Molecular genetic aspects of hereditary hearing impairment

Molekularne aspekty dziedzicznie uwarunkowanych niedosłuchów

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Privatdozent **Dr med. Markus Pfister** – pracownik Kliniki Otolaryngologii Uniwersytetu w Tybindze w Niemczech. Kierownik zespołu Biologii Molekularnej w *Tuebingen Research Hearing Centre*. W swoich pracach koncentruje się na klinicznych i eksperymentalnych aspektach patofizjologii upośledzenia słuchu; identyfikacji podłoża genetycznego dziedzicznie uwarunkowanych niedosłuchów, analizach funkcjonalnych mutacji, wprowadzaniu nowoczesnych metod regeneracji komórek rzęsatych opartych na terapiach genowych, wykorzystaniu komórek macierzystych. Współpracuje z wieloma ośrodkami w USA, Belgii, Turcji, Węgrzech i Polsce. Przebywał na licznych stypendiach, m.in. na uniwersytetach Harvarda, Stanford, w Michigan, San Francisco, Antwerpii. Autor oraz współautor wielu publikacji poświęconych zagadnieniom biologii molekularnej upośledzenia słuchu, jak i chirurgii z zakresu głowy i szyi.

Dr n. med. Maciej Wróbel jest pracownikiem Kliniki Otolaryngologii i Onkologii Laryngologicznej Akademii Medycznej w Poznaniu. Po ukończeniu studiów na Wydziale Lekarskim Akademii Medycznej w Poznaniu w 1999 r. rozpoczął studia doktoranckie, zakończone obroną pracy doktorskiej na temat genetycznie uwarunkowanych niedosłuchów. W ramach współpracy z Instytutem Genetyki Człowieka PAN w Poznaniu oraz innymi ośrodkami polskimi i zagranicznymi prowadzi badania związane z genetycznymi uwarunkowaniami rodzinnie występujących zaburzeń słuchu, zagadnieniami ototoksyczności oraz rolą komórek macierzystych w patologii ucha wewnętrznego. Dwukrotnie przebywał na stażach naukowych w ramach stypendium Niemieckiej Fundacji Nauki (DAAD) oraz Unii Europejskiej.

A b s t r a c t

Hereditary hearing impairment (HI) is a clinically and genetically heterogeneous entity, which assumedly present at least one third of all hearing impairment in humans. The incidence of severe to profound hearing loss in newborns is 1-2 in 1000 live births. At least 50% of these cases can be attributed to genetic causes. Depending on the clinical appearance, syndromic and non-syndromic hereditary hearing impairment can be distinguished. Up to 33% of all hereditary hearing impairment is syndromic. This entity includes 1168 syndromes with otologic manifestations. The remaining 67% represents non-syndromic hearing impairment with no additional clinical features besides HI. Non-syndromic HI is further classified by the mode of inheritance in autosomal-dominant (18%), autosomal-recessive (80%), X-linked (1-2%) and mitochondrial (<1%) hearing impairment.

Identification of the molecular background of hearing loss is a first step to understand mechanisms which form the knowledge basis for a causal treatment of the loss of hearing based on new technologies such as gene therapy or cell regeneration

The authors present a brief overview on current status on gene identification, the diagnostic considerations when evaluating a patient who might have a hereditary hearing impairment as well as briefly describe treatment options based on gene therapy.

Key words: *hereditary hearing loss, deafness, syndromic hearing loss, non-syndromic hearing loss, gene therapy, stem cells.*

Streszczenie

Dziedzicznie uwarunkowane zaburzenia słuchu są klinicznie i genetycznie bardzo heterogenną grupą, do której zalicza się ok. 1/3 wszystkich przypadków upośledzenia słuchu u ludzi. Dane epidemiologiczne wskazują, że 1–2:1000 dzieci jest głuchych od urodzenia lub głuchnie w pierwszym okresie życia, aż połowa z nich może być zaliczona do grupy z genetycznym uwarunkowaniem wady.

