Molecular Neurobiology
Copyright © 1998 Humana Press Inc.
All rights of any nature whatsoever reserved.
ISSN0893-7648/98/17(1–3): 17–31/$11.75

Therapeutic Potential of Neurotrophins for Treatment of Hearing Loss

Wei-Qiang Gao

Department of Neuroscience, Genentech, Inc., MS#72, 1 DNA Way, South San Francisco, CA 94080

Abstract

Degeneration of spiral ganglion neurons (SGNs) and hair cells in the cochlea induced by aging, injury, ototoxic drugs, acoustic trauma, and various diseases is the major cause of hearing loss. Discovery of growth factors that can either prevent SGN and hair-cell death or stimulate hair-cell regeneration would be of great interest. Studies over the past several years have provided evidence that specific neurotrophins are potent survival factors for SGNs and protect these neurons from ototoxic drugs in vitro and in vivo. Current research focuses more on understanding the mechanism of hair-cell regeneration/differentiation and identification of growth factors that can stimulate hair-cell regeneration. SGNs are required to relay the signal to the central nervous system even when a cochlear implant is used to replace hair-cell function or in the case that cochlear sensory epithelium can be stimulated to regenerate new hair cells successfully. Therefore, neurotrophins may have their therapeutic value in prevention and treatment of hearing impairment.

Index Entries: Neurotrophin; spiral ganglion neurons, auditory, ototoxicity, hearing impairment; hair cells; regeneration; cochlear implant; immortalized cell line.

Introduction

Hearing loss is among the most common neurological deficits. The majority of hearing loss is attributable to damage to the peripheral auditory system. This system is composed of two key components: spiral ganglion neurons (SGNs), which are the primary auditory neurons, and hair cells, which are the auditory receptor cells in the organ of Corti. Whereas the hair cells convert sound into electrical signals, the SGNs deliver signals from the periphery to the brain through the cochlear nerve. Damage or loss of either the SGNs or the hair cells can block the auditory-signaling pathway and lead to hearing impairments. In general, hearing loss can be caused by aging, loud sound, therapeutic drugs, chemicals, mechanical injury, infections, tumors, and genetic diseases. Some sensorineural hearing impairments result from

SGN degeneration, some are caused by hair cell loss, and others are attributable to damage to both SGNs and hair cells. Because hearing impairment is a serious handicap affecting millions of people in the world, any agent that can protect SGNs and/or hair cells from damage would be highly beneficial.

Neurotrophins are good candidate molecules for prevention of SGN-loss-mediated hearing impairments because they have been intensively demonstrated to regulate neuronal differentiation and survival during development (Korsching, 1993; Gao et al., 1995b), and to protect neurons from injury and neurotoxicity of certain chemicals in adults (for example, Hefti, 1986; Gao et al., 1995a). Indeed, because of their profound trophic effects on the nervous system, specific neurotrophins are currently in clinical trials for some neurodegenerative diseases (*see* a recent review by Gao et al., 1997). The mammalian neurotrophin family contains four members: nerve-growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). Each of the neurotrophins has a specific high-affinity functional receptor, a member of the trk family (Barbacid, 1993; Snider, 1994). For example, NGF specifically signals through trkA, BDNF, and NT-4/5 through trkB, and NT-3 selectively activates trkC. All of the neurotrophins also bind to the NGF low-affinity receptor, p75 (Chao, 1994). Although the exact function of p75 is still not well-known, recent studies indicate that the binding of NGF to p75 can enhance the trkA-mediated signal transduction pathway (Davies et al., 1993a; Clary and Reichardt, 1994; Verdi et al., 1994) or induce apoptosis when the neurons do not express trkA (Casaccia-Bonnefil et al., 1996; Cortazzo et al., 1996; Frade et al., 1996).

In the auditory system, *in situ* hybridization studies have revealed the expression of neurotrophins in hair cells and neurotrophin receptors, trkB, trkC, and p75, in SGNs (Pirvola et al., 1992; Ylikoski et al., 1993; Pirvola et al., 1994; Schecterson and Bothwell, 1994; Wheeler et al., 1994; Zheng et al., 1995). Furthermore, analyses of BDNF/ NT-3 and trkB/trkC knockout mice indicate a substantial loss of SGNs (*see* a recent review by Fritzsch et al., 1997b). These observations together provide strong evidence for the idea that neurotrophins can be retrogradely transported from hair cells to the SGNs to influence the survival and maintenance of SGNs in normal, physiological situations and opens the possibility that exogenously applied neurotrophins could promote SGN survival and enhance neuronal function following acoustic trauma and ototoxic insults.

This article will describe in vitro and in vivo assay systems that have been used to identify SGN and hair-cell survival factors and review data emphasizing neuroprotection by specific neurotrophins. This article will also briefly provide an overview of studies on hair-cell regeneration/differentiation. In addition, the potential methods of delivering neurotrophins and/or other growth factors into the cochlea for treatment of hearing loss will be discussed.

Use of In Vitro Culture Systems to Screen for SGN and Hair-Cell Survival Factors

The cochlea is a complicated bony structure, and performing in vivo studies to screen for SGN and hair-cell growth factors is difficult and has a low throughput. The establishment of dissociated SGN-cell culture has provided a more accessible system (Lefebvre et al., 1994; Zheng et al., 1995). The SGN-cell culture can be done by dissociating the spiral ganglion tissue into single-cell suspension following enzymatic digestion and placing these cells in culture dishes in serum-free medium. Under these culture conditions, the survival of SGNs is poor. Identification of survival factors can be achieved by inclusion of a particular testing molecule in the culture medium and examination of the increased number of SGNs in the culture. However, the dissociated SGN-cell culture does not allow one to screen for hair-cell-growth factors. Development of organotypic culture of postnatal rat cochlear explants solves

this problem because it contains intact hair cells and maintains the afferent neuronal innervation (Sobkowicz et al., 1975; Kelley et al., 1993; Rastel et al., 1993; Zheng and Gao, 1996). The organotypic culture of cochlear explants has now proven to be useful for understanding the mechanism for actions of ototoxins (Richardson and Russell, 1991; Zheng and Gao, 1996), to discover protective agents for SGNs and hair cells (Richardson and Russell, 1991; Zheng and Gao, 1996), and to search for hair-cell growth factors (Kelley et al., 1993; Lefebvre et al., 1993; Chardin and Romand, 1995).

Recently a novel, rapid method of organotypic culturing of postnatal cochlear explants in a three-dimensional (3-D) collagen matrix in well-defined, serum-free medium has been developed (Zheng and Gao, 1996), without using a previously reported Maximov slide assembly (Sobkowicz et al., 1975) or a floating-drop method (Rastel et al., 1993). Embedding the cochlear explants in the 3-D collagen and culturing them in serum-free medium can keep the tissue unfolded and limit proliferation and migration of nonneuronal cells. Laminar architecture of the explant is well-maintained in the culture (Fig. 1A,B). Using double histochemical labeling with a monoclonal antibody against neurofilament protein and a phalloidin-FITC conjugate (Rastel et al., 1993; Zheng and Gao, 1996), surviving SGNs and hair cells in the cochlear explants can be easily identified in the explants (*see* Fig. 1). Whereas the neurofilament antibody stains the SGNs and their peripheral projections to the hair-cell region (Fig. 1D), the phalloidin labels the surviving hair cells in the organ of Corti (Fig. 1C).

