Contributions of Contemporary Human Temporal Bone Histopathology to Clinical Otology

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Abstract

Contemporary techniques have greatly enhanced the contributions of human temporal bone (HTB) histopathology to our understanding of the mechanisms of human otologic disease and disease treatment. Herein, we review some of the most salient contributions of this research to disease management. The field of HTB histopathology is challenged by limited resources as applies to trained investigators, infrastructure, and well-equipped laboratories. This research provides insights into clinical otology that cannot be obtained by any other means. Measures should be taken to preserve and extend the contributions of HTB research.

Keywords

otology, histopathology, temporal bone, neuropathology

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Contemporary techniques in human temporal bone (HTB) histopathology have allowed researchers to make significant contributions to our understanding of human otologic and neurotologic disease and treatment. These advances in our understanding have been integral to the practice of modern otology and neurotology but may be underrecognized by clinicians. Although animal models may be invaluable for some investigations of human disease, for many diseases, there are no animal models, or animals differ from humans in significant ways. In some cases, initial discoveries are made in animals and then confirmed in HTB studies.¹,²

New techniques of analysis allow integration of information obtained from a variety of methods using a single HTB. Methodological integration of techniques may include information accessed through traditional light microscopy, as well as by more contemporary means of analysis, including transmission electron microscopy (TEM), immunohistochemistry (IHC), nonradioactive in situ hybridization, and DNA and proteomics analysis.³ These techniques allow for multilevel investigations of tissue, from the synaptic and molecular level to larger structural changes. Advances in radiologic assessment of otologic disease allow for noninvasive diagnostic studies that can be validated by comparison with pathological tissue.⁴

A key example of this application is the identification of the herpes varicella-zoster virus (VZV) in the geniculate ganglion of archived HTB from patients who had Ramsey-Hunt syndrome (herpes zoster oticus) in life. The technique of DNA extraction and amplification of the VZV gene via polymerase chain reaction (PCR) from the affected side, using the unaffected side as a control, confirmed the etiology of this syndrome,⁵,⁶ which was previously debated.

Of importance, maximal information is derived from this valuable resource when 2 conditions are met: the HTB tissue is of good quality, as well as processed competently and expeditiously, and when tissue is accompanied by a well-documented history of otologic disease. Hence, it is of grave concern that resources for procurement, processing, and analysis of this tissue appear to be dwindling.⁷

The purpose of this review is to present to the clinician a brief overview of some of the most salient contributions of this field of scientific inquiry to clinical practice. Contributions are numerous, and this review is not exhaustive or comprehensive as much as illustrative of the potential to contribute to diagnosis and management of patients with clinical otologic disease.

Otosclerosis

HTB studies have been instrumental in defining the fundamental histopathology of this disease, describing correlation with hearing phenotype, explaining the variability in disease expression, and defining parameters of surgical outcomes. The most salient clinical feature of otosclerosis is adult-onset progressive conductive hearing loss due to fixation of the stapedial footplate along the anterior annulus.

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Discovery could inform the identification of a potential cochlear extension and spiral ligament hyalinization; this is integral to the disease process in otosclerotic HTBs with et al13 identified transforming growth factor with confirmatory immunohistochemistry, a study by Richard et al.12 Furthermore, transcriptomic analysis by shotgun mass spectrometry combined with confocal immunohistochemistry, a study by Richard et al.13 demonstrated transforming growth factor β1 (TGFβ1) as a potential therapeutic target to prevent the development and extension of otosclerosis in some individuals. Changes in the concomitant levels of tumor necrosis factor-α and osteoprotegerin in otosclerotic HTB ankylosis suggest a possible etiopathogenetic mechanism of osteosclerotic lesions. Studies that reflect alteration in bony metabolism in patients with otosclerosis also lend credence to the potential benefit of therapeutic agents that target these pathways, including bisphosphonates.7