Na podstawie obrazu klinicznego, towarzyszących dodatkowych patologii lub ich braku, u osoby niesłyszącej możemy rozróżnić niedosłuch izolowany (ok. 67%) lub występujący w zespołach wad (ok. 33%). Dalej, w odniesieniu do niedosłuchów izolowanych, podział dotyczy sposobu dziedziczenia wady – 18% dziedziczonych jako cecha autosomalnie dominująca, 80% – autosomalnie recesywna, 1–2% sprzężona z chromosomem X. Pozostały ułamek procenta to niedosłuchy dziedziczone w linii matczynej związane z mitochondrialnym DNA.

W miarę ulepszania metod diagnostyki molekularnej oraz stopniowego wprowadzania testów skryningowych do praktyki klinicznej, w coraz większym odsetku identyfikuje się defekt genetyczny leżący u podłoża wady. Przełożenie zdobytych informacji w zakresie badań podstawowych na codzienną praktykę kliniczną nabiera jeszcze większego znaczenia, a identyfikacja podłoża genetycznego upośledzenia słuchu to podstawowy krok do zrozumienia reguł odpowiedzialnych za proces słyszenia oraz podstawowy element do opracowywania nowych metod leczenia opartych na terapiach genowych czy regeneracji komórek.

Autorzy przedstawiają krótki przegląd dotyczący aktualnej wiedzy na temat genetycznego podłoża upośledzenia słuchu, zagadnień diagnostyki molekularnej głuchoty oraz opcji leczenia opartych na terapii genowej.

Słowa kluczowe: *dziedzicznie uwarunkowany niedosłuch, głuchota wrodzona, terapia genowa, regeneracja.*

(Postępy w chirurgii głowy i szyi 2005; 2: 13–20)

Introduction

Around 80% of those affected by hearing loss suffer from this sensory deficit of the inner ear. Causal treatment options for this are not available in current clinical practice. Loss of hearing is often caused by the irreversible loss of sensory cells located in the inner ear. Sensory cells convert physical acoustic signals into electrical and chemical signals which are transferred to the central nervous system. A loss of sensory cells results in an irreversible loss of hearing, also known as sensorineural hearing loss or perception hearing loss. This form of deafness can currently only be alleviated by providing prosthetic hearing aids. This solution is, however, often unsatisfactory for those concerned due to a lack of speech discrimination and means that hearing aids are actually only used by a relatively small proportion of those with a hearing impairment. Furthermore, there is a negative attitude to hearing devices amongst those concerned due to social stigmatism and cosmetic considerations. There are additional limitations for specific jobs.

Due to a lack of knowledge about the molecular basis of hearing loss, our present diagnostic and therapeutic tools are limited and make hearing impairment one of the most poorly treatable human health problems. With already a fifth of the total population affected and an expected increase as a result of current demographic and lifestyle changes, the severe impact on social and professional life will make hearing loss the major health concern of the communication age. This will particularly affect the social integration of the aging population in the future. Causal treatment options can only be expected by improving the scientific basis at the molecular level. Understanding these mechanisms forms, subsequently, the knowledge basis for a causal treatment of hearing loss based on gene therapy.

Epidemiology

Hearing loss is the most frequently occurring sensory deficiency and the second most common chronic illness in humans after arthritis (National Institute of Health Statistics, 1994). According to epidemiological studies by the British MRC Institute of Hearing Research, the total number of persons with hearing loss of at least 25 dB in 2005 is over 560 million worldwide. Around 190 million hearing-impaired people are reckoned to live in the industrialised countries: 80 million in Europe and over 30 million in the USA and Canada [1]. These figures will continue to rise. It is estimated that in 2015 there will be over 700 million people with a significant

loss of hearing worldwide [1]. Exposure to noise in the workplace together with the noise trauma associated with stationary recreational activities will lead to hearing damage amongst the younger population [2, 3]. According to statistical studies in Sweden, more than 50% of people with a hearing impairment today are under 65 and thus of working age (Hearing Research Foundation, 1999). The imminent demographic crisis of an aging population in industrial societies will aggravate this problem since more than a third of the population in these countries is over 65 [4](The National Institute of Health Statistics, 1994).