Specific Neurotrophins Promote the Survival of SGNs and Protect Them from Ototoxic Drugs in Cultures

In a series of experiments designed to screen for molecules that promote the survival of SGNs (Zheng et al., 1995), a panel of growth factors that have been reported to promote cell survival in the nervous system have been tested in the SGN-dissociated cell culture. These experiments clearly demonstrate that inclusion of NT-4/5 in the cultures greatly enhances the survival of cultured SGNs. The survival-promoting effect of NT-4/5 is dose-dependent with a maximal activity at a range of 10–20 ng/mL. NT-4/5 is as potent as BDNF, but stronger than NT-3. NGF and other growth factors examined include epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), transforming growth factor-β1 (TGF-β1), TGF-β2, TGF-β3, and TGF-β5 do not show any survival effects on cultured SGNs. The survival effects of BDNF and NT-3 are also observed in the SGN cultures prepared from adult rats (Lefebvre et al., 1994).

Clinically, there are generally four classes of therapeutic ototoxins: salicylates such as aspirin, aminoglycosides such as gentamicin, chemotherapeutic agents such as cisplatin, and diuretic drugs such as ethacrynic acid. To determine if these ototoxins can induce degeneration of SGNs and hair cells in the culture, my colleague and I (Zheng and Gao, 1996) have extended our work from the dissociated SGN-cell culture to the organotypic culture of cochlear explants by addition of each of three classes of ototoxins (sodium salicylate, gentamicin, and cisplatin) in the culture medium. These experiments yield some novel findings regarding the toxic actions of sodium salicylate, gentamicin, and cisplatin. Whereas gentamicin preferentially kills hair cells, sodium salicylate specifically damages auditory neurons without apparent damage to hair cells. Cisplatin, however, induces degeneration of both auditory neurons and hair cells with a more profound damage to SGNs at low concentrations. Whereas most previous studies tend to emphasize toxic effects on hair cells by these agents, the observations obtained in the organ culture suggest that some of the ototoxic insults could directly damage SGNs (Zheng and Gao, 1996). Consistent with the work on dissociated SGN-cell cultures (Zheng et al.,

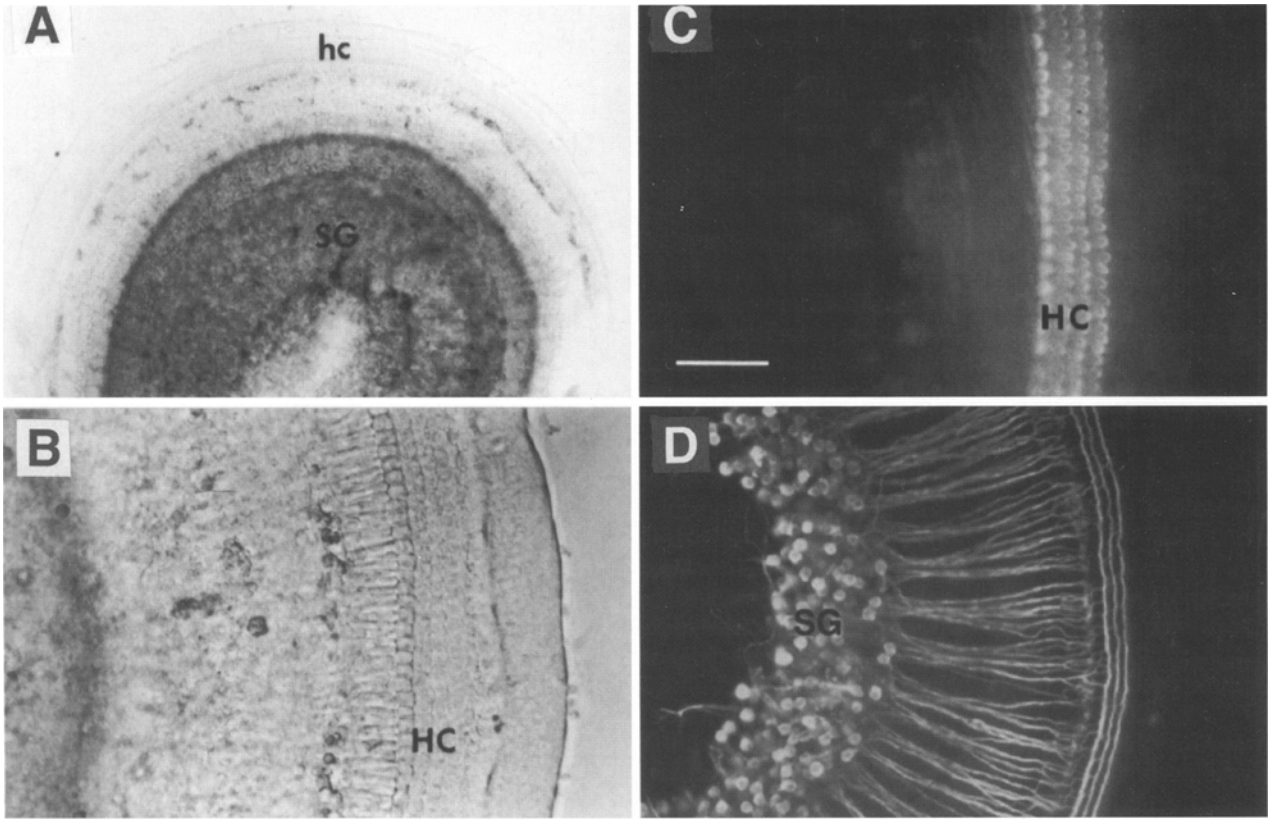

Fig. 1. Organotypic culture of cochlear explants provides a good assay for identification of SGN and hair-cell growth factors. **(A, B)** Nomarski micrographs of cochlear explants dissected from P3 rats and grown for 2 d in vitro at low and high magnifications, respectively. **(C, D)** Fluorescence microscopy shows phalloidin staining of stereociliary bundles of hair cells and neurofilament antibody labeling of SGNs in cochlear explants cultured for 4 d, respectively. Note that the cultured cochlear explants maintained normal laminar structures including the spiral ganglion, one row of inner hair cells, three rows of outer hair cells **(C),** and the neuronal projections to the hair cells in the organ of Corti **(D).** Abbreviation: SG, spiral ganglion; hc, hair cells. Bar, 200 μm for **A,** 50 μm for **B, C,** and **D.**

1995), when specific neurotrophins are included along with one of the ototoxins, SGN degeneration is either completely prevented or ameliorated (Zheng and Gao, 1996). NT-4/5 and BDNF show equivalent potency and are stronger than NT-3. NGF does not show any protective effects. Other growth factors examined include EGF, FGF-2, FGF-5, FGF-7, insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), TGF-α, TGF-β1, TGF-β2, TGF-β3, TGF-β5, or retinoic acid all failed to show any protective effects on SGNs or hair cells in the organ culture. These observations are mainly quantified by analyses of the density of neurofilament-antibody-stained SGN peripheral projections and phalloidin-labeled hair cells in the whole mount cochlear explant tissues. In addition, these results are confirmed and validated with total cell counts of surviving SGN cell bodies in serial cryostat sections of the cultured cochlear explants (Zheng and Gao, 1996). Representative examples of the cryostat sections of the cochlear explant cultures are shown in Fig. 2: the preservation of SGNs in the cultures cotreated with cisplatin and NT-4/5 is evident, based on the

neurofilament-antibody labeling. The protective effects of exogenous NT-4/5, BDNF, and NT-3 but not NGF on SGNs are apparently attributable to the fact that SGNs express trkB and trkC, but not trkA.

