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Nerve Maintenance and Regeneration in the Damaged Cochlea

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Abstract

Following the onset of sensorineural hearing loss, degeneration of mechanosensitive hair cells and spiral ganglion cells (SGCs) in humans and animals occurs to variable degrees, with a trend for greater neural degeneration with greater duration of deafness. Emergence of the cochlear implant prosthesis has provided much needed aid to many hearing impaired patients and has become a well-recognized therapy worldwide. However, ongoing peripheral nerve fiber regression and subsequent degeneration of SGC bodies can reduce the neural targets of cochlear implant stimulation and diminish its function. There is increasing interest in bio-engineering approaches that aim to enhance cochlear implant efficacy by preventing SGC body degeneration and/or regenerating peripheral nerve fibers into the deaf sensory epithelium. We review the advancements inmaintaining and regeneratingnerves indamaged animal cochleae, with an emphasis on the therapeutic capacity of neurotrophic factorsdelivered to the inner ear after an insult. Additionally, we summarize the histological process of neuronal degeneration in the inner ear and describe different animal models that have been employed to study this mechanism. Research on enhancing the biological infrastructure of the deafened cochlea in order to improve cochlear implant efficacy is of immediate clinical importance.

Keywords

Nerve regeneration; Neurotrophic factors; *BDNF*; *NTF-3*; Cochlear implant; Spiral ganglion cell

1. Introduction

Injury or loss of sensory cells in the peripheral nervous system (PNS) results in detachment and withdrawal of nerve endings, also known as Wallerian degeneration. The soma of the detached neurons may survive, and at times, reconnect with a new target by regenerative growth of nerve fibers. During this process, regrowth of a single axon occurs spontaneously in the PNS at a slow rate of 1mm/day (Schnell et al., 1994). Regenerated axons or dendrites reinnervate either a new target or their original target, leading to partial to complete recovery of function (Brushart et al., 1998; Fu et al., 1997; Soileau et al., 1987). The regenerative response of nerve fibers varies between species and types of injury (Torvik et al., 1975). The molecules that participate in facilitating or inhibiting nerve fiberregeneration have been

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characterized in both the PNS and the central nervous system (CNS) (Bomze et al., 2001; Caroni et al., 1988a; Caroni et al., 1988b; Savio et al., 1989; Schwab et al., 2005; Schwab et al., 1985). Knowledge of these molecular players helps in designing therapeutic approaches to initiate or enhance nerve regeneration in many tissues, including the inner ear.

Several pathologies in the inner ear involve degeneration of auditory nerve fibers from the area of the sensory epithelium. This usually is due to loss of the target, the inner hair cell. In some cases, degeneration of the spiral ganglion cell (SGC) bodies ensues. With the loss of these bipolar neurons responsible of conveying the sensory signals produced by hair cells into the brain, the ability to treat deafness with a cochlear implant is diminished. One way to enhance the benefits derived from the cochlear implant is to maximize the physiological condition of remaining SGCs. An alternative therapeutic goal is to induce regeneration of nerve fibers back into the basilar membrane area (BMA). Such regeneration could have several important benefits: enhancing function of the cochlear implant due to greater proximity of the nerve endings to the electrodes, indirectly improving the physiological status of the SGCs, and providing a neural substrate for future therapies such as hair cellregeneration via transdifferentiation or stem cell implantation. This chapter discusses nerve regeneration in the traumatized cochlea with emphasis on two main strategies of improving the efficacy of the cochlear prosthesis: (a) development of preventive therapy to preserve the SGC population, and (b) bio-engineering approaches which could induce axonal regrowth of the peripheral nerve fibers and increase their proximity to the electrode. This would potentially improve the sensitivity to electrical stimulation, channel selectivity and even temporal properties of cochlear implant stimulation.