Nadol16 performed a histopathologic study of HTBs from 22 patients who had undergone stapedectomy, with subsequent identification of residual and recurrent conductive hearing loss. Results of evaluation of these ears provide valuable insight into surgical management. The most common findings included resorptive osteitis of the incus at the site of prosthesis attachment (64%), otosclerotic obliteration of the round window (23%), displaced prosthesis onto a fragment of the footplate (23%), prosthesis abutting the bony margin of the oval window rather than centered in the fenestration (18%), and the presence of postoperative new bone formation (neo-osteogenesis) in the oval window (14%), with multiple factors seen in most specimens. Malleus fixation was found in only 1 specimen, contradicting some assessments in which it has been thought to be relatively common. As discussed by Nadol,16 incus osteitis (incus necrosis) is thought to be secondary to pressure necrosis, a loose crimp, or damage to the incus mucosal envelope. Related to the finding of round window obliteration, Nadol16 encourages examination of this structure during surgery but recommended proceeding with stapes surgery, as it is not possible to do a complete assessment of the round window at the time of surgery. Based on the finding of new bone formation at the footplate, an extensively obliterated footplate must be drilled to saucerize, and the final fenestra should be comfortably larger than the piston prosthesis to accommodate for possible neo-osteogenesis. Thus, evidence from these HTB studies has informed otosclerosis surgical management.

Middle Ear Pathology: Otitis Media

There is a continuum of ear infection that is present even in the presence of an intact tympanic membrane. The term *otitis media* (OM) refers to a group of inflammatory diseases that affect the middle ear. *Chronic otitis media* is a term that has been used to indicate an inflammatory process within the middle ear that is associated with irreversible tissue pathology. Disease may be active, with perforation and suppuration, or inactive, with sequelae of previous infections, such as hypertrophic middle ear mucosa (Figure 2A) and even development of cholesteatoma (Figure 2B). Chronic “silent” otitis media is a disease concept that was first reported by Kaya et al.17 based on a study of HTBs that showed prominent granulation tissue in some HTBs without perforation. Studies of HTBs from infants who had meningitis demonstrate evidence of silent OM in the
absence of diagnosed ear infection. Affected ears contained bacteria within fibrous matrices; free-floating bacteria along with scattered neutrophils, monocytes, and other inflammatory cells were found infiltrating fibrous networks and were often seen in large areas of the middle and inner ears. HTB research has demonstrated that OM may cause sensory cell damage in the inner ear. Monsanto et al.19 studied HTBs of 40 ears with a premortem otitis media diagnosis and a medical history consistent with a graded continuum of disease, progressing through the following stages: serous OM, serous-purulent OM, mucoid and mucoid-purulent OM, and chronic OM. Investigators identified significant inner and outer hair cell loss in the basal turn of the cochlea in all but the serous OM group, and a significant vestibular hair cell loss was identified in the latter 2 groups compared with control, non-diseased HTBs. Results suggest that advancement in degenerative cochlear changes from base to apex is associated with disease progression, while vestibular sensory cells appear to be affected only in the later disease stages. Furthermore, the mean area of stria vascularis was found to be significantly decreased in the basal turn of the cochlea in all OM groups. Authors suggest that the stria vascularis may be directly affected by the inflammatory process associated with OM. Consistent with this finding, Kaya et al.17 demonstrated a significant increase in degree of endolymphatic hydrops as well as outer hair cell loss in HTB with silent OM-associated serous labyrinthitis. These histopathological findings are consistent with clinical findings of increased high-frequency hearing loss as well as balance disorders in patients with OM.

**Inner Ear**

Histopathology of HTBs with known genetic defects has facilitated understanding of the pathophysiology of these disorders as well as the potential for hearing restoration gene therapy.17,20,21 Importantly, studies in HTB have revealed significantly different histopathologic changes associated with genetic defects in humans compared with mice,22 highlighting the fact that animal studies alone are insufficient to fully inform effective treatment development for genetic hearing loss.