In Vivo Effects of Specific Neurotrophins

Several laboratories have conducted experiments to determine the neuroprotective effects of neurotrophins by introducing NT-3 and BDNF into the cochlea using osmotic minipumps (Ernfors et al., 1996; Staecker et al., 1996; Miller et al., 1997). In all cases, systemic treatment with a combination of an aminoglycoside (either kanamycin or amikacin) and a diuretic drug (ethacrynic acid) in guinea pigs can completely destroy all cochlear hair cells and lead to a significant or nearly complete loss of SGNs. Cotreatment with NT-3 or BDNF in these guinea pigs can greatly attenuate the loss of SGNs and result in a large preservation of SGNs. Whereas one group shows that NT-3 is either stronger or as potent as BDNF in preventing SGNs from death caused by aminoglycoside insult (Staecker et al., 1996), the other group reports that BDNF is more effective than NT-3 in the same animal model (Miller et al., 1997). The latter observation is in agreement with in vitro data. In addition, minipump infusion of NGF into the cochlea has also been reported to have protective effects on auditory nerve against neomycin-induced neuronal degeneration (Schindler et al., 1995). In any case, these in vivo results reinforce the idea that specific neurotrophins may be useful in preventing auditory neuronal loss induced by ototoxins and injury.

Hair-Cell Survival and Hair-Cell Regeneration/Differentiation

Although both in vitro and in vivo studies demonstrate clearly that specific neurotrophins have strong protective effects on SGNs, they do not protect hair cells from the ototoxins. Consistent with these findings, whereas there is a dramatic loss of SGNs in transgenic mice in which genes encoding for neurotrophins and their receptors are deleted, hair cell number is unchanged (Ernfors et al., 1995; Fritzsch et al., 1995; Minichiello et al., 1995; Bianchi et al., 1996; Fritzsch et al., 1997a). Although minor abnormality of vestibular hair-cell morphology and thickness of the cochlear and vestibular sensory epithelia are reported in BDNF/NT-3 and trkB/trkC double mutant mice, respectively, in earlier studies (Ernfors et al., 1995; Minichiello et al., 1995), more recently others have not found these deficits and observed normally maturing hair cells without any afferent or efferent innervation in these-mutant animals (Fritzsch et al., 1997c). These results together suggest that neurotrophins are not crucial for the survival and differentiation of hair cells. In addition, none of the many presently known growth factors shows protective effects on hair cells in the study done in my laboratory (Zheng and Gao, 1996). However, others have reported that at a relatively low concentration of neomycin, FGF-2 significantly protects hair cells from neomycin damage in vitro (Low et al., 1996). In the latter study, TGF-α and retinoic acid also showed a moderate protective effect on hair cells. Moreover, glial-derived neurotrophic factor (GDNF) is recently reported in abstract form to protect hair cells from ototoxins or acoustic trauma in guinea pig models (Magal et al., 1997; Shoji et al., 1997).

Since both hair cells and SGNs are critical for delivery of the auditory signals to the central nervous system, the lost hair cells need to be replaced for effective recovery of hearing loss. Recent studies suggest that although the frequency is relatively low, mammalian inner ears have the capacity to regenerate new hair cells following ototoxic insults (Forge et al., 1993; Warchol et al., 1993; Yamane et al., 1995; Zheng and Gao, 1997). These studies represent an extension of earlier work on avian and lower vertebrate inner ears. Over the past several years, accumulating evidence has indicated that

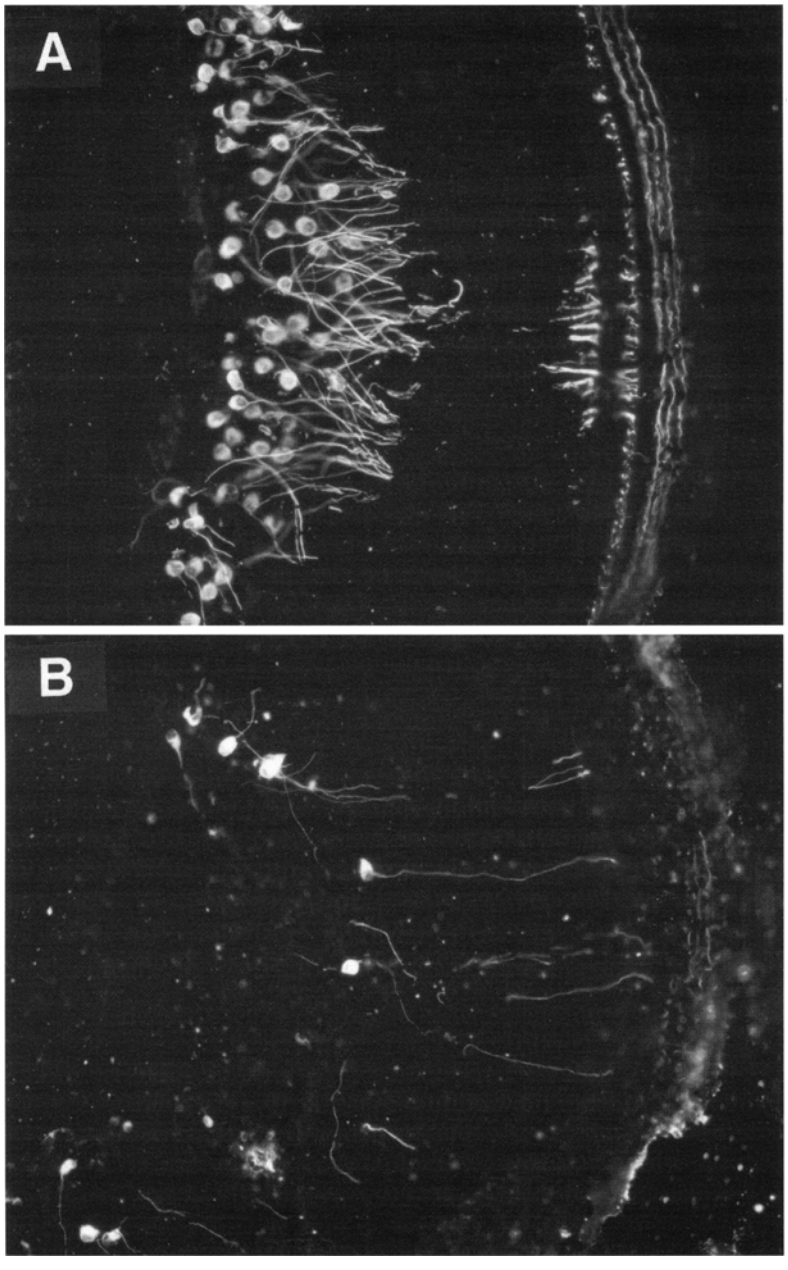

Fig. 2. Typical examples of neurofilament immunostained sections of a normal cochlear explant culture **(A),** a culture treated with 4 µg/mL cisplatin **(B),** and a culture cotreated with 4 µg/mL cisplatin and 20 ng/mL NT-4/5. *(continued)*

supporting cells in the inner ear are most likely hair-cell progenitors (Corwin and Cotanche, 1988; Ryals and Rubel, 1988; Balak et al., 1990; Weisleder and Rubel, 1992; Jones and Corwin, 1996; Stone et al., 1996; Warchol and Corwin, 1996). Following noise or aminoglycoside insults, significant proliferation of supporting cells is observed in the avian inner-ear epithelium (Hashino et al., 1992; Raphael, 1992; Hashino and Salvi, 1993; Stone and Cotanche,