2. Rationale/impetus for enhancing SGC survival and inducing nerve fiber regeneration

Despite significant advances in cochlear implant outcomes, the rate of improvement has slowed over the past decade(O'Leary et al., 2009), and there remain limitations in the benefit of cochlear implantation, particularly in restoring hearing in noise and in music appreciation. The concept behind the design of multichannel cochlear implants is the stimulation of a discrete neural population by each electrode along the length of the cochlea, allowing the specific and tonotopic stimulation of the auditory nerve fibers and spatial separation of frequency-specific temporal envelope information. If each electrode stimulates a specific and discrete neural population, each electrode should correspond to a specific sound percept and render a distinct perceptual channel (O'Leary et al., 2009). Currently, despite most devices having over 20 electrodes in the array, the actual number of perceptual channels achieved is closer to 8 (Friesen et al., 2001). This is likely due to current spread between electrodes and longitudinally along the cochlea via the perilymph, thereby causing non-specific stimulation of neurons adjacent to the target neuronal population. A greater number of effective perceptual channels are needed: as many as 20 to hear well in noise (Dorman et al., 1998) and over 30 to appreciate music and lexical tone (Kong et al., 2004; Xu et al., 2003). Not only are a greater number of perceptual channels necessary to appreciate music, but also they must function according to a specific and finely tuned temporal and spectral scale (Kong et al., 2004). In order to accomplish this, the implant's electrode array must stimulate a more discrete population of auditory neuronal fibers, and it must be able to do so in a way that retains the fine spatiotemporal cues of the original acoustic signal.

Various methods of accomplishing this goalhave been proposed, including bringing the electrodes and peripheral neuronal elements into closer approximation. The greater the distance of the electrode from the target neural element, the greater current is needed to activate the target. With greater current, there is greater spread of the electric signal via the surrounding perilymph (Snyder et al., 2008); thus, to avoid overlapping signals, fewer more widely spaced electrodes must be used. In addition, because current spreading also causes

the strength of the signal to decay exponentially as a function of distance (Black et al., 1981; Black et al., 1983), more power must be used to stimulate those broader, more distant targets. If electrodes are brought into closer contact with the neural elements, a smaller current is needed to activate the neurons, and less current spread will occur (Snyder et al., 2008). Presently, this goal is accomplished by the use of perimodiolar electrode arrays. These arrays are designed such that, rather than lying along the outer cochlear bony wall as with traditional straight arrays, they curve to approximate the modiolus. Furthermore, the electrode contacts are isolated to the medial surface of the array. Studies of perimodiolar devices have demonstrated a significantly lower threshold, increased dynamic range, increased specificity of neural excitation, and a maintained tonotopic map along the length of the array (Briaire et al., 2006; Cohen et al., 2006; Frijns et al., 2001; Snyder et al., 2008). However, these improvements have not translated to improved speech perception outcomes (Cohen et al., 2003; Cohen et al., 2005; Fitzgerald et al., 2007; Hughes et al., 2006), let alone improved music appreciation abilities.

One potential limitation of perimodiolar arrays, as demonstrated in computational models of the human cochlea, is the potential to stimulate across cochlear turns in the apex, leading to a false pitch percept (Briaire et al., 2006; Frijns et al., 2001), although little evidence of this has been found in human perimodiolar implant recipients (Cohen et al., 2006). Additional possible reasons for the limited efficacy of the perimodiolar array in eliciting improved speech perception is that the electrodes may still be too far from their targets and there is still significant volume of intervening perilymph allowing the longitudinal spread of current (O'Leary et al., 2009). Neuronal fiber regeneration may bring the target peripheral fibers in closer proximity to the electrode, thereby allowing more specific neural activation at lower thresholds (Staecker et al., 2010).

3. Normal innervation of the cochlea

The sensory epithelium of the inner ear is composed by a mosaic of sensory and non-sensory cells. Sensory hair cells transduce vibratory mechanical energy into neuronal impulses which are then conveyed by SGCs to the brain and produce auditory perception. SGCs are bipolar nerve cells with central axons that connect with the auditory brain stem and peripheral fibers that synapse with the hair cells (Fig. 1A) (Anniko, 1983; Raphael et al., 2003; Spoendlin, 1981; Webster et al., 1981). The larger Type I afferents synapse with inner hair cells and the smaller Type II afferents synapse with outer hair cells (Kiang et al., 1982; Liberman et al., 1990; Spoendlin, 1969). Efferent peripheral nerve fibers arise from the lateral superior olive in the auditory brain stem (Warr et al., 1979). Efferent fibers synapse with outer hair cells and with Type I afferent nerve fibers (before they reach the inner hair cells) (Liberman, 1980; Liberman et al., 1986; Smith et al., 1963). Myelination of SGCs and nerve fibers is provided by Schwanncells. The extent and areas of myelin presence vary between species (Romand et al., 1990; Romand et al., 1982). In the osseous spiral lamina, one Schwann cell encloses each nerve fiber, but afferent nerves lose their myelin sheaths just before entering the habenula perforata. Once in the habenula perforata, extensions of a single satellite cell surround a nerve axon. Past the habenula, axons continue as unmyelinated fibers (Spoendlin, 1969). It is possible that maintenance and support of these fibers within the auditory epithelium is provided by the supporting cells in the sensory epithelium (Spoendlin, 1966).