Tab. 1. Syndromic hearing impairment

Syndrome	additional symptoms	mode of inheritance	localisation	gene	OMIM
Alport	Nephropathy	X-chromosomal	Xq22.3	*COL4A5*	301050
		autosomal recessive	2q36-37	*COL4A3/COL4A4*	203780
BOR	Renal anomaly, ear malformations, cervical fistulas	autosomal dominant	8q13.3 1q31	*EYA1* unknown	113560 –
LQT	Cardiac arrhythmia	autosomal dominant (Romano-Ward -Syndrome) and	11p15.5 7q35-36 3p21-24	*KCNQ1* (LQT1) *HERG* (LQT2) *SCN5A* (LQT3)	192500 152427 603830
		recessive (Jervell-Lange-Nielson -Syndrome	4q25- 27 21q22.1 21q22.1	unknown (LQT4) *KCNE1* (LQT5) *KCNE2* (LQT6)	600919 176261 603796
Norrie	Ocular symptoms mental disturbance	X-chromosomal	Xp11.4	*NPD* (Norrin)	310600
Pendred	Diffuse thyroid enlargement (goiter), developmental abnormalities of the cochlea	autosomal recessive	7q31	*SLC26A4* (Pendrin)	274600
Stickler	Vitreoretinal degeneration, premature joint degeneration with abnormal epiphyseal development,	autosomal dominant	12q13.11-13.2	*COL2A1* (STL1)	108300
	midface hypoplasia, irregularities of the vertebral bodies,		1p12	*COL11A1* (STL2)	604841
	cleft palate deformity		6p21.3	*COL11A2* (STL3)	184840
Treacher Collins	Coloboma of the lower eyelid, micrognathia, microtia, hypoplasia of the zygomatic arches, macrostomia, inferior displacement of the lateral canthi with respect to the medial canthi	autosomal dominant	5q32-q33.1	*TCOF1*	154500
Usher	Retinitis pigmentosa	autosomal recessive	14q32 11q13.5 11p15.1 10q 21q 10q21-22 1q41 3p23-24.2 5q14.3-21.3 3q21-25	unknown (USH1A) *MYO7A* (USH1B) *USH1C* (USH1C) *CDH23* (USH1D) unknown (USH1E) *PCDH15* (USH1F) *USH2A* (USH2A) unknown (USH2B) unkown (USH2C) *USH3* (USH3)	276900 276903 276904 601067 602097 602083 276901 276905 605472 276902
Waardenburg	Dystopia canthorum, pigmentary abnormalities	dominant and recessive	2q35 3p14.1-12.3 2q35 13q22 20q13.2-13.3 22q13	*PAX 3* (WS1) *MITF* (WS2) *PAX 3* (WS3) *EDNRB* (WS4) *EDN3* (WS4) *SOX10* (WS4)	193500 193510 148820 277850 277580 277580

Molecular genetics of hereditary hearing impairment

Hereditary hearing impairment is a clinically and genetically heterogeneous entity and it is thought that at least one third of all hearing impairment in humans has a genetic basis [5]. About 1 to 2 in 1000 newborn children are estimated to have a prelingual, severe to profound hereditary hearing impairment [6].

Clinically, syndromic and non-syndromic hereditary hearing impairment (HI) can be differentiated. Up to 33% of all hereditary hearing impairment is syndromic. This entity includes 1168 syndromes with otologic manifestations (Online London Dysmorphology Database). The remaining 67% represents non-syndromic hearing impairment with no additional clinical features besides hearing impairment. Non-syndromic HI is further classified according to the mode of inheritance in autosomal-dominant (DFNA) (18%), autosomal-recessive (DFNB) (80%), X-linked (DFN) (1-2%) and mitochondrial (<1%) hearing impairment [7].

Syndromic hearing impairment

Syndromic hearing impairment is associated with other clinical symptoms e.g. blindness, cardiac arrhythmia or pigment abnormalities. These syndromes are based on mutations in genes that commonly function in other tissues as well as the cochlea [8]. More than 100 genes have been identified since 1990, showing a large heterogeneity even in the same type of syndromic hearing impairment, e.g. Usher-Syndrome type I a-f with 6 different genetic loci [7]. However, the molecular genetic basis of most syndromes is still unknown. In view of the large number of syndromes it is impossible to embrace them all in the review. The clinically most important and common ones are listed in table 1.

