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Chapter 22

CURRENT THERAPEUTIC STRATEGIES FOR HUMAN USHER SYNDROME

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22.1. INTRODUCTION

The human Usher syndrome (USH) is the most frequent cause of combined deaf-blindness in man. It is clinically and genetically heterogeneous and at least twelve chromosomal loci are assigned to three clinical USH types. USH1, USH2 and USH3 differ in the severity of the symptoms: hearing loss, balance problems and retinal degeneration namely *retinitis pigmentosa* (RP), as well as in the progression of the disease.

Mutations in all USH genes result in similar symptoms. Nevertheless, molecular analyses of the corresponding gene products surprisingly revealed that USH proteins belong to diverse protein classes and families: myosin VIIa (USH1B) functions as a molecular motor; harmonin (USH1C), SANS (scaffold protein containing ankyrin repeats and SAM domain, USH1G) and whirlin (USH2D) are scaffold proteins; cadherin 23 (USH1D) and protocadherin 15 (USH1F) represent cell-cell adhesion proteins; USH2A isoform b (USH2A) and the largest receptor GPR98/VLGR1b (very large G-protein coupled receptor 1b, USH2C) with seven transmembrane folds and clarin-1 (USH3A) with four transmembrane domains are transmembrane proteins (Reiners *et al.* 2006, Ebermann *et al.*, 2007). Protein interaction assays demonstrated that all USH proteins are part of protein networks present in the sensory cells of the inner ear and eye. The main organizers of the networks are the three scaffolds harmonin, SANS and whirlin. Alternative splicing further increases the complexity of the USH networks by the presence of various isoforms, mainly of the scaffold harmonin, but also

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isoforms of other USH proteins, like cadherin 23 do exist (Table 22.1) (Reiners *et al.*, 2003, 2006, Lagziel *et al.*, 2009, Overlack *et al.*, 2010).

Table 22.1. Currently evaluated USH therapy strategies

Type	Gene	Protein	Function	Size / isoforms	Cell	Virus	ZFN	Read-through
1B	*MYO7A*	Myosin VIIa	Molecular motor	3.6 - 6.6 kb / yes		Lenti X [2] AAV X [3]		
1C	*USH1C*	Harmonin	Scaffold protein	1.6 - 2.7 kb / yes		AAV ∅ [4]	∅ [6] p.R31X	∅ / X [7,8] p.R31X
1D	*CDH23*	Cdh23	Cell-cell adhesion	1.5 - 10 kb / yes				∅[9]
1E	--	--	--					
1F	*PCDH15*	Pcdh15	Cell-cell adhesion	- 5.8 kb / yes				X [10] p.R3X; p.R245X; p.R643X; p.R929X
1G	*SANS*	SANS	Scaffold protein	1.4 kb / no				
1H	*USH1H*	--	--					
2A	*USH2A*	USH2A (usherin)	Matrix, cell adhesion	4.6 - 15.6 kb / yes	X [1]			
2B								
2C	*VLGR1b*	GPR98 (VLGR1b)	GPCR, cell adhesion	5. 9 - 19.3 kb / yes				
2D	*DFNB31*	whirlin	Scaffold protein	1.5 - 2.7 kb / yes		AAV X [5]		
3A	*CLRN-1*	Clarin-1	Cell adhesion	0.3 - 0.7 kb / yes				
3B	--	--	--					

X: published; ∅: currently in progress; [1]Lu et al., 2010; [2]Hashimoto et al., 2007; [3]Allocca et al., 2008; [4]currently evaluated in our lab; [5]Zou et al 2010; [6]currently evaluated in our lab; [7]Goldmann et al. 2010; [8]Goldmann et al. submitted; [9]Ben-Yosef, personal communication; [10]Rebibo-Sabbah et al., 2007.
Cell: cell replacement; Lenti: lentivirus; AAV: recombinant adeno-associated virus; ZFN: zinc finger nuclease; read-through: translational read-through.

Additional reports indicate that USH also affects tissues and organs other than the ear and eye, which is explainable by the rather wide expression profiles of all USH proteins, for example in the nasal epithelium, brain, trachea, and sperm cells (Arden and Fox 1979, Hunter *et al.*, 1986, Petrozza *et al.*, 1991, Barrong *et al.*, 1992, Baris *et al.*, 1994, van Aarem *et al.*, 1999, Tosi *et al.*, 2003). Nonetheless, USH patients suffer mostly from the loss of the most important senses namely the ear and eye required for human social contacts and the quality of life.