4. Hair cell loss and SGC degeneration

Various etiologies, hereditary, environmental or combined, can result in sensorineural hearing loss. Regardless of the causes of sensorineural hearing loss, the typical pathological outcome usually involves hair cell degeneration. Once lost, hair cells in the mammalian

cochlea are not spontaneously replaced (Hawkins, 1973; Roberson et al., 1994). Once hair cells are lost, they are replaced by phalangeal scars generated by supporting cells (Forge, 1985; Raphael et al., 1991).

With the degeneration of the hair cells, the afferent fibers lose their target and eventually start to die back (Fig. 1B). Their regression leaves the SGCs with a short peripheral process that does not reach the BMA. In some cases, the peripheral process dies back all the way to the SGC soma leaving the cell body and central process intact and functional (Hardie et al., 1999; Spoendlin, 1975). In addition to regression from the BMA nerve fibers usually also undergo demyelination in the osseous spiral lamina, starting from the distal end (Dodson et al., 2000; Lawner et al., 1997b). The central connections of the SGCs in the cochlear nucleus are maintained, although the characteristics of this connection change in deafened ears (Bledsoe et al., 1995; Ryugo et al., 1998; Zeng et al., 2009).

The SGC bodies can survive for many years in human ears devoid of hair cells, although degeneration can take place at a slow or even rapid pace. In animal models of severe hair cell lesions, SGCs degenerate in a faster pace than in human ears. The exact mechanism of SGC death is not fully understood, although earlier SGCs death after intracochlear injection of gentamicin is thought to be caused by glutamate toxicity (Dodson, 1997), and later SGC degeneration may be due to depletion of neurotrophic factors or lack of activity in the SGC, which normally would be provided by the hair cells. Early changes in the synaptic region after noise exposure, observed without hair cell loss, may also result on SGC degeneration (Kujawa et al., 2009). Studies detecting the precise signaling cascades that promote SGC injury and death will be valuable for future therapies that enhance SGC survival.

5. Animal models for auditory nerve degeneration and regeneration

Examining the pathological process of auditory nerve degeneration requires a variety of models that encompass the different conditions and time points of auditory nerve degeneration. Both moderate and severe SGC lesions in animal models can be created by systemic or local application of various ototoxic reagents, including but not limited to aminoglycosides, loop diuretics, carboplatin, and ouabain. Aminoglycosides can cause partial to complete degeneration of the organ of Corti and secondary degeneration of the SGCs (Johnsson, 1974; Leake et al., 1988). Systemic application of kanamycin and ethacrynic acid or local application of neomycin via perilymph injection can cause severe hair cell and SGC lesions (Raphael et al., 1991; Shibata et al., 2010; Wise et al., 2010). Carboplatin can induce a lesion restricted to inner hair cells in chinchilla (Sugawara et al., 2005; Wang et al., 2003). Ouabain, a cardiac glycoside, attenuates the action of Na^+-K^+ ATPase and leads to selective degeneration Type I SGCs in gerbils and mice (Lang et al., 2010; Matsuoka et al., 2007; Schmiedt et al., 2002). Mechanical damage caused by traumatic acoustic overstimulation can create a varying degree of hair cell and/or nerve degeneration (Abrashkin et al., 2006; Bohne et al., 2000; Borg et al., 1983). Axotomy of the VIIIth nerve in the inner auditory meatus causes a secondary degeneration of the majority of the afferent nerve fibers and most of the efferent nerve fibers (Spoendlin, 1979). Sectioning the nerve fibers at the osseous spiral lamina can cause degeneration of auditory nerve fibers with an intact organ of Corti (Spoendlin, 1979; Sugawara et al., 2005).

If the insult is mild or recent, the supporting cells can retain their normal morphological characters. In cases where the insult is severe or a prolonged period of time has passed since the insult, the supporting cells may degenerate as well and form or are replaced by a flat layer of polymorphic cuboidal cells, which we refer to as the “flat epithelium” (Fig. 1C) (Kim et al., 2007). The flat epithelium can be observed frequently as the outcome of severe hearing loss caused by various etiologies including hereditary deafness (Pawlowski et al.,

2006), aminoglycoside insult in guinea pigs (Izumikawa et al., 2008; Shibata et al., 2010), and in humans with prolonged history of deafness (Nadol et al., 2006). This highly degenerated condition of sensory epithelium that remains in the deaf ears will likely be the substrate to first receive any of the future therapies.