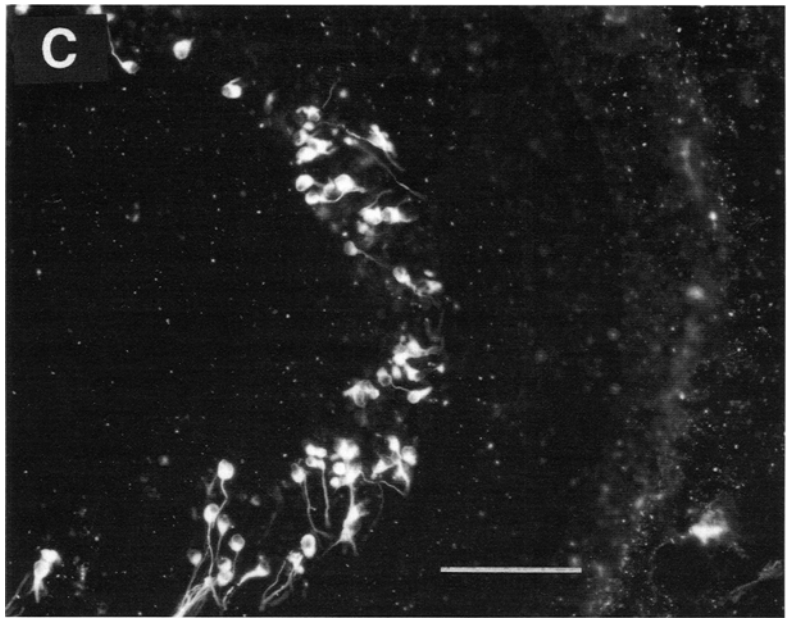

Fig. 2. *Continued* **(C).** The explant cultures were prepared from middle turns of postnatal day 3 rat cochleae. Note that cisplatin induced a massive loss of SGNs and NT-4/5 greatly prevented it from happening. Some of the peripheral projections of SGNs could not be seen in panel **C** because of a tilted cutting angle. Bar, 100 μm.

1994). Experiments using DNA synthesis tracers indicate that some new hair cells derive from the dividing cells (Corwin and Cotanche, 1988; Ryals and Rubel, 1988; Weisleder and Rubel, 1992; Warchol et al., 1993; Yamane et al., 1995; Stone et al., 1996; Zheng and Gao, 1997).

Because the proliferation of supporting cells is an early step during the hair-cell regeneration process, efforts have been made over the past 4 yr to identify supporting-cell mitogens. These studies are done with a belief that supporting-cell mitogens may potentially facilitate hair-cell regeneration processes and eventually be used as agents for treatment of hearing impairments (Fig. 3). Toward this end, inner-ear organ cultures (Warchol et al., 1993; Lambert, 1994; Yamashita and Oesterle, 1995) and pure inner-ear epithelial cell cultures that contain only supporting cells and hair cells (Gu et al., 1996; Zheng et al., 1997a) have been established. Studies using these types of cultures have identified several growth factors as supporting-cell mitogens. These include epidermal EGF, TGF-α, several FGF family members, IGF-1, IGF-2, and human-glial growth factor (hGGF) (Lambert, 1994; Yamashita and Oesterle, 1995; Gu et al., 1996; Oesterle et al., 1997; Zheng et al., 1997a). However, whether these growth factors can directly stimulate hair-cell regeneration still remains to be determined.

Because hair-cell regeneration might replicate the process of hair-cell differentiation, it is important to understand the mechanism of the initial hair-cell differentiation during normal development (Fig. 3). Recent studies have identified some specific genes such as transcription-factor genes involved in mammalian hair-cell differentiation (Ryan, 1997). In particular, a POU domain transcription factor, Brn3.1, plays an important role at the initial differentiation step of hair cells. When this gene is knocked out, hair-cell differentiation fails (Erkman et al., 1996). Several other genes, such as bone morphogenic proteins (BMPs), appear to be involved in early development and formation of inner-ear structures (Oh et al., 1996; Wu and Oh, 1996; Whitfield et al., 1997). Lateral inhibition mediated by notch-notch ligand interaction is

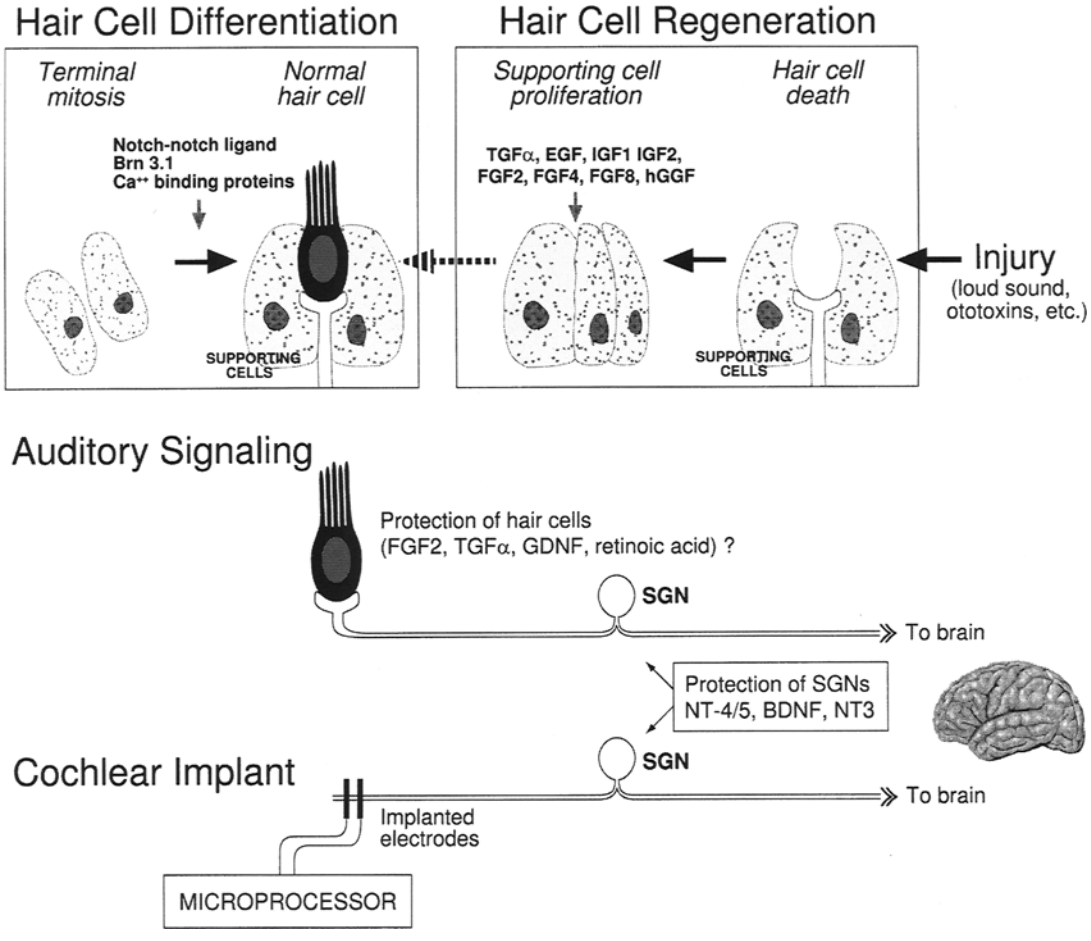

Fig. 3. A schematic drawing of therapeutic roles of various growth factors, specific genes, and cochlear implants in treatment of hearing loss. Note that survival of SGNs, which can be promoted by specific neurotrophins, is crucial for relaying the auditory signals from normal hair cells, cochlear implants, or regenerated hair cells, to the brain. Studies on hair-cell regeneration/differentiation have discovered several mitogens for supporting cell proliferation and demonstrated involvement of a few genes in hair-cell differentiation. Future studies are needed to identify molecules that can directly stimulate regeneration of new hair cells following hair-cell loss caused by injury. A combination of specific neurotrophins and cochlear implants or hair-cell growth factors would be more competent for recovery of hearing function.