Non-syndromic hearing impairment

The first locus was detected by Wallis and co-workers in 1988 resulting in a rapid rise of genetic HI research [9]. In non-syndromic hearing impairment, until now, 54 autosomal dominant (DFNA), 60 autosomal recessive (DFNB), 8 X-linked loci (DFN) and 2 mitochondrial loci

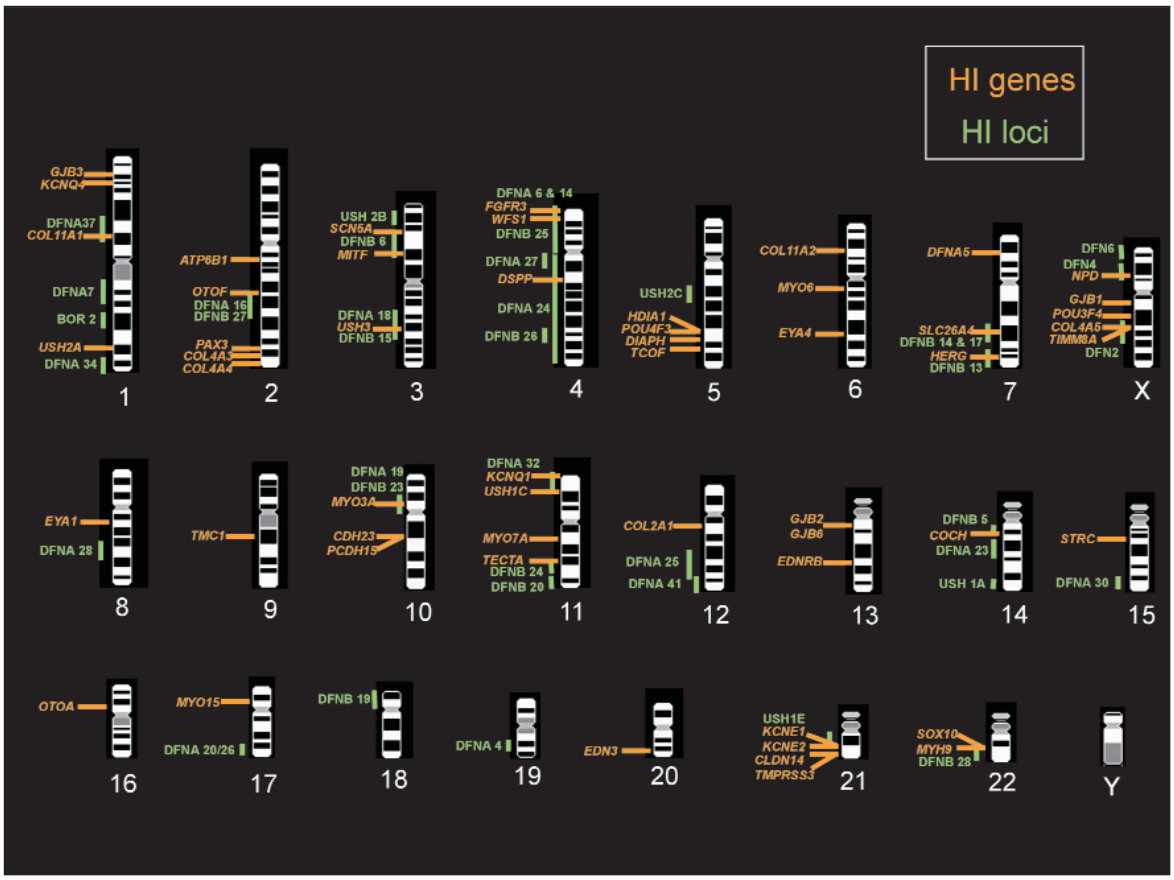

Fig. 1. Localisation of genes and loci involved in Hereditary Hearing Loss

have been mapped on the human genome. In total, 37 non-syndromic genes have been identified since 1994, and more than 150 functionally-important genes of the inner ear are predicted to be identified [7].