The time course of secondary neuronal degeneration subsequent to hair cell loss appears to vary between species. In mice, rat, and guinea pigs the loss of SGCs can occur in weeks or months (Bichler et al., 1983; Dodson et al., 2000; Staecker et al., 1998) while in humans the process is much slower and can take years (Nadol, 1997; Nadol et al., 1989). Postmortem human temporal bone studies have shown that in some cases, many years after a severe cochlear lesion leaving a flat epithelium, the SGCs are still intact in both numbers and morphology (Nadol et al., 2006; Nadol et al., 1989). It is not clear whether this is a pure species-dependent phenomenon or a manifestation of the sub-optimal animal models where the initial lesion is often very severe. Currently there is no ideal animal model which would mimic the same degeneration process seen in humans. As we argued in our KHRI 50th anniversary paper written by Dr. Pfingst, perhaps the histological picture we create by deafening and providing neurotrophins (with better SGC survival) will more closely resemble human cochlear implant users, especially now that guidelines for cochlear implant use have broadened.

Supporting cells can play an important role in the survival of peripheral nerve fibers. In a lesion where the supporting cells remain, degeneration of SGCs may proceed much slower than in the absence of supporting cells (Sugawara et al., 2005). These results suggest that neuronal degeneration may be a complex process that depends on more factors than just hair cell survival. In addition to supporting cells, the roles of other non-sensory cells in the inner ear should be assessed, including the mesothelium and Schwann cells.

The survival of SGCs and other morphological features related to SGC survival has been correlated with several measures of cochlear implant function. Thus, maintaining healthy and functional population of SGC bodies and associated structures would seem to be crucial for the performance of the cochlear implant (Chikar et al., 2008; Hartshorn et al., 1991; Kang et al., 2010; Pfingst et al., 1981; Shepherd et al., 1997). As such, studies which investigate the factors that contribute to slowing down or preventing SGC degeneration are likely to enhance outcome of the cochlear implant therapy in the future. The functional outcomes of enhanced SGC survival on cochlear implant stimulation in animals are discussed in detail in the review by Pfingst et al., in this issue of Hearing Research.

6. Spontaneous regeneration of the cochlear nerve

Spontaneous resprouting of SGC nerve fibers has been seen following the induction of various inner ear lesions, including severing of the eighth cranial nerve at the internal acoustic meatus (Spoendlin et al., 1976), aminoglycoside toxicity (Johnsson et al., 1972; Terayama et al., 1977; Terayama et al., 1979; Webster et al., 1982), and noise induced hearing loss (Bohne et al., 1992; Lawner et al., 1997a; Strominger et al., 1995). After severing the eighth cranial nerve there is initially a near total loss of SGCs and their associated peripheral processes (Spoendlin et al., 1976). However, months following this injury Spoendlin and Suter found many new large fibers in the region between the habenula perforata and inner hair cells. They noted that this occurred in the absence of a corresponding increase in SGC bodies and concluded that these new fibers were likely sprouting from the few surviving neurons (Spoendlin et al., 1976).

Regenerated nerve fibers were found in the auditory epithelium in chinchillas exposed to a severe acoustic trauma, leading to elimination of hair cells and damage to supporting cells (Bohne et al., 1992; Lawner et al., 1997a; Strominger et al., 1995). Presence of relatively

well preserved areas of the organ of Corti adjacent to the focal lesion appears to correlate with the presence of regenerated fibers. The spontaneous regeneration of nerve fibers in the traumatized cochleae was limited to the area adjacent to the habenula perforata (Bohne et al., 1992; Lawner et al., 1997a; Spoendlin et al., 1976; Terayama et al., 1977; Terayama et al., 1979). Similarly, more recent studies of the guinea pig ear following neomycin deafening demonstrated that a few looping neuronal fibers remain in the BMA close to the habenula perforata (Fig. 2A) (Shibata et al., 2010). The looping fibers exit and return back to the habenula perforata while weaving their way between the cuboidal cells of the flat epithelium. Similar results have been observed in other reports in the deafened inner ear (Wise et al., 2005). These results suggest that the nerve fibers may be sensitive to gradients of neurotrophin concentrations in the flat epithelium. This lack of significant nerve fiber regeneration in the weeks following aminoglycoside toxicity is likely related to the complete absence of hair cells and supporting cells to provide structural and neurotrophic support to the residual SGCs. In the longer term, most SGCs in severely deafened animals degenerate so fiber regeneration is not expected to occur.