The currently evaluated USH treatment strategies can be divided into two major sections:
1. Non-USH specific approaches, which include cell replacement, neuroprotective factors as well as cochlear and retina implants.

2. Contemporary gene-based therapeutic approaches, specific for each USH subtype. The identification of USH causing genes allows the development of gene specific therapies, including gene augmentation by non-viral or viral vectors, gene repair induced by homologous recombination mediated by zinc finger nucleases (ZFNs) and translational read-through therapy.

Animal models that accurately reflect the disease present in patients are imperative for the evaluation of therapeutic strategies (Smith *et al.*, 2009). Up to now, most USH animal models recapitulate the human deafness phenotype (Williams 2008), making these mice useful models for the evaluation of therapeutic protocols for the inner ear. In contrast, USH mice typically do not suffer from retinal degeneration as seen in human patients. Without representative animal models the translation of treatment strategies to clinical trials will be difficult. Of all the USH mice generated to date, only the *Ush2a*-null mouse (Liu *et al.*, 2007, Lu *et al.*, 2010), whirlin null mouse (Yang *et al.* 2010) and *Ush1c* knock-in mouse (Lentz *et al.*, 2007, Lentz *et al.*, 2010) showed a mild retinal phenotype. The *Ush1c* knock-in mouse has been created by introducing the human *Ush1c* gene containing a specific mutation, into the mouse genome. Thus, generating new mouse models with human mutations might overcome the problem of the lack of retinal phenotype in mice. However, animal models of larger mammals, particularly pigs, show probably the same or at least a more similar phenotype to human than rodent models. Such models may be a necessary option for the evaluation of therapeutic strategies.

In this chapter we want to summarize the different therapeutic approaches and possibilities for the auditory and ophthalmic phenotype of USH patients. We will explain the therapy options in more detail and provide insights into the state of the art. However, one has to keep in mind that the hearing impairment of USH is caused by developmental defects occurring during embryogenesis *in utero*. Thus with exception of cochlear implants and functional replacement strategies, all inner ear treatments discussed below, need prenatal treatment which bear high risk of miscarriage. So far cochlear implants and hearing aids are the only feasible methods for the alleviation and treatment of the auditory component of USH.

22.2. NON-USH SPECIFIC TREATMENT OPTIONS FOR THE EAR AND THE EYE

The non-USH specific treatment options for the ear and eye include the functional replacement of the damaged sensory cells with either *trans*-differentiated cells or stem cells. In the retina, the application of neurotrophic factors could prolong the vision of USH patients. Furthermore, cochlear implants and to some extent visual implants are useful for the improvement of hearing and vision in USH patients. In addition, some ophthalmologists believe in a nutritional therapy. This is based on a clinical study indicating that in some RP and USH2 patients high dose of vitamin A supplement can slow, but not halt, retinal degeneration (Berson *et al.*, 1993). Since in our opinion the data of this study are not convincing the nutritional therapy and its modifications will not be discussed in the present review.

22.2.1. Functional Replacement of the Degenerated Sensory Cells in the Inner Ear and Retina

At the time USH is typically diagnosed, a large fraction of sensory cells are either maldeveloped as is the case of inner ear hair cells or degenerated as is the case for photoreceptor cells in the retina. At this stage, functional replacement of sensory cells might be the most practical solution. Such therapies include the transplantation of stem cells or genetically modified *trans*-differentiated cells. In both cases non-sensory cells will differentiate into respective sensory cells of the cochlea or retina.

In non-mammalian vertebrates (as for example fish) injured hair cells of the cochlea and photoreceptor cells of the retina can be regenerated, leading to the near complete restoration of hearing and vision (Kesser and Lalwani 2009, Karl and Reh 2010). In humans, there seems to be no recovery of lost sensory cells in the cochlea and retina possible. To circumvent this lack of recovery in the field of regenerative medicine two strategies are currently followed namely stem cell transplantation and the *trans*-differentiation or reprogramming of existing differentiated cells.

Identification of genes that induce sensory cell differentiation is instrumental for inducing *trans*-differentiation. In the inner ear, a promising candidate is the basic helix loop helix transcription factor *Atoh1* (formally called *Math1*). Data gathered in guinea pigs showed that induction of *Atoh1* expression offers the possibility to *trans*-differentiate supporting cells into new hair cells in the organ of Corti and thereby improve auditory thresholds (Izumikawa *et al.*, 2005, Batts and Raphael 2007).