HTB studies have also elucidated changes that occur with aging and presbycusis, again pointing to mechanisms of change and potential therapeutic targets. Hao et al.23 examined Sox10 (transcription factor) expression in the lateral cochlear wall of young and aged mice (CBA/CaJ) and in HTBs prepared for analysis by either paraffin embedding/sectioning or frozen sectioning. Use of both Sox10 expression models allowed for a more complete picture given the low expression in human postmortem cochlear tissues. A similar combined mouse/human investigation used immunostaining for myelin basic protein (MBP) in aging mice and HTBs from older (aged 63-91 years) donors to elucidate possible presbycusis mechanisms related to spiral ganglion cell (SGC) degenerative changes. Results showed significant declines in MBP immunoreactivity and losses of MBP-positive auditory nerve fibers in the spiral ganglia of both older human and aged mouse ears, and they suggest that myelin degeneration may play a critical role in SGC loss and subsequent decline in auditory nerve function in presbycusis.

This combined approach has been used with success by many investigators. Important discoveries of inner ear pathological mechanisms have been identified first in animals and later confirmed as human pathology using HTB studies, including cochlear synaptopathy and cochlear macrophages, described below.

**Cochlear Synaptopathy**

Seminal findings by Kujawa and Liberman24-26 detail the loss of synapses between inner hair cells (IHCs) and the peripheral processes of the SGCs, in the absence of hair cell loss, in animals exposed to noise levels that produce only temporary threshold shifts. Such findings provide possible pathological correlates of significant clinical conditions, including understanding speech in noise, tinnitus, and hyperacusis. Documentation of synaptopathy is dependent on the use of techniques that include immunostaining or
serial-section electron microscopy. Application of quadruple-immunostaining protocols that allowed synaptic counts, hair cell counts, neuronal counts, and differentiation of afferent and efferent fibers in mouse models in the original work can be applied also in HTBs, when harvested within 9 hours postmortem and prepared appropriately. Viana et al performed quantitative analysis in this manner from 5 HTBs of subjects with no history of otologic disease, aged 54 to 89 years. Findings included evidence of cochlear synaptopathy and degeneration of cochlear nerve peripheral axons, despite a near-normal hair cell population. Results suggest that cochlear synaptopathy, or primary neural degeneration, is an important correlate of human presbycusis.

**Cochlear Macrophages**

Several agents and circumstances that produce hair cell damage, including noise, aminoglycoside antibiotics, aging, and diphtheria toxin, have all been demonstrated to induce macrophage migration into the inner ear. Phagocytosis of injured hair cells is accomplished by a combination of resident and migratory macrophages as well as cochlear supporting cells. Other possible roles for cochlear macrophages are still under investigation and include maintenance of cochlear homeostasis as well as antigen presentation and induction of adaptive immunity. The role of macrophages in the human inner ear was addressed in an archival HTB by O’Malley et al. The authors used immunohistochemistry techniques with antibodies specific for cells of monocytic lineage (monocytes, macrophages, and microglia) to examine HTBs from donors with no known otologic disorders, aged 52 to 88 years. The study identified a cell class, large in number and consistent in morphology and protein expression with macrophages/microglia, throughout the inner ear. The authors note that they have no proof that these are “resident” rather than “recruited” macrophages. However, their presence in abundance in the absence of any known otologic disease seems consistent with a role in the inner ear homeostasis. Some have postulated that these cells may have a role in otologic disorders such as idiopathic sudden sensorineural hearing loss and endolymphatic hydrops.