7. Neurotrophic factors for inner ear treatment

Different molecules have been employed to prevent SGC degeneration after an insult. Neurotrophins, glia derived neurotrophic factor (GDNF), and fibroblast growth factors (FGF) were found to be potent. Neurotrophins play multiple roles in the mammalian CNS and PNS, such as promotion of cell survival, neurogenesis, maintenance of neurons and synaptogenesis. The family of neurotrophins includes nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4). Neurotrophin function is regulated by intercellular signaling pathways which are activated by the membrane receptors which include the tyrosine kinase receptor family (TrkA, B, and C) or p75 neurotrophin receptors. During inner ear development, BDNF and NT-3 and their respective receptors TrkB and C are necessary for the differentiation and survival of the cochlea and vestibular neurons. BDNF and/or NT-3 are secreted from hair cells and are thought to play a crucial role in synaptogenesis of these cells (Despres et al., 1994; Medd et al., 2000). BDNF or NT-3 knockout mice exhibit partial or complete loss of afferent and efferent neurons in the cochlea (Ernfors et al., 1995; Fritzsch et al., 1997; Fritzsch et al., 2004). Additionally, neurotrophins have been employed in regenerative studies in other systems of the CNS and PNS (Bellamkonda, 2006; Di Polo et al., 1998; Tuszynski et al., 2005). Therefore, attempting to deliver neurotrophins into the inner ear fluids to prevent SGC degeneration was a logical choice. Indeed, exogenous application of neurotrophins (e.g. BDNF, NGF and NT-3) into the cochlear fluids have proven to be beneficial in neuro-protection against insults (Glueckert et al., 2008; Hildebrand et al., 2008; Kanzaki et al., 2002; Nakaizumi et al., 2004; Staecker et al., 1996; Wise et al., 2005). GDNF and FGF have been demonstrated to be equally neuro-protective in the inner ear (Glueckert et al., 2008; Shibata et al., 2007; Yagi et al., 1999).

Delivery of trophic factors such as neurotrophins into the inner ear has been accomplished by several methods. Studies have shown enhanced survival of neurons by delivering neurotrophins via mini-osmotic pumps (Glueckert et al., 2008; Miller et al., 1997; Wise et al., 2005) or employing forced expression of neurotrophins by introducing the genes for these growth factors into cells that line the perilymph or endolymph (Bowers et al., 2002; Miller et al., 1997; Nakaizumi et al., 2004; Shibata et al., 2010; Staecker et al., 1998; Wise et al., 2010). Both methods have been shown to significantly improve SGC survival against ototoxic drugs or traumatic noise exposure. However, SGC degeneration is thought to be a continuous process after an initial insult, thus, sustained levels of neurotrophins are required throughout life. The withdrawal of exogenous neurotrophins can lead to a rapid degeneration of SGCs (Gillespie et al., 2003; Shepherd et al., 2008). However, when brief electrical

stimulation for eABR recording is provided, the effects of neurotrophin withdrawal are less traumatizing (Agterberg et al., 2009). Thus, selecting the method for delivery of neurotrophins may depend on additional considerations and improvements of the technology.

Several lines of evidence have shown that electrical stimulation can promote enhanced spiral ganglion survival (Miller, 2001; Miller et al., 1995; Mitchell et al., 1997) and thus when combined with cochlear implant, a sustained application of neurotrophins may not be required, although this notion is still controversial (Agterberg et al., 2010; Chen et al., 2010; Li et al., 1999; Shepherd et al., 1994). If indeed prolonged administration of neurotrophins is required, gene transfer technology seems to be a promising option given its efficacy in the inner ear. Some viral vectors such as adenovirus provide gene expression lasting for a month or two (Lalwani et al., 1998; Li Duan et al., 2002; Praetorius et al., 2002; Yagi et al., 2000) but pose the potential risks of cytotoxicity and immunogenicity, such that risks of their use in the ear outweighs their benefits. However advancement in viral vectors such as the adeno-associated virus, which has few side effects and induces gene expression lasting many years (Bankiewicz et al., 2006) may increase the clinical feasibility of gene transfer technology.