likely to play a part in cell fate determination and hair-cell differentiation following terminal mitosis of hair-cell progenitors (Lewis, 1991; Lanford et al., 1997; Lewis et al., 1997; Whitfield et al., 1997). Combined with previous tritiated thymidine hair-cell birth date studies (Ruben, 1967; Sans and Chat, 1982), a developmental time course of hair-cell development has been outlined for both cochlear (Kelley et al., 1993) and vestibular systems (Zheng and Gao, 1997). It has now been clearly demonstrated that expression of specific calcium-binding proteins coincides well with the initial hair-cell differentiation and these calcium-binding proteins can

be used as hair-cell markers for future hair-cell survival and regeneration studies (Zheng and Gao, 1997).

Another approach related to hair-cell replacement therapy is to conditionally establish immortalized inner-ear supporting-cell lines and hair-cell lines. Using the immortomouse that carries the large T-antigen oncogene in every cell, conditionally immortalized cells have been generated from inner-ear tissues. Some of the immortalized cells derived from the E15 cochlea exhibit hair-cell features, although they are not successfully subcloned (Holley and Lawlor, 1997; Holley et al., 1997). Several cell lines derived from either postnatal cochlea or embryonic otocysts have been subcloned, and some of them display neuron-like features but none of the cell lines show hair-cell phenotypes (Kalinec et al., 1996; Barald et al., 1997). With a slightly different approach, my laboratory has established utricular epithelial-cell lines by transferring the tsA58 allele of the SV40 large T-antigen oncogene (Gao and Hatten, 1994) into neonatal rat utricular supporting cells using retrovirus (Zheng et al., 1997b). These established cell lines are stably maintained and exhibit many features similar to primary utricular supporting cells (Zheng et al., 1997b). They grow in patches and assume a polygonal morphology. These cells express epithelial cell markers, but not fibroblast, glial, or neuronal antigens. More promising is the finding that these cells can assume a morphological differentiation when cultured in serum free medium at non-permissive temperature in the presence of bFGF. Under these conditions, the cells shrink in size, become elongated, and even express early hair-cell markers such as calretinin and calmodulin (Zheng and Gao, 1997), although stereociliary bundles, a phenotype for mature hair cells, are not formed in the cultures. The established epithelial-cell line may potentially be useful for hair-cell differentiation/regeneration studies and for possible replacement of lost hair cells by implantation of these cells back to the inner ear epithelium.

Combination Therapy by Using Neurotrophins Together with Hair-Cell Growth Factors or Cochlear Implants

Stimulating hair-cell regeneration by growth factors, or by using the conditionally immortalized inner ear cells that might differentiate into hair cells, may be a way to replace the lost hair cells following acoustic trauma or ototoxic damage. However, hair-cell regeneration takes time and SGNs may die before the lost hair cells are replaced. Therefore, neurotrophins will likely be necessary for prevention of SGN death following injury.

Similarly cochlear implants, which bypass hair-cell function by electrical stimulation of the SGNs, have proven clinically effective in a number of patients, but survival of a critical number of the SGNs are required for the implanted electrodes to function appropriately (Fig. 3). Neurotrophins have been shown not only to exert neurotrophic effects but also neurotropic effects (Song et al., 1997). Application of neurotrophins prior to or at the time of cochlear implant surgery may not only prevent the SGNs from death, but also attract the remaining SGN peripheral terminals to grow to implanted electrodes by promoting neurite outgrowth, which would enhance the efficacy of cochlear implants. Thus, it is expected that before discovery of hair-cell regeneration growth factors, the first application of neurotrophins in the cochlea could be in combination with cochlear implants (*see below*).

Methods for Delivering Neurotrophins or Other Growth Factors into the Cochlea

The use of neurotrophins as therapeutic molecules is a novel and sound approach to neurodegenerative disorders with a strong scientific rationale and experimental data. As summa-

rized in a recent review article (Gao et al., 1997), some of the neurotrophins are currently in clinical trials. For example, NGF is in clinical trials for treatment of diabetic neuropathy. For the peripheral nervous system, systemic administration such as subcutaneous injections is appropriate as the nerve terminals can access and retrogradely transport the neurotrophins to the neuronal cell body. However, delivery of neurotrophins into the brain has been a critical issue because neurotrophins are proteins that cannot pass through the blood–brain barrier. For treatment of hearing loss, on one hand, the cochlea is similar to the peripheral nervous system and does not have the blood–brain barrier. A preliminary study from my laboratory suggests that proteins are capable of reaching the cochlea after systemic administration (unpublished observations). On the other hand, in order to have sufficient amounts of neurotrophins reach the cochlea through systemic administration, high dose is required, which could potentially induce undesired side effects. As a solution to this problem, local application of neurotrophins appears to be an alternative way to deliver neurotrophins into the inner ear. Indeed, several studies have demonstrated that osmotic minipumps can successfully deliver neurotrophins to the scala tympani of the cochlea. In guinea pig models of hair-cell degeneration, minipump infusion of neurotrophins have preserved SGNs following aminoglycoside insults (Schindler et al., 1995; Ernfors et al., 1996; Staecker et al., 1996; Miller et al., 1997). One shortcoming of this method is, however, that it requires surgical opening of the inner ear, risking perilymphatic infection during the course of minipump infusion. An alternative way is to apply a piece of gelfoam that has been presoaked with a high concentration of neurotrophins to the round-window membrane. Preliminary studies in my laboratory indicate that gelfoam soaked with a 10,000-fold or higher concentration of a protein placed on the round-window membrane can allow the molecule to passively diffuse into the cochlear perilymph down the concentration gradient. A major advantage of this method is that surgery in the middle ear is relatively less invasive and does not directly disturb the perilymph in the inner ear.

As mentioned above, an attractive possibility would be to combine neurotrophin delivery to the cochlea with cochlear-implant surgery. With a neurotrophin present at the site of implanted electrodes, the peripheral terminals of remaining SGNs may be attracted to grow toward and terminate in close proximity to the implanted electrodes because of the neurotrophin's possible tropic effect. Once the cochlear implant is in effect, the remaining neurons can be maintained because of the electric stimulation (Leake et al., 1992), which can upregulate the endogenous neurotrophin (Victoria et al., 1997). Since the cochlear implant is already established clinically, this combination therapy does not require additional surgery. Thus, new cochlear-implant devices allowing application of neurotrophins at the time of cochlear-implant surgery need to be developed and are theoretically expected to have better efficacy.