In the retina, transplantation of photoreceptor precursor cells from a stage corresponding to the onset of expression of the transcription factor *Nrl*, which is involved in photoreceptor differentiation, are being studied (MacLaren *et al.*, 2006). However, the availability of material for transplantation is a major limitation. Alternatively, cells isolated from bone marrow, the ciliary body and embryonic tissues have the potential to develop into photoreceptor cells and can be implanted (Stone 2009). Additional approaches demonstrate that four transcription factor genes from mouse (*Oct3/4, Sox2, c-Myc, Klf-4*, Takahashi and Yamanaka 2006) and human (*OCT4, SOX2, NANOG, LIN28*, Yu *et al.*, 2007) tissues can *trans*-differentiate cells of adult tissues into induced pleuripotent stem cells (iPSC) by transfection. These cells might be useful for the creation of patient specific stem cells (Stone 2009).

Cells for the applications described above with a perfect immunologic match could be achieved by deriving the cells from the USH patients themselves. These cells would still contain the genetic defect responsible for USH. Consequently, the non-mutated USH gene has to be applied in parallel of the cell generation for transplantation otherwise neither hair cells nor photoreceptor cells will acquire functions. Taking cells from healthy donors on the other hand would provide the correct gene but will require long term immune suppression to prevent host versus graft reactions.

First results on transplantation of stem cells for USH were recently published (Lu *et al.*, 2010). In their treatment approach forebrain-derived progenitor cells were transplanted into the *Ush2a*-null mouse line (Liu *et al.* 2007). Mice that received transplanted cells performed significantly better in tests assessing visual acuity and contrast sensitivity, compared to control mice. In addition, the mislocalization of red/green cone opsin, observed in untreated *Ush2a*-null mice was reversed in treated mice.

In conclusion, the current results of cell replacement strategies did not come up to high expectations of neither patients nor researchers. All strategies must be scrutinized in more detail until they are applicable in the clinic.

22.2.2. Neurotrophic Factors

USH3 and some USH2 patients have some remaining functional hair cells in the cochlea, which could be protected by neurotrophic (~ survival) factors. So far little is known about the ability of neurotrophic factors to enhance hair cell survival. Over expression of glial cell derived neurotrophic factor (GDNF) in the inner ear can protect hair cells against degeneration induced by aminoglycoside ototoxicity (Kawamoto *et al.*, 2003). Several data indicate that the application of apoptosis inhibitors (Atar and Avraham 2010) or the over expression of anti-apoptotic proteins may protect hair cells of the inner ear from degeneration (Staecker *et al.*, 2007).

In contrast to the inner ear, vision loss in USH patients is not caused by developmental defects but by degeneration of photoreceptor cells. At the time of diagnosis there are still functional photoreceptors remaining in the retina. Sustained release of neurotrophic factors could prolong the survival of sensory cells of the eye, by preventing or slowing down the degenerative processes and thereby extend the visual capacity of USH patients (Stieger *et al.*, 2007, Leveillard and Sahel 2010). The proof of concept that neurotrophic factors can slow down retinal degeneration was shown by several laboratories (Faktorovich *et al.*, 1990, La Vail *et al.*, 1992, Bok *et al.*, 2003, LaVail 2005, Stieger *et al.*, 2007, Leveillard and Sahel 2010). To date several neurotrophic factors have been analyzed for their ability to induce cell survival enhancement in the retina, for example ciliary neurotrophic factor (CNTF), rod-derived cone viability factor (RdCVF) or the fibroblast growth factor (FGF) (La Vail 2005, Sahel 2005, Leveillard and Sahel 2010). Adeno-associated virus-mediated transfer of the gene encoding the neurotrophic factor into any retinal cell type is suitable. Subsequently, the retinal cells themselves serve as factories for the production of secreted survival factors (Auricchio 2003, Sahel 2005, Leveillard and Sahel 2010). Alternatively neurotrophic factors were introduced by transgenic human cells, enclosed in semi-permeable capsules (Sieving *et al.*, 2006). A completed Phase I trial revealed that the delivery of CNTF using encapsulated cells is safe for the human retina (Sieving *et al.*, 2006). Currently, a Phase II/III study to evaluate the effectiveness of CNTF implants on vision in persons with *retinitis pigmentosa*, including patients suffering from USH2 and USH3 is being carried out (www.clinicaltrials.gov). Improvement of vision in USH patients of this study would be an important step forward for the treatment of USH.