**Ménière’s Disease**

Study of HTBs from patients with Ménière’s disease (MD) has provided valuable insight into MD pathophysiology. HTB histopathology has consistently shown evidence of endolymphatic hydrops (EH) in patients with MD, with relative sparing of sensory and neural structures. Because EH has been the most commonly observed histopathologic feature of MD, it has been assumed that EH represents the culmination of MD symptoms. The HTB study by Merchant et al demonstrated that, while all MD cases showed evidence of hydrops, EH was also evident in 9 HTB specimens from patients who did not have symptoms of vertigo associated with MD (Figure 3). However, these cases did have documented fluctuating and/or progressive sensorineural hearing loss in 7 of 9 cases. Hence, EH is a histological marker for MD and not directly responsible for the symptoms of vertigo but is commonly associated with other otologic symptoms.

Monsanto et al identified significant structural differences in the endolymphatic drainage system in MD patients’ HTBs compared with those from EH patients without MD and normal HTBs. The authors hypothesize that these structural differences, which they suggest could result from developmental, environmental, infectious, or genetic disorders, could predispose patients to developing symptoms of MD in the event of abnormalities of endolymph homeostasis. Another HTB study demonstrated a significantly higher incidence of cupular and free-floating deposits in the posterior semicircular canals of MD-affected HTBs than without; this incidence correlated with the duration of symptoms of MD rather than age, consistent with association of these disorders.

**Cochlear Implantation and Postimplant Pathology**

A number of studies of post–cochlear implantation (CI) HTBs provide important information, some with surgical implications. HTB studies have allowed us to answer the following clinically important questions:

1. What is the relationship between SGC number and speech performance post-CI, and are therapies that enhance survival of SGCs potentially important targets in efforts to improve implant performance?

Early work by Nadol et al showed a strong correlation between SGC population and deafness etiology in HTBs. Otte et al demonstrated a positive correlation between SGC number and speech discrimination scores in unimplanted HTBs. Therefore, it was hypothesized that better post-CI performance would be correlated with higher SGC survival. However, 3 HTB studies have appeared to refute that hypothesis. Given the known wide variability in
SGC survival that can be related to a variety of mitigating factors in addition to deafness etiology—and the findings of Seyyedi et al\textsuperscript{39} that show no statistically significant difference in SGC survival between the 2 ears of patients with hearing loss attributed to the same etiology—Seyyedi et al\textsuperscript{39} performed an analysis of 12 temporal bones from 6 bilaterally implanted patients for whom post-CI speech recognition data are available, controlling for factors such as hearing loss etiology, deafness duration, age at implantation, duration of implant use, age at death, and insertion depth. Their findings strongly supported the positive correlation between post-CI word recognition scores and residual population of SGCs, and they lend further importance to efforts to preserve SGC populations in implanted patients through delivery of neurotrophins and other agents.

2. Analysis of implanted HTBs: what are the implications and recommendations for surgical technique?

An inflammatory response is commonly identified in the cochlea of implanted HTBs, with infiltration of type B and T lymphocytes, foreign body giant cells, and macrophages, in proximity to the electrode track\textsuperscript{40} (see Figure 4). This reaction is greatest near the base and lessens toward the apex, indicating that insertion trauma may exacerbate the inflammatory response.\textsuperscript{41} Richard et al\textsuperscript{42} compared the extent of neo-ossification and fibrosis following CI performed via a round window, round window extended, and cochleostomy approach, and they found significantly less evidence of cochlear trauma in HTBs implanted using a round window approach (compare Figure 5A,B). Interestingly, the extended round window approach was associated with more new tissue deposition than the other approaches, which the authors hypothesize is due to more drilling in the hook region and greater lateral wall trauma. Li et al\textsuperscript{43} documented lateral cochlear wall damage following CI and found a significant correlation between new bone and fibrous tissue and degree of lateral wall damage (see Figure 5C,D). Those cochleae with higher grades of damage demonstrated significantly greater fibrous and bony tissue deposition near the electrode, greatest in the base.