8. Peripheral nerve fiber re-growth

Neurotrophins are useful not only for SGC body protection but also for inducing the outgrowth of peripheral nerve fibers, as initially shown in culture condition (Avila et al., 1993; Van De Water et al., 1996). Re-growth of peripheral nerve fibers in a deaf ear may provide several benefits. First it may enhance SGC survival, which would be a benefit by itself. Second, peripheral fiber regrowth may improve cochlear implant outcomes if it can be directed into the BMA and the underlying connective tissue and remain there in the long term; this would place neurons closer to the cochlear implant electrodes and enhance the spatial selectivity of stimulation. Third, such regrowth would provide similar enhancement of the functional outcomes of other restorative methods, including such novel techniques as cell replacement by stem cell therapy.

Several groups have investigated whether enhanced re-growth of peripheral nerve fibers could be induced by osmotic pump injection of neurotrophins in guinea pigs with deafened ears (Glueckert et al., 2008; Wise et al., 2005). Injection of neurotrophin proteins into the perilymph resulted in a disorganized fashion of peripheral nerve fiber re-growth, probably because the highest concentration of neurotrophins was in the perilymph at the tip of the cannula. For the re-grown nerve fibers to be meaningful, they need to be distributed preferentially in the BMA, in a tonotopic fashion. Ectopic nerve fibers may be counter-productive and diminish the cochlear implant performance outcome.

We investigated whether it is possible to direct nerve fibers more locally into the BMA of the deaf epithelium via forced expression of transgenic BDNF in cells lining the cochlear fluids. We determined that robust growth of fibers into the deafened epithelium occurred after inoculating with either adenovirus carrying *BDNF* reporter gene insert (Ad.*BDNF*) or adeno-associated virus carrying *GFP-BDNF* reporter gene insert (AAV.*GFP-BDNF*) (Shibata et al., 2010). In ears treated via scala media or scala tympani with Ad.*BDNF*, a robust regrowth of nerve fibers was observed in the BMA of the deaf epithelium (Fig. 1D, 1E and 2B). Furthermore, in ears inoculated with AAV.*GFP-BDNF*, we were able to visualize cells that were transduced and expressed GFP, which served as focal targets for nerve fibers growth. Similarly, Wise et al. have shown localized re-growth of nerve fibers in deafened cochlear tissue using adenovirus with *GFP-BDNF* or *GFP-NTF-3* reporter gene inserts (Wise et al., 2010). Thus, gene transfer technology to force neurotrophin transgene expression in the cells lining the cochlear fluids, allows us to guide re-growth of peripheral

nerve fibers into the BMA of the deaf epithelium. Further research is needed on the safety of this method, long term survival of the regenerated fibers, the identification of the type of nerve fibers that grow into the BMA and the outcome on the performance of the cochlear implant. Although many questions remain concerning neural regeneration in the inner ear, its potential clinical applications are promising and exciting, and research is ongoing.

9. Potential role of Schwann cells in inner ear axonal re-growth

Recently, there has been growing interest in the role of Schwann cells in the cochlea (Bohne et al., 1992; Glueckert et al., 2008; Hansen et al., 2001; Morris et al., 2006; Whitlon et al., 2009; Wise et al., 2005). Schwann cells can exist in one of two phenotypes; in the immature state they proliferate, but do not myelinate axons, whereas in the mature state they are myelinating but do not undergo mitosis. Connexin-29 has been identified as a marker expressed exclusively in myelinating Schwann cells, in their cell body and along the myelin sheath surrounding the neural fibers (Eiberger et al., 2006; Tang et al., 2006), and has been harnessed as a tool for investigating the importance of the Schwann cells and myelination. In the Connexin-29 null mouse, Tang and colleagues found delayed hearing development, increased sensitivity to otoacoustic trauma, and prolonged ABR latencies in spite of normal hair cell morphology, attributable to defects in myelination of SGC bodies (Tang et al., 2006). These findings can be explained by the role of Schwann cells in providing myelin layers that increase the speed of propagation of action potentials, as well as their putative role in energy conservation, neurotrophin support, and tonotopic organization via facilitation of path finding.

