PubMed: 1787360]
7. Urpegui Garcia A, et al. Malignant melanoma of the middle ear, a rare site. *Acta Otorrinolaringol Esp*. 1999; 50(7):559–62. [PubMed: 10619884]
8. Uchida M MT. Malignant amelanotic melanoma of the middle ear. *Archives of Otolaryngology—Head & Neck Surgery*. 2001; 127(9):1126–1128. [PubMed: 11556865]
9. Ozturk O, et al. Primary malignant melanoma of the middle ear mucosa: a case report. *Kulak Burun Bogaz Ihtis Derg*. 2006; 16(2):83–6. [PubMed: 16763422]
10. Mehta B, Maniyar H. Primary mucosal malignant melanoma of middle ear—A case report. *Indian Journal of Otolaryngology and Head & Neck Surgery*. 2007; 59(1):71–72. [PubMed: 23120394]
11. Huellner MW, et al. Melanoma of the middle ear: initial presentation, Fluoro-2-deoxy-dglucose positron emission tomography/computed tomography imaging and follow up. *The Journal of Laryngology & Otology*. 2011; 125(05):536–539. [PubMed: 21272419]
12. Gratton MA, Wright CG. Hyperpigmentation of Chinchilla Stria Vascularis Following Acoustic Trauma. *Pigment Cell Research*. 1992; 5(1):30–37. [PubMed: 1631019]
13. Shaddock LC, Hamernik RP, Axelsson A. Cochlear vascular and sensory cell changes induced by elevated temperature and noise. *American Journal of Otolaryngology*. 1984; 5(2):99–107. [PubMed: 6496868]
14. Gratacap B, Charachon R, Stoeber P. Results of an ultrastructural study comparing stria vascularis with organ of Corti in guinea pigs treated with kanamycin. *Acta Otolaryngol*. 1985; 99(3–4):339–42. [PubMed: 4013723]

15. Shaddock LC, Wright CG, Hamernik RP. A morphometric study of microvascular pathology following experimental rupture of Reissner's membrane. *Hear Res.* 1985; 20(2):119–29. [PubMed: 4086379]
16. Olszewska E WM, Goon P, Shamaa A, Upile T, Rogowski M, Steinsaesser L, Sudhoff HH. Melanocyte localization and distribution in human cholesteatoma. *Histology and Histopathology.* 2008; 23:6.
17. Kaya M, et al. Augmentation of intraocular inflammation by melanin. *Investigative Ophthalmology & Visual Science.* 1992; 33(3):522–31. [PubMed: 1531974]
18. Burkhart CG, Burkhart CN. The mole theory: primary function of melanocytes and melanin may be antimicrobial defense and immunomodulation (not solar protection). *International Journal of Dermatology.* 2005. 44(4):340–342.
19. Rosas AL, et al. Activation of the alternative complement pathway by fungal melanins. *Clin Diagn Lab Immunol.* 2002; 9(1):144–8. [PubMed: 11777844]
20. Avramidis N, et al. Anti-inflammatory and immunomodulating properties of grape melanin. Inhibitory effects on paw edema and adjuvant induced disease. *Arzneimittelforschung.* 1998; 48(7):764–71. [PubMed: 9706378]
21. Boman HG, Hultmark D. Cell-Free Immunity in Insects. *Annual Review of Microbiology.* 1987; 41(1):103–126.
22. Larsen HA, et al. Pigment-producing granulomatous myopathy in Atlantic salmon: a novel inflammatory response. *Fish Shellfish Immunol.* 2012; 33(2):277–85. [PubMed: 22634154]
23. Le Poole IC, et al. A novel, antigen-presenting function of melanocytes and its possible relationship to hypopigmentary disorders. *The Journal of Immunology.* 1993; 151(12):7284–92. [PubMed: 8258725]

Figure 1.
Dark pigment within the mastoid cavity subsequently demonstrated to be extracellular melanin.