22.2.3. Application of Implants: Cochlear Implants, Hearing Aids, Retinal Implants

In the following part we discuss the application of implants into the ear and eye as well as hearing aids. Implants for the ear or the retina electrically stimulate surviving neuronal cells in the affected organ and subsequently evoke a pseudo stimulus, which is transmitted to the respective auditory or visual processing centers in the brain (Figure 22.1A-C).

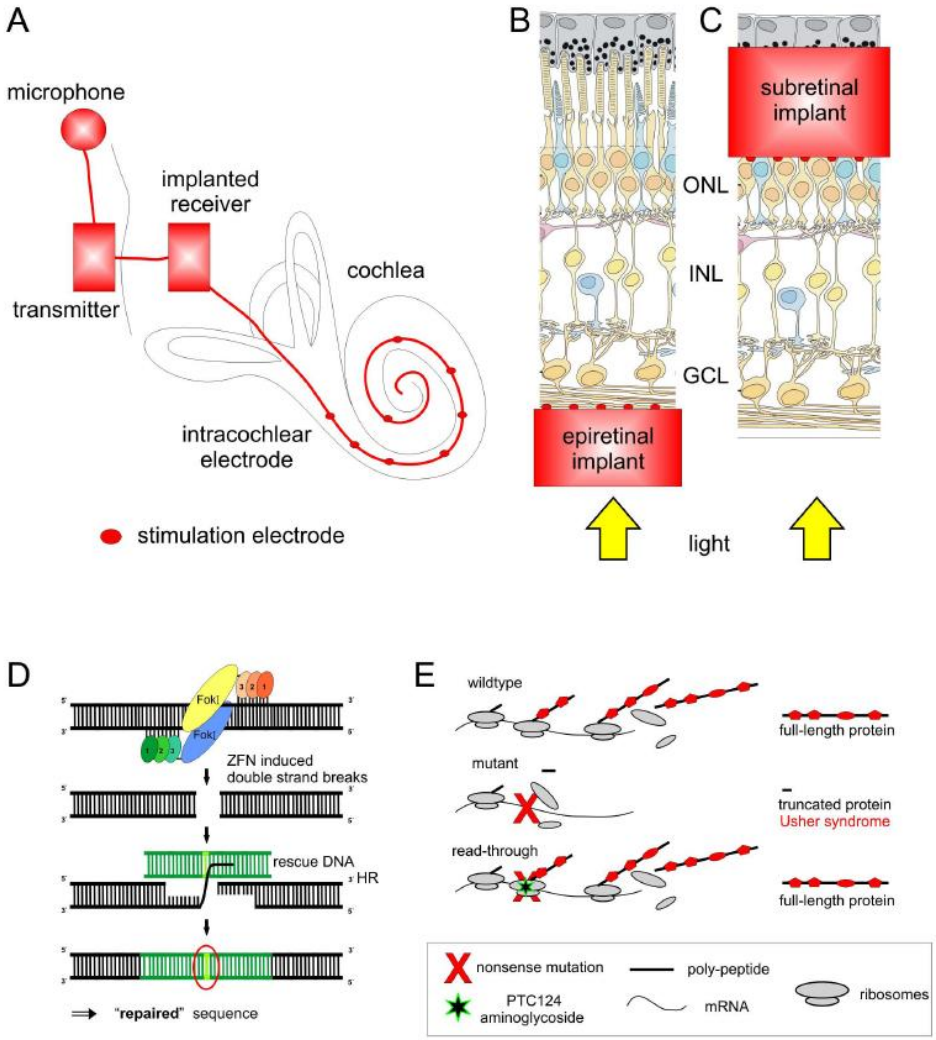

Figure 22.1. Schematic representation of different treatment approaches for USH. A. Cochlear implant. An external microphone collects sound waves and transduces them into electrical impulses. These are transmitted to the intracochlear electrode, which stimulates the corresponding auditory region within the cochlea. B. Epiretinal implant. A camera outside the eye receives light, transduces it into electrical impulses and transmits them to the epiretinal implant. The epiretinal implant is located on the cells of the ganglion cell layer (GCL), which are directly stimulated by the electrical impulse. C. Subretinal implant. The subretinal implant is packed with light sensitive microphotodiodes, which replace lost photoreceptors of the outer nuclear layer (ONL). The light is converted into an electrical stimulus, which activates the remaining secondary neurons in the inner nuclear layer (INL). D. Gene repair. A pair of zinc finger nucleases (ZFN) binds to the DNA close to the mutation, cause a double strand break and thereby activate the cell autonomous homologous recombination machinery. For DNA repair an introduced nonmutated rescue plasmid is used as template. Red circle indicates repaired mutation site. E. Translational read-through. Functional full-length protein is generated by the translation of mRNA. A premature termination codon (PTC; red cross) induces an early interruption of protein translation leading to a truncated and non functional poly-peptide. Aminoglycosides and PTC124 allow the incorporation of an amino acid at the PTC of the mutant mRNA and the generation of full-length protein.