Schwann cells are also essential to creating an environment favorable to nerve fiber regrowth (Jessen et al., 2005). Recent studies using Schwann cells co-cultured with neurons and neurotrophic factors by Whitlon and colleagues revealed the intimate relationship between neurite growth and Schwann cell proliferation. Growth cones were absent in cultures without Schwann cells, and Schwann cells only appeared to migrate to and around areas of neurite growth, suggesting an important reciprocal relationship between these two phenomena (Whitlon et al., 2009). Schwann cells certainly provide their own neurotrophic factors, but Wise suggested that Schwann cells also bind exogenously delivered neurotrophins, thus providing a neurotrophin-laden pathway for neurite growth (Wise et al., 2005). This role for Schwann cells in regeneration should be considered when designing methods for neurotrophin delivery and peripheral fiber regeneration in the deafened cochlea.

Multiple studies have looked at the ultrastructure of SGCs, peripheral processes and their myelin sheaths after noise-induced and ototoxic damage to the cochlea. In the chinchilla, spontaneous nerve regeneration after noise exposure has been observed, and included re-growth of myelin sheaths, but the pattern and thickness of those myelin sheaths differed from normal (Bohne et al., 1992; Lawner et al., 1997b). In these cases, Schwann cell-like cells were seen extending through the habenula and into the basilar membrane, in association with regenerating nerve fibers. In the guinea pig, it has been shown that after ototoxic damage, there is widespread hair cell loss and subsequent degeneration of SGCs. The remaining neurons have fewer mitochondria, decreased cell circularity, fewer layers of myelin, and resemble a gestational state of Type I SGCs (Agterberg et al., 2008; Dodson et al., 2000). In cases of VII nerve transection, Schwann cells dedifferentiate into their immature phenotype allowing for proliferation and migration along with axons during the process of regeneration (Cheng et al., 2002).

The recent efforts to regenerate SGCs using neurotrophic factors such as BDNF and NT-3 are explained in section 7 above. In one such study by Wise, guinea pigs were deafened with systemic kanamycin and furosemide, and subsequently implanted with a mini-osmotic pump

for BDNF delivery. Results at one month suggest BDNF helps preserve SGCs, increasing numbers of Schwann cells and peripheral fibers (Wise et al., 2005). However Agterberg and colleagues used comparable methods as Wise, and examined specimens under the electron microscope, noting altered pattern and reduced thickness of myelin surrounding surviving neurons compared to normal, contributable either to the initial damage, or the transient nature of the neurotrophin delivery (Agterberg et al., 2008). It is likely that improved pattern and thickness of myelination in regenerated nerve fibers would enhance action potential propagation and SGC functioning in ears that receive cochlear implant.

10. Nerve fiber regeneration versus auditory neuropathy

While the ultimate goal of regenerative medicine in the field of neurotology is the restoration of hearing via regeneration of sensory hair cells and auditory nerve fibers in the deafened human cochlea, nerve fiber regeneration has the potential for more immediate use in the clinical setting. One potential application for isolated auditory neuronal fiber regeneration is in the treatment of auditory neuropathy (AN), which is found in approximately 8% of the sensorineural hearing loss population (Madden et al., 2002). AN is a relatively recently recognized clinical entity in which the outer hair cells remain intact and functional until late in the disease process, and the hearing loss is secondary to pathology at the level of the synapse of the inner hair cell and auditory neuronal fiber, or in the auditory nerve itself (Starr et al., 1996; Worthington et al., 1980). Audiologic findings include an absent or abnormal auditory brainstem response (ABR), intact otoacoustic emissions (OAE), variable results on pure tone audiometry and disproportionately poor speech understanding, particularly in noise (Kraus et al., 2000; Starr et al., 1996). This spectrum of findings has been attributed to abnormal temporal processing along the auditory neuronal pathway in these patients (Kraus et al., 2000; Zeng et al., 1999).

Hearing aids frequently are not significantly beneficial to this patient population, and currently the best method of auditory rehabilitation for AN patients is in the form of cochlear implantation (Buss et al., 2002; Madden et al., 2002; Mason et al., 2003; Shallop et al., 2001; Trautwein et al., 2000). However, significant variability in cochlear implant therapy outcomes remains in this population, and this likely is related to inter-patient variability in the exact site of the pathologic lesion (Madden et al., 2002; Mason et al., 2003; Rance et al., 2008; Rance et al., 2009; Teagle et al., 2010; Trautwein et al., 2000). Some data have suggested that a normal appearing electrically evoked ABR may predict improved outcomes following cochlear implantation in this population (Gibson et al., 2007), while cochlear nerve deficiency may predict poorer performance (Teagle et al., 2010; Walton et al., 2008). These findings suggest that restoration of a functional auditory nerve is key to the optimal hearing rehabilitation in auditory neuropathy.