Kamakura and Nadol\textsuperscript{44} found that post-CI word recognition is negatively correlated with the percent volume of new bone formation (but not fibrous tissue formation) within scala tympani, scala vestibule, and scala media. Percent new bone formation was positively correlated with the degree of intracochlear insertional trauma, particularly as reflected in trauma to the basilar membrane. The authors suggest, based on their findings, that tissue formation may result from a foreign body response to the electrode and that trauma alone is not a critical determinant of formation of fibrous tissue. However, further work by Ishai et al\textsuperscript{45} found that cochlear damage as evidenced by fracture of the osseous spiral lamina and/or displacement of the basilar membrane was associated with development of thickened fibrous tissue surrounding the electrode, especially basally. They examined the pattern and degree of fibrous sheath formation following CI in patients who were implanted with 2 different devices and identified greater intracochlear damage associated with a larger diameter electrode. They also found no relationship between extent of fibrous tissue deposition and word recognition but suggest that fibrous tissue formation may affect ability to conserve residual hearing in some circumstances and that understanding the mechanical and molecular causes of tissue deposition may lead to improved electrode designs and surgical practices, with enhanced conservation of cochlear function.\textsuperscript{45}

3. What are the reasons for delayed hearing loss after initially successful hearing preservation CI for hybrid implantation?

Advances in surgical technique have permitted atraumatic electrode insertions that enhance preservation of residual hearing in patients with significant low-frequency hearing. One frustrating and often puzzling outcome of intended CI hearing preservation surgery is the initial conservation of low-frequency hearing followed by delayed loss. HTB histopathology reported by Ishiyama et al\textsuperscript{46} and Linthicum et al\textsuperscript{47} demonstrate that CI insertion via cochleostomies that involve scala vestibule lateral wall trauma (compare Figure 5C,D) incites fibrosis and neo-ossification, which is likely to compromise the ductus reuniens with resultant endolymphatic hydrops due to the limitation in egress of endolymphatic fluid. Hydrops results in delayed low-frequency hearing loss. In HTBs for which the cochleostomy involves only the scala tympani, there was no finding of hydrops. It is recommended that surgeons use the round window approach, taking care not to injure the endosteum of the scala tympani during electrode insertion. Hydrops may not be the only cause of delayed hearing loss. Quesnel et al\textsuperscript{48} examined a pair of HTBs from a unilateral hybrid implant patient who experienced delayed hearing loss. Findings of cochlear hair cell and SGC counts were
similar from side to side. The scala tympani and part of scala vestibuli on the implanted side were filled with loose fibrous tissue and neo-ossification and exhibited hydrops. The authors speculate that decreased compliance at the round window and increased damping of responses from scala tympani due to the tissue formation may explain the postimplant hearing loss.

**Vestibular Schwannoma**

HTB histopathology studies can help us to answer the following questions:

1. Is the pathology associated with vestibular schwannoma (VS) confined primarily to neural structures in the internal auditory canal (IAC)?
2. Can neurofibromatosis type 2 (NF2) VS be completely resected with preservation of hearing function?

HTB studies reveal that VS is associated with cochlear degenerative changes in addition to neural degeneration, although the exact mechanism(s) are unknown. Mahmud et al found evidence of inner ear degenerative changes—including hair cell loss and pathologic changes in stria vascularis and the spiral ligament—in all temporal bones from VS patients with speech discrimination scores less than 50%. Roosli et al performed an analysis of temporal bones from 32 patients with unilateral sporadic VS arising within the IAC (Figure 6A), using the opposite ear as a control, with the goal of comparatively assessing cochlear pathology on the side ipsilateral to the VS. As in the Mahmud et al study, most patients with VS in this series were found to have significant cochlear pathology, including degeneration of inner hair cells (in 75%), outer hair cells (88%), stria vascularis (69%), and cochlear neurons (85%). Almost half were demonstrated to have an acidophilic precipitate in the endolymphatic or perilymphatic...
spaces, and a quarter were identified as having endolymphatic hydrops. There was no significant relationship between tumor size and cochlear degenerative changes, with the exception of inner hair cell loss being more common with large tumors ($P = .009$). No significant correlations were found between tumor volumes and air and bone conduction hearing thresholds or speech discrimination scores. Roosli et al. postulated that schwannoma cell generation of cytokines may be responsible for the degenerative changes; it is assumed that the acidophilic precipitate identified in these temporal bones represents high protein concentration. Other possible etiologies include vascular compromise (not seen in this series) or other biochemical alterations.