The genes associated with the development of non-syndromic hearing loss exclusively affect the mechanism of hearing, play a critical role in the function of the cochlea, displaying new insights into the human auditory system. That is why identified functional important genes [10] of the inner ear can be categorized according to their presumed function as:

- Genes encoding for structural proteins: *DIAPH, TECTA, COCH, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, NDP, USH2A, OTOG, OTOA, DSPP, USH1C, DSPP*
- Stereociliary/mechanoelectrical transduction channel genes: *MYO7A, MYO15, MYH9, MYO6, MYO3A, STRC*
- Genes involved in ion transport: *GJA1, GJB1, GJB2, GJB3, GJB6, KCNQ4, KCNQ1, HERG, SCN5A, KCNE1, KCNE2, CLDN14, SLC26A4* and *ATP6B1*
- Genes encoding for transcription factors and developmental regulation: *POU3F4, POU4F3, EYA1, EYA4, PAX3, MITF, SOX10, EDNRB, EDN3, FGFR3* and *TFCP2L3*
- Unknown function: *CDH23, PCDH15, DFNA5, OTOF, TCOF, TIMM8A, TMC1, TMPRSS3, USH3A, WFS1* and *TMIE*
- Genes encoded by the mitochondrial genome: *tRNALeu, tRNALys, tRNASer, tRNAGlu* and *12SrRNA*
- Hearing impairment genes in cooperation with modifier genes: *DFNB26* and *DFNM1*

Phenotype – genotype correlations

The clinical classification of non-syndromic hearing impairment takes the following criteria into account [11, 12]: severity of hearing impairment, age of onset, type of HI, frequencies involved, unilateral/bilateral, stable/progressive, syndromic/non-syndromic.

Recessive inherited non-syndromic hearing impairment (DFNB)

In contrast to the syndromic hearing impairment, which is mostly inherited in an autosomal dominant mode, the non-syndromic one has an autosomal recessive transmission in 70 to 80% of cases. The phenotype of these patients is prelingual in onset, severe to profound in severity, stable and affects all frequencies. Therefore highly affected children usually do not acquire speech and have to use sign language for communication. The detection of the underlying gene mutations is complicated by extreme genetic heterogeneity, intermarriages of hearing impaired persons and the impossibility to associate different gene defects to different forms of hearing impairment

Tab. 2. Clinical classification of hearing impairment

Criteria	Category
syndromic/ non-syndromic	– syndromic: associated with other clinical anomalies – non–syndromic: isolated hearing impairment
grade of hearing impairment	– normal: <20 dB – mild: 21-40 dB – moderate: 41-70 dB – severe: 71-95 dB – profound: >95 dB
age of onset	– prelingual – postlingual
type	– conductive: outer or middle ear defect – sensorineural: cochlea and retrocochlea defect – combined
frequencies involved	– lower frequencies: 250-500 Hz – middle frequencies: 500-2000 Hz – high frequencies: 2000-8000 Hz
unilateral/bilateral	– right/left ear – symmetric/asymmetric: >10 dB difference between both ears for at least two different frequency ranges
stable/progressive	– progressive – stable

Dominant inherited non-syndromic hearing impairment (DFNA)

Ten to fifteen percent of non-syndromic cases are inherited in a dominant mode. In contrast to recessive ones, patients with the dominant mode show a milder form of disease, which is usually less severe, delayed in onset, and progressive. The mapping of involved genes is facilitated in comparison to recessive forms, because normally no silent carrier is found in a pedigree. Therefore the main strategy for the identification of dominant inherited non-syndromic hearing loss associated genes is the traditional linkage analysis. Thereby the major problem is to evaluate large-sized, suitable families.

In general, recessive hearing impairment tends to be more severe than dominant HI. This might be due to the fact that in recessive cases both alleles harbor the disease causing mutation in comparison to only one allele in dominant HI. Recessive hearing impairment tends to be stable whereas dominant hearing impairment is usually progressive. So far, only families with mutations in *TECTA* show a non-progressive type of autosomal dominant hearing impairment [13]. Moreover, recessive

Tab. 3. Differences between autosomal dominant and recessive inherited hearing impairment

Classification of HI	autosomal recessive	autosomal dominant
distribution of both types	70-80%	15-20%
grade of HI	severe to profound	mild to moderate
age of onset	congenital in most cases	variable
type	sensorineural	sensorineural
frequencies involved	all	variable, high frequencies in most cases
localisation	bilateral	bilateral
progression	stable	variable, progressive

HI is characterized predominantly by a congenital or prelingual onset as opposed to dominant HI which usually has a postlingual onset [14].