Utilization of viral vectors and genetically engineered cells is another way to introduce neurotrophins to the cochlea. Initial experiments have used herpes simplex virus and adenovirus in experimental settings (Geschwind et al., 1996; Dazert et al., 1997). In vitro viral transduction of the spiral-ganglion explants with a specific neurotrophin gene can successfully lead to an enhanced SGN survival (Geschwind et al., 1996). Lalwani et al. (1996) have also shown that in vivo, expression of a reporter gene in the cochlear is achieved by using adeno-associated viral infection. Similar viral-gene transfer has been conducted with a replication-deficient adenoviral vector in guinea pigs (Raphael et al., 1996). The latter two studies suggest that efficient viral-gene transfer into multiple types of cochlear cells in vivo can be achieved without apparent morphological signs of pathology or toxicity. However, viral infections may still risk a possibility of some unpredictable adverse effects such as tumorigenesis and overgrowth of nonneural tissues. Using polymer capsules that hold genetically engineered neurotrophin-producing cells, but

allow proteins to come out the capsule, may be a better method for this type of gene therapy (Hoffman et al., 1990; Hoffman et al., 1993).

One potential, but less invasive way to deliver neurotrophins or other molecules to the cochlea is by injection through the tympanic membrane (Magal et al., 1997). In treatment of some severe middle-ear infections, penetration of the tympanic membrane with a thin needle is clinically practiced, and the tympanic membrane is capable of sealing itself afterwards. However, the exact amount of neurotrophins that can successfully reach the cochlear perilymph with the middle-ear injection may vary because the injected neurotrophin might leak away through the Eustachian tube into the nose and mouth instead of passing the oval and round windows into the inner ear. Further studies evaluating this method need to be done before it can be considered for use in human patients.

Finally, an even less invasive potential method is to identify small molecules that can upregulate neurotrophic activity in spiral ganglion in vivo. For example, systemic administration of GM1 ganglionside can significantly ameliorate the atrophy of spiral-ganglion neurons in mice with conductive hearing loss, presumably through enhancing endogenous growth-factor activity in spiral ganglion (Walsh and Webster, 1994).

Conclusions

Compelling evidence indicates that specific neurotrophins promote survival of SGNs and protect them from ototoxins. This evidence is obtained by experiments performed not only in vitro including dissociated SGN-cell cultures and organotypic cultures of cochlear explants, but also in mammalian animal models. Cochlear implant has been effective in a number of patients with profound hearing loss and its efficacy is likely dependent upon its capacity to stimulate the peripheral terminal of the remaining SGNs which relay the signals produced by the cochlear implants to the central nervous system. Studies of hair-cell regeneration/differentiation and the influence of growth factors on the survival and regeneration of hair cells following injury are promising for possible hair-cell renewal and recovery. It is predicted that a combination of specific neurotrophins with cochlear implant or hair-cell growth factors would eventually be beneficial for those suffering from hearing loss (*see* Fig. 3).

Acknowledgments

I wish to thank J. Lisa Zheng, Annette K. Lewis, and Randall R. Stewart for their contribution to the work described in the article. I also thank Jay Han for critical reading of the manuscript and Wayne Anstine for preparation of the figures.

References

Balak K. J., Corwin J. T., and Jones J. E. (1990) Regenerated hair cells can originate from supporting cell progeny: evidence from phototoxicity and laser ablation experiments in the lateral line system. *J. Neurosci.* **10,** 2502–2512.

Barald K. F., Lindberg K. H., Hardiman K., Kavka A. I., Lewis J. E., Victor J. C., Gardner C. A., and Poniatowski A. (1997) Immortalized cell lines from embryonic avian and murine otocysts: tools for molecular studies of the developing inner ear. *Int. J. Devel. Neurosci.* **15,** 523–540.

Barbacid M. (1993) The trk family of neurotrophin receptors: molecular characterization and oncogenic activation in human tumors, in *Molecular Genetics of Nervous System Tumors,* (Levin, A. and Schmidek, H., ed.) Wiley-Liss, New York, pp. 123–136.

Bianchi L. M., Conover J. C., Fritzsch B., DeChiara T., Lindsay R. M., and Yancopoulos G. D. (1996) Degeneration of vestibular neurons in late embryogenesis of both heterozygous and homozygous BDNF null mutant mice. *Development* **122,** 1965–1973.

Casaccia-Bonnefil P., Carter B. D., Dobrowsky R. T., and Chao M. V. (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature* **383,** 716–719.

Chao M. V. (1994) The p75 neurotrophin receptor. *J. Neurobiol.* **25,** 1373–1385.

Chardin S. and Romand R. (1995) Regeneration and mammalian auditory hair cells [letter; comment]. *Science* **267,** 707–711.

Clary D. O. and Reichardt L. F. (1994) An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. *Proc. Natl. Acad. Sci. USA* **91,** 11,133–11,137.

Cortazzo M. H., Kassis E. S., Sproul K. A., and Schor N. F. (1996) Nerve growth factor (NGF)-mediated protection of neural crest cells from antimitotic agent-induced apoptosis: the role of the low-affinity NGF receptor. *J. Neurosci.* **16,** 3895–3899.

Corwin J. and Cotanche D. (1988) Regeneration of sensory hair cells after acoustic trauma. *Science* **240,** 1772–1774.

Davies A. M., Lee K. F., and Jaenisch R. (1993a) p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. *Neuron* **11,** 565–574.

Dazert S., Battaglia A., and Ryan A. F. (1997) Transfection of neonatal rat cochlear cells in vitro with an adenovirus vector. *Int. J. Devel. Neurosci.* **15,** 595–600.

Erkman L., McEvilly R. J., Luo L., Ryan A. K., Hooshmand F., O'Connell S. M., Keithley E. M., Rapaport D. H., Ryan A. F., and Rosenfeld M. G. (1996) Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development. *Nature* **381,** 603–606.

Ernfors P., Duan M. L., ElShamy W. M., and Canlon B. (1996) Protection of auditory neurons from aminoglycoside toxicity by neurotrophin-3. *Nature Med.* **2,** 463–467.

Ernfors P., Van De Water T., Loring J., and Jaenisch R. (1995) Complementary roles of BDNF and NT-3 in vestibular and auditory development. *Neuron* **14,** 1153–1164.

Forge A., Li L., Corwin J. T., and Nevill G. (1993) Ultrastructural evidence for hair cell regeneration in the mammalian inner ear [see comments]. *Science* **259,** 1616–1619.

Frade J. M., Rodriguez-Tebar A., and Barde Y. A. (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. *Nature* **383,** 166–168.

Fritzsch B., Sarai P. A., Barbacid M., and Silos-Santiago I. (1997a) Mice with a targeted disruption of the neurotrophin receptor trkB lose their gustatory ganglion cells early but do develop taste buds. *Int. J. Devel. Neurosci.* **15,** 563–576.

Fritzsch B., Silos-Santiago I., Bianchi L. M., and Farinas I. (1997b) The role of neurotrophic factors in regulating the development of inner ear innervation. *Trends Neurosci.* **20,** 159–64.

Fritzsch B., Silos-Santiago I., Smeyne R., Fagan A., and Barbacid M. (1995) Reduction and loss of inner ear innervation in trkB and trkC receptor knockout mice: a whole-mount DiI and scanning electron microscopic analysis. *Auditory Neurosci.* **1,** 401–417.