In cases of AN, the sensory hair cells function normally and serve as a prime target for regenerated functional auditory neurons. Animal models for the study of auditory neuropathy include various genetic defects such as mutations inotoferlin, pejvakin, and diaphanous homolog 3 (Delmaghani et al., 2006; Grati et al., 2009; Kim et al., 2004; Romanos et al., 2009; Schoen et al., 2010; Starr et al., 2004; Varga et al., 2003). Animal models have also been created with the use of ouabain, a Na/K ATPase pump toxin that selectively kills Type I afferent neurons and preserves the hair cells (Lang et al., 2005; Lang et al., 2010; Schmiedt et al., 2002). Development of these animal models will allow further research into the role of regenerative technologies in the treatment of this disorder.

11. Conclusions

Means of improving the efficacy of the cochlear prosthesis are being investigated.

Hair cell loss and SGC degeneration can occur in varying levels and rates pertaining to the various insults and species.

Limited spontaneous regeneration of nerve fibers can be seen after insults.

Neurotrophic factors have been demonstrated to induce peripheral nerve fiber re-growth in animal models in parallel with enhanced survival of SGCs.

Neuronal fiber regeneration may bring the target peripheral fibers in closer proximity to the cochlear implant electrode, thereby allowing more specific neural activation at lower thresholds.

Schwann cells may play a potential role in designing methods for nerve fiber regeneration.

Neuronal fiber regeneration may be a potential approach for treatment in isolated cases of AN.

Research emphasizing neuronal re-growth will likely be beneficial for future clinical goals.

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Research Highlights

- Nerve fibers regress from the sensory epithelium after hair cell death
- Transgene expression of BDNF or NTF-3 in the basilar membrane area rescues spiral ganglion cell bodies and promotes outgrowth of peripheral nerve fiberss

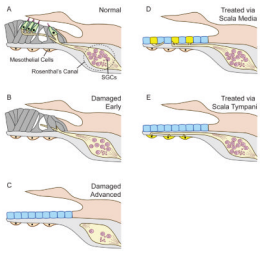

Figure 1. Schematic figures depictingauditory epithelium and nerve in normal versus deafened organ of Corti (A through C) and Ad. neurotrophin treated auditory epithelium via scala media (D) or scala tympani (E)

Inner and outer hair cells are innervated by peripheral nerve fibers extending from the SGC in the Rosenthal's canal (A). Shortly after an insult, the hair cells have degenerated and supporting cells form pharyngeal scars. The peripheral nerve fibers become disorganized and regress back to the SGC soma. SGC loss begins subsequently (B). In severe cases, the organ of Corti is replaced by single layer of cuboidal cells, the "flat epithelium". The peripheral nerve fibers have regressed completely and SGC loss is more substantial (C). Treatment of the flat epithelium treated with viral vectors carrying neurotrophic factors via scala media leads to secretion of neurotrophins by cells of the flat epithelium. Nerve fibers re-sprout and grow towards the source of the neurotrophin, such that the epithelial cells serve as hot spots and attract peripheral nerve fibers. SGC survival is also enhanced (D). When the neurotrophin expressing viral vector is placed in scala tympani, the mesothelial cells are transduced and serve as hot spots that attract peripheral fibers to the basilar membrane area (E).

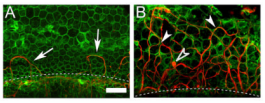

Figure 2. Whole mount of a flat epithelium of a guinea pig cochlea with (B) or withoutAd.*BDNF*treatment (A), stained for actin (phalloidin, green) and neurofilaments (red)

The flat epithelium, in the area where the organ of Corti used to reside, is composed of polymorphic cuboidal cells. The intercellular adherens junctions are actin rich. Nerve fibers are mostly absent, except for a few looping fibers (arrow) next to the habenula perforata (dashed line) (A). In a cochlea with flat epithelium treated with Ad.*BDNF*a large number of re-grown peripheral nerve fibers is present. The fibers appear to traverse the epithelium between the cuboidal cells (arrowheads). Fibers are of different diameter and their orientation in the flat epithelium varies from longitudinal to radial. Some bulging regions that resemble terminals are seen (open arrowhead) (B). Scale bar = 50 μm, for (A) and (B).