X-linked hearing impairment (DFN)

In X-linked hearing impairment, sex difference in hearing loss can be detected, because affected males are homozygous and affected females are heterozygous for the disease causing allele [15]. Thus, affected females show a less severe phenotype in comparison to affected males, who carry only the disease causing allele (hemizygosity). X-linked hearing impairment (DFN3) may present additional characteristic features [16]. Frequent findings may include a bulbous internal auditory canal and a cochlear hypoplasia.

Consequently, the further development of a phenotype database will allow the collection of epidemiological data for genetic studies and phenotype/genotype correlation. The achievement of this data is the basic and most important tool in the identification and characterization of new genetic risk factors, e.g., hearing loss genes and their loci [14].

Molecular diagnostics

All identified hearing loss loci can be analyzed and risk calculations can be performed in families with hereditary hearing impairment in which significant cosegregation to a hearing loss locus was established. A major limitation of this analysis is often the size of a family. For this reason, a reference center should be contacted before such an analysis is intended.

Mutation analysis is in principle possible for all identified functionally important genes of the inner ear. However, in daily practice, only mutation analysis of Connexin 26 is currently suitable due to the epidemiological importance of this gene as well the established low cost screening techniques (e.g. SSCP, restriction test, sequencing). DFNB1, the hearing loss locus for Connexin 26 as hearing loss gene, accounts for 50 to 80% of all autosomal recessive hearing impairment. Up to 70% of these cases are due to one single mutation, a deletion of a single guanine nucleotide in position 30 to 35, called 35delG [17]. In addition, up to 10% of sporadic cases of severe to profound hearing impairment harbors the 35delG mutation in both alleles [18].

Therefore this test is the first genetic test clinically available for children affected by sporadic non syndromic hearing impairment.

Treatment options – gene therapy

The term gene therapy describes any procedure intended to treat or alleviate disease by genetically modifying the cell of a patient. A major motivation for gene therapy has been the need to develop novel treatments for diseases for which there is no effective conventional treatment.

As the essential component of classical gene therapy is that cloned genes have to be introduced and expressed in the cells of a patient in order to overcome the disease. It means that it is possible to develop new molecular-based therapeutic strategies on the sensory organ of the inner ear.

Gene transfer systems

The first successful transfection of the inner ear *in vivo* was described in 1996 [19, 20]. As transfer systems, viral vectors adeno-associated viruses or adenoviruses were used in both studies and applied through the round window.

The expression of the reporter gene could thus be detected in various cell types of the inner ear. In particular, an expression was detected in receptors, the hair cells and neurones, the spiral ganglion cells as well as in the spiral ligamentum.

The general strategies of gene therapy for the sensory system of the ear are based on:

- gene amplification for loss of hearing caused by recessive genes or correction of a mutation for loss of hearing caused by dominant genes,

- specific gene amplification in otoprotection,
- specific influence of gene expression in regeneration.

Genetic hearing loss

A number of new hearing-loss genes and illness-causing mutations have been identified in the past few years when investigating hearing loss. In many cases, the physiological and epidemiological significance of these genes in the hearing process is still unclear. However, Connexin 26, for example, was found to play a significant role in profoundly deaf patients. For this reason, an early therapeutic approach appears worthwhile for this type of hearing loss (DFNB1). This development of new strategies is, however, currently restricted by the lack of animal models for this gene. Using tests for deafness on a mouse model it was, however, successfully proven that inserting the myo15 gene in zygotes leads to normal inner ear morphology in hearing mice [21]. This strategy is thus potentially useable.

Otoprotection

Models for otoprotection are different. These include animal models with which loss of hearing is due to noise exposure or administering ototoxic substances (e.g. aminoglycoside antibiotics). Intensive neuroprotective factors with different application forms and vector systems are currently being introduced to the inner ear of animal models in order to prevent or at least reduce apoptotic cell death of sensory cells and neurones [22]. In the auditory system, GDNF, BDNF and NT-3 in particular are described as active neurotrophic factors which contribute to the development of the neuritogenesis of auditory projections and the survival of neurones [23-25]. These factors can prevent ototoxic damage in *in vivo* experiments [26-29].