Fritzsch B., Silos-Santiago I., Bianchi L., and Farinas I. (1997c) Effects of neurotrophin and neurotrophin receptor disruption on the afferent inner ear innervation. *Sem. Cell Dev. Biol.* **8,** 277–284.

Gao W.-Q., Dybdal N., Shinsky N., Murnane A., Schmelzer C., Siegel M., Keller G., Hefti F., Phillips H. S., and Winslow J. W. (1995a) Neurotrophin-3 reverses experimental cisplatin-induced peripheral sensory neuropathy. *Annals Neurol.* **38,** 30–37.

Gao W.-Q., Weil R., Dugich-Djordjevic M., and Lu B. (1997) The therapeutic potentials of neurotrophic factors for diseases of the nervous system. *Exp. Opin. Ther. Patents,* **7,** 345–338.

Gao W.-Q., Zheng J. L., and Karihoaloo M. (1995b) Neurotrophin-4/5 (NT-4/5) and brain-derived neurotrophic factor (BDNF) act at later stages of cerebellar granule cell differentiation. *J. Neurosci.* **15,** 2656–2667.

Gao W. Q. and Hatten M. E. (1994) Immortalizing oncogenes subvert the establishment of granule cell identity in developing cerebellum. *Development* **120,** 1059–1070.

Geschwind M. D., Hartnick C. J., Liu W., Amat J., Van De Water T. R., and Federoff H. J. (1996) Defective HSV-1 vector expressing BDNF in auditory ganglia elicits neurite outgrowth: model for treatment of neuron loss following cochlear degeneration. *Human Gene Ther.* **7,** 173–182.

Gu R., Marchonni M., and Corwin J. T. (1996) Glial growth factor enhances supporting cell proliferation in rodent vestibular epithelia cultured in isolation. *Soc. Neurosci. Abstr.,* **22,** 520.

Hashino E. and Salvi R. J. (1993) Changing spatial patterns of DNA replication in the noice-damaged chick cochlea. *J. Cell. Sci.,* **105,** 23–31.

Hashino E., Tanaka Y., Salvi R. J., and Sokabe M. (1992) Hair cell regeneration in the adult budgerigar after kanamycin ototoxicity. *Hear. Res.* **59,** 46–58.

Hefti F. (1986) Nerve growth factor (NGF) promotes survival of septal cholinergic neurons after fimbrial transections. *J. Neurosci.* **6,** 2155–2162.

Hoffman D., Breakefield X. O., Short M. P., and Aebischer P. (1993) Transplantation of a polymer-encapsulated cell line genetically engineered to release NGF. *Exper. Neurol.* **122,** 100–106.

Hoffman D., Wahlberg L., and Aebischer P. (1990) NGF released from a polymer matrix prevents loss of ChAT expression in basal forebrain neurons following a Fimbria-Fornix lesion. *Exp. Neurol.* **110,** 39–44.

Holley M. C. and Lawlor P. (1997) Production of conditionally immortalised cell lines from a transgenic mouse. *Audiol. Neuo-Otol.* **2,** 25–35.

Holley M. C., Nishida Y., and Grix N. (1997) Conditional immortalization of hair cells from the inner ear. *Int. J. Devel. Neurosci.* **15,** 541–52.

Jones J. E. and Corwin J. T. (1996) Regeneration of sensory cells after laser ablation in the lateral line system: hair cell lineage and macrophage behavior revealed by time-lapse video microscopy. *J. Neurosci.* **16,** 649–662.

Kalinec F., Kalinec G., and Kachar B. (1996) Development of organ of Corti and stria vascularis cell line from transgenic mice. *Assoc. Res. Otolaryngol. Abstr.* **19,** 145.

Kelley M. W., Xu X. M., Wagner M. A., Warchol M. E., and Corwin J. T. (1993) The developing organ of Corti contains retinoic acid and forms supernumerary hair cells in response to exogenous retinoic acid in culture. *Development* **119,** 1041–1053.

Korsching S. (1993) The neurotrophic factor concept: a reexamination. *J. Neurosci.* **13,** 2739–2748.

Lalwani A. K., Walsh B. J., Reilly P. G., Muzyczka N., and Mhatre A. N. (1996) Development of in vivo gene therapy for hearing disorders: introduction of adeno-associated virus into the cochlea of the guinea pig. *Gene Ther.* **3,** 588–592.

Lambert P. (1994) Inner ear hair cell regeneration in a mammal: identification of a triggering factor. *Laryngoscope* **104,** 701–717.

Lanford PJ, Raz Y., Lindshell C., Weinmaster G., and Kelley M. (1997) Expression of notch and jagged correlates with development of hair cells in the organ of Corti. *Soc. Neurosci. Abstr.* **23,** 1968.

Leake P. A., Snyder R. L., Hradek G. T., and Rebscher S. J. (1992) Chronic intracochlear electrical stimulation in neonatally deafened cats: effects of intensity and stimulating electrode location. *Hear. Res.* **64,** 99–117.

Lefebvre P. P., Malgrange B., Staecker H., Moghadass M., Van de Water T. R., and Moonen G. (1994) Neurotrophins affect survival and neuritogenesis by adult injured auditory neurons in vitro. *NeuroReport* **5,** 865–868.

Lefebvre P. P., Malgrange B., Staecker H., Moonen G., and Van de Water T. R. (1993) Retinoic acid stimulates regeneration of mammalian auditory hair cells [see comments]. *Science* **260,** 692–695.

Lewis A., Frantz G., Radtke F., and Gao W.-Q. (1997) Expression patterns of notch family receptors and ligands during development of the mouse inner ear. *Soc. Neurosci. Abstr.* **23,** 1968.

Lewis J. (1991) Rules for the production of sensory cells. *Ciba Foundation Symposium* **160,** 25–39; 40–53.

Low W., Dazert S., Baird A., and Ryan A. F. (1996) Basic fibroblast growth factor (FGF-2) protects rat cochlear hair cells in organotypical culture from aminoglycoside injury. *J. Cell. Physiol.* **167,** 443–450.

Magal E., Kuang R., Hever G., Collins F., and Louis J.-C. (1997) GDNF protects cochlear hair cells in animal models of ototoxicity. *Assoc. Neurosci. Abstr.* **23,** 1822.

Miller J. M., Chi D. H., O'Keeffe L. J., Kruszka P., Raphael Y., and Altschuler R. A. (1997) Neurotrophins can enhance spiral ganglion cell survival after inner hair cell loss. *Int. J. Devel. Neurosci.* **15,** 631–43.

Minichiello L., Piehl F., Vazquez E., Schimmang T., Hokfelt T., Represa J., and Klein R. (1995) Differential effects of combined trk receptor mutations on dorsal root ganglion and inner ear sensory neurons. *Development* **121,** 4067–4075.

Oesterle E. C., Tsue T. T., and Rubel E. W. (1997) Induction of cell proliferation in avian inner ear sensory epithelia by insulin-like growth factor-I and insulin. *J. Compar. Neurol.* **380,** 262–274.

Oh S. H., Johnson R., and Wu D. K. (1996) Differential expression of bone morphogenetic proteins in the developing vestibular and auditory sensory organs. *J. Neurosci.* **16,** 6463–6475.

Pirvola U., Arumäe U., Moshnyakov M., Palgi J., Saarma M., and Ylikoski J. (1994) Coordinated expression and function of neurotrophins and their receptors in the rat inner ear during target innervation. *Hear. Res.* **75,** 131–144.