Clinical evidence also points to a multifactorial cause of hearing loss in patients with VS. The absence of otoacoustic emissions suggests more than retrocochlear pathology alone. Sudden hearing loss is occasionally observed in patients with VS and is often responsive to steroid therapy. Roosli et al. argue that hearing loss recovery is more consistent with impact of anti-inflammatory treatment on biochemical changes within the cochlea than on the tumor-neural interface. Furthermore, tumor removal does not result in hearing recovery, and long-term hearing outcomes typically demonstrate progressive hearing loss, consistent with continued cochlear degenerative changes, particularly in the setting of incomplete resection of tumor or treatment with gamma knife or other stereotactic radiation.

In addition to cochlear degenerative changes, Hizli et al. identified peripheral vestibular pathology; they found a 40% to 60% reduction in ipsilateral vestibular hair cells densities, evidence of hydropic changes, and inner ear fluid precipitate, analogous to findings in the cochleae of patients with VS. Again, the authors point to the protein in the fluids of the vestibule as being either representative of proteins that are responsible for degenerative changes or an epiphenomenon of another process that contributes to this damage. Findings have significant implications for VS management in patients with chronic vestibular symptoms. While most patients with VS remain asymptomatic due to slow tumor growth and central compensation, some have disabling symptoms that seem most likely secondary to peripheral degenerative changes. Ongoing degeneration of vestibular sensory epithelium may occur, creating an unstable peripheral vestibular lesion that is amenable to chemical or surgical labyrinthectomy.

Like sporadic VS, schwannomas associated with NF2 are often associated with cochlear degenerative changes. However, a study of 26 temporal bones from 16 NF2 cases showed clear differences in the biologic behavior of these tumors. In NF2 cases, tumors were found to be predominantly cellular, Antoni type A. They show aggressive behavior and are often multicentric. There was a propensity to involve the labyrinth—including the cochlea and vestibular end organs. Total removal and removal with hearing preservation is more difficult with these tumors, for reasons demonstrated in these temporal bones, including evidence of infiltration between the fibers of the cochlear nerve (compare Figure 6B,C), propensity to develop new tumors, and significant degenerative changes observed in the cochlea of these temporal bones.

State of HTB Research

Studies of HTBs were conducted in Europe during the late 19th and early 20th centuries and provided the first information on the etiology of common ear diseases. Subsequent years saw the development of resources and funding to support up to 28 temporal bone laboratories in the United States by 1976. Since that time, funding and research activity have diminished to a handful of laboratories that are operational today. The National Temporal Bone, Hearing, and Balance Resource Registry has been supported by the National Institute of Deafness and Other Communication Disorders since 1992. The registry provides support for tissue and data acquisition and tissue processing for investigators around the world, and it is an invaluable resource that must be preserved.

The study of human temporal bone tissue is a critically important activity that uniquely advances our understanding of human otologic disease and treatment. There is no substitute for this resource, which provides the primary means of understanding the critical links between experimental animal work and human disease. The viability and contribution of HTB research depend on access to high-quality tissue, associated complete medical history documentation of otologic disease, availability of highly trained scientists who can apply contemporary and innovative techniques of tissue analysis, and funding sources to support tissue acquisition and processing, infrastructure, research, and training.

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Author Contributions

Debala L. Tucci, substantial contributions to the conception and design of the work and the analysis of data for the work, drafting the work for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; Joni K. Doherty, substantial contributions to the conception or design of the work and the analysis of data for the work, revising the work critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that
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