In pre-clinical application studies, Staecker et al. [30] used a herpes simplex virus-1 (HSV-1) vector to deliver BDNF to the inner ear and assessed its protective effect against neomycin. The gene therapy group demonstrated significantly higher salvage rate for spiral ganglion neurons (SGNs), in contrast to loss of SGNs in control animals (without the BDNF transgene).

Neurotropin-3 (NT-3) mediated protection against cisplatin-induced ototoxicity has been documented using an HSV-1 derived viral vector [31, 32]. Chen et al. [32] established that efficacy of the vector in an in vitro study, where HSV-1-mediated transfer of NT-3 conferred increased survival to cochlear explants after cisplatin exposure. Bowers et al. [31] confirmed these effects in an *in vivo* model, where HSV-1 mediated transfer of NT-3 to SGNs suppressed cisplatin-induced apoptosis and necrosis. The authors suggest that these findings may not only be useful to prevent cisplatin-related injury, but may also provide preventative treatment for hearing degeneration due to normal aging.

Several studies have established the efficacy of an Ad vector carrying the GDNF gene (Ad.GDNF) to protect against a variety of ototoxic insults. When administered prior to aminoglycoside challenge, Ad.GDNF significantly protects cochlear [33] and vestibular hair cells from cell death [34]. Pretreatment with Ad.GDNF also provides significant protection against noise-induced trauma. Finally, Ad.GDNF enhances SGN survival when administered 4 to 7 days after ototoxic deafening with aminoglycosides [35].

Regeneration

Regeneration represents another significant therapeutic approach. Unlike in other vertebrae, in humans and other mammals, hair cells are not replaced once they have been lost. Their loss and the restriction of function connected with this are permanent and irreversible. The only causal approach to treatment is replacing lost hair cells through the biological process of hair cell regeneration.

The aim of regeneration biology for hearing is to throw light upon cellular and molecular mechanisms which permit a regeneration of hearing by creating sensory cells *de novo* in the inner ear. Unlike the situation with humans and mammals, other vertebrae, and warm-blooded birds in particular, are able to regenerate hair cells spontaneously. In these cases, hair cell regeneration involves forming new hair cells from cell division in neighbouring support cells. Traditional thinking has, up until now, rated the chances of such regeneration based on the renewal of cells in the acoustic organ of mammals as very low. Acoustic organ cells were not considered capable of entering the cell cycle and thus regeneration based on cell renewal was inconceivable. However, it was possible to overcome this biological dogma, whereby the identification and the corresponding influence of a relevant cell cycle regulator, cyclin-dependent kinase inhibitor $p27^{Kip1}$, could be used to show on a molecular level that cell division is also possible in the adult sensory acoustic organ [36]. From a therapeutic point of view, the influence of the expression of this gene could be thought of as an induction of the hair cell regeneration process.

The recent discovery of stem cells in the adult inner ear that are capable of differentiating into hair cells, as well as the finding that embryonic stem cells can be converted into hair cells, open an additional exciting possibility for the future development of a stem-cell-based regeneration of the inner ear [37]. However potential obstacles have to be overcome before these treatment options can be used in humans.

The future

The ability to hear and thus communicate has profound effects on quality of life in nearly all

professional and social areas and makes hearing loss the one of the main problems for health care in a society dependent on communication (NCOA Research, 1999). The economic costs incurred due to loss of productivity caused by untreated hearing loss are currently estimated at 75 mld € a year in Europe alone. This is expected to increase to 87 mld € for 2005. These costs could be compared to those incurred in building a motorway five times all the way around the German border (Better Hearing Institute, 1999; Maastricht Report, 1999).

New exciting research data on regeneration aspects of the inner ear based on stem cells or genes, however, will trigger the research to overcome the current obstacles and to develop at the end a molecular based therapy for this most common sensory deficit in humans. That is why the possibility of successfully introducing genes into the periphery auditory system using various application forms and viral and non-viral transfer systems (vectors) is the first significant step towards a possible molecular-genetic therapeutic strategy for diseases of the inner ear.

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