Pirvola U., Ylikoski J., Palgi J., Lehtonen E., Arumäe U., and Saarma M. (1992) Brain-derived neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of developing inner

ear ganglia. *Proc. Natl. Acad. Sci. USA* **89,** 9915–9919.

Raphael Y. (1992) Evidence for supporting cell mitosis in response to acoustic trauma in the avian inner ear. *J. Neurocytol.* **21,** 663–671.

Raphael Y., Frisancho J. C., and Roessler B. J. (1996) Adenoviral-mediated gene transfer into guinea pig cochlear cells in vivo. *Neurosci. Lett.* **207,** 137–141.

Rastel D., Abdouh A., Dahl D., and Romand R. (1993) An original organotypic culture method to study the organ of Corti of the newborn rat in vitro. *J. Neurosci. Methods* **47,** 123–131.

Richardson G. P. and Russell, I. J. (1991) Cochlear cultures as a model system for studying aminoglycoside induced ototoxicity. *Hear. Res.* **53,** 293–311.

Ruben R. J. (1967) Development of the inner ear of the mouse: a radioautographic study of terminal mitosis. *Acta Otolarynhol. Suppl.* **220,** 1–44.

Ryals B. M. and Rubel E. W. (1988) Hair cell regeneration after acoustic trauma in adult Coturnix quail. *Science* **240,** 1774–1776.

Ryan A. (1997) Transcription factors and the control of inner ear development. *Sem. Cell Devl. Biol.* **8,** 249–256.

Sans A. and Chat M. (1982) Analysis of temporal and spatial patterns of rat vestibular hair cell differentiation by tritiated thymidine radioautography. *J. Compar. Neurol.* **206,** 1–8.

Schecterson L. C. and Bothwell M. (1994) Neurotrophin and neurotrophin receptor mRNA expression in developing inner ear. *Hear. Res.* **73,** 92–100.

Schindler R. A., Gladstone H. B., Scott N., Hradek G. T., Williams H., and Shah S. B. (1995) Enhanced preservation of the auditory nerve following cochlear perfusion with nerve growth factors. *Am. J. Otol.* **16,** 304–309.

Shoji F., Yamasoba T., Miller J., Altschuler R., Schacht J., Louis J.-C., and Magal E. (1997) The prevention of noise-induced deafness with growth factors: GDNF, BDNF, NT-3. *Soc. Neurosci. Abstr.* **27,** 619.

Snider W. D. (1994) Functions of the neurotrophins during nervous system development: What the knockouts are teaching us. *Cell* **77,** 627–638.

Sobkowicz H. M., Bereman B., and Rose J. E. (1975) Organotypic development of the organ of Corti in culture. *J. Neurocytol.* **4,** 543–572.

Song H. J., Ming G. L., and Poo M. M. (1997) cAMP-induced switching in turning direction of nerve growth cones. *Nature* **388,** 275–279.

Staecker H., Kopke R., Malgrange B., Lefebvre P., and Van de Water T. R. (1996) NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells. *NeuroReport* **7,** 889–894.

Stone J. S. and Cotanche D. A. (1994) Identification of the timing of S phase and the patterns of cell proliferation during hair cell regeneration in the chick cochlea. *J. Compar. Neurol.* **341,** 50–67.

Stone J. S. Leano S. G., Baker L. P., and Rubel E. W. (1996) Hair cell differentiation in chick cochlear epithelium after aminoglycoside toxicity: in vivo and in vitro observations. *J. Neurosci.* **16,** 6157–6174.

Verdi J. M., Ip N., Yancopoulos G. D., and Anderson D. J. (1994) Expression of trk in MAH cells lacking the p75 low-affinity nerve growth factor receptor is sufficient to permit nerve growth factor-induced differentiation to postmitotic neurons. *Proc. Natl. Acad. Sci. USA* **91,** 3949–3953.

Victoria L. V., Hansen M. R., Wu X., Abbas P. J., Gantz B. J., and Green S. H. (1997) Electrical stimulation increases neurotrophin expression in rat spiral ganglion neurons in vivo. *Assoc. Otolaryngol. Res. Abstr.* **20,** 55.

Walsh M. E. and Webster D. B. (1994) Exogenous GM1 ganglioside effects on conductive and sensorineural hearing losses. *Hear. Res.* **75,** 54–60.

Warchol M. E. and Corwin J. T. (1996) Regenerative proliferation in organ cultures of the avian cochlea: identification of the initial progenitors and determination of the latency of the proliferative response. *J. Neurosci.* **16,** 5466–5477.

Warchol M. E., Lambert P. R., Goldstein B. J., Forge A., and Corwin J. T. (1993) Regenerative proliferation in inner ear sensory epithelia from adult guinea pigs and humans. *Science* **259,** 1619–1622.

Weisleder P. and Rubel E. W. (1992) Hair cell regeneration in the avian vestibular epithelium. *Exper. Neurol.* **115,** 2–6.

Wheeler E. F., Bothwell M., Schecterson L. C., and Von Bartheld C. S. (1994) Expression of BDNF and NT-3 mRNA in hair cells of the organ of corti: quantitative analysis in developing rats. *Hear. Res.* **73,** 46–56.

Whitfield T., Haddon C., and Lewis J. (1997) Intercellular signals and cell-fate choices in the developing inner ear: origins of global and of fine-grained pattern. *Sem. Cell Dev. Biol.* **8,** 239–247.

Wu D. K. and Oh S. H. (1996) Sensory organ generation in the chick inner ear. *J. Neurosci.* **16,** 6454–6462.

Yamane H., Nakagawa T., Iguchi H., Shibata S., Takayama M., Nishimura K., and Nakai Y. (1995) In vivo regeneration of vestibular hair cells of guinea pig. *Acta Otolaryngol. (Stockh) Suppl.* **520,** 174–177.

Yamashita H. and Oesterle E. C. (1995) Induction of cell proliferation in mammalian inner-ear sensory epithelia by transforming growth factor alpha and epidermal growth factor. *Proc. Natl. Acad. Sci. USA* **92,** 3152–3155.

Ylikoski J., Pirvola U., Moshnyakov M., Palgi J., Arumae U., and Saarma M. (1993) Expression patterns of neurotrophin and their receptor mRNAs in the rat inner ear. *Hear. Res.* **65,** 69–78.

Zheng J. L. and Gao W.-Q. (1996) Differential damage to auditory neurons and hair cells by ototoxins and neuroprotection by specific neurotrophins in rat cochlear organotypic cultures. *Eur. J. Neurosci.* **8,** 1897–1905.

Zheng J. L. and Gao W.-Q. (1997) Analysis of rat vestibular rat hair cell development and regeneration using calretinin as an early marker. *J. Neurosci.* **17,** 8270–8282.

Zheng J. L., Helbig C., and Gao W.-Q. (1997a) Induction of cell proliferation by fibroblast and insulin-like growth factors in pure rat inner ear epithelial cell cultures. *J. Neurosci.* **17,** 216–226.

Zheng J. L., Lewis A., and Gao W.-Q. (1997b) Establishment of conditionally immortalized rat utricular epithelial cell lines using a retrovirus-mediated gene transfer technique. *Hear. Res.*, **117,** 13–23.

Zheng J. L., Stewart R. R., and Gao W.-Q. (1995) Neurotrophin-4/5 enhances survival of cultured spiral ganglion neurons and protects them from cisplatin neurotoxicity. *J. Neurosci.* **15,** 5079–5087.