Long-term studies indicate that cochlear implantation is beneficial in USH1 patients because they are profoundly deaf (Pennings *et al.*, 2006). In addition, these patients will develop progressive visual impairment in puberty. The decision for a cochlear implant has to be taken as early as possible, preferably within the first decade of life. The earlier the implantation occurs, the more beneficial it is for the patient. Different studies show that early employment of cochlear implants in USH1 patients results in an improved audiologic performance and better speech perception (Damen *et al.*, 2006, Pennings *et al.*, 2006). This is most likely due to a relatively high plasticity in the young auditory cortex. The cochlear implantation helps USH1 patients to remain independent during daily life and therefore greatly improve their quality of life. Hearing aids which are worn behind the patient's ear, amplify the sound stimulus. However, they can only be helpful for USH2 and USH3 patients with residual hair cell function.

The success of cochlear implants for the treatment of hearing deficiencies has inspired scientists to invent an analogous treatment for visual impairments. However, the retina is a multilayered tissue that does not only transduce visual stimuli into electrical signals but also processes them in a complex way. Thus the healthy retina gathers and transmits more than 100 times more information to the brain than the cochlea does (Stone 2009). Despite these challenges different tools have been developed and are being evaluated (Zrenner 2002, Hornig *et al.*, 2005, Yanai *et al.*, 2007, Besch *et al.*, 2008).

In general, retinal prostheses make use of surviving retinal neurons (amacrine, bipolar, horizontal cells and ganglion cells) to restore vision (Humayun *et al.* 1999). Some devices stimulate the ganglion cells at the epiretinal side by a flexible micro contact film, whereas others electrically stimulate the subretinal side of the retina (Figure 22.1B, C) (Hornig *et al.*, 2005, Besch *et al.*, 2008). In some systems special goggles project bright images into the eye stimulating subretinal photodiode arrays. Alternatively, external cameras capture the images and transmit the information to the retina (summarized in Stone 2009). The major problem for the application of retinal implants is that their technology is not yet fully developed. To date the number of electrodes is far too low to result in the perception of a real image of the object in view; and only black and white differentiation will be possible. Therefore, in the future even more sophisticated devices will be the only option for patients suffering from complete blindness. Nonetheless, this approach is certainly promising for USH patients in the late phase of disease progression, and has to be further improved and evaluated (Winter *et al.*, 2007).

22.2.4. Gene-Based Therapy

USH is a monogenetic recessive disease. Since all USH proteins are organized in USH related networks, the lack of a specific protein may result in the disruption of the entire USH protein network, causing sensorineuronal degeneration in the inner ear and retina. Consequently, restoring the natural protein expression should reverse the USH related phenotype.

Patient screening conducted over the last few years has provided detailed knowledge about mutations in the genes causing USH. This makes it possible to follow approaches specifically designed and adjusted to the affected gene or specific mutations for the treatment of USH. Such personalized gene-based therapies include gene augmentation and gene repair

by homologous recombination mediated by zinc finger nucleases (ZFN). These strategies could provide a cure of USH. In addition, specifically for nonsense mutations the application of translational read-through inducing drugs, like modified aminoglycosides or PTC124 are promising treatment options.

22.2.4.1. Gene Augmentation

USH is caused by the loss of specific USH gene expression, and consequently should be amenable to treatments by gene augmentation. Gene augmentation is based on the exogenous replacement of a mutated gene by a functional copy in the affected organ.

A well known approach to rescue functional protein expression is gene augmentation or gene replacement strategy by viral vectors, mostly by lentivirus and adeno-associated virus. Alternatively, non-viral approaches, like nanoparticles, can be applied for the delivery of non-mutated genes into the affected organs. Both the inner ear and eye affected in USH are small, compartmentalized and enclosed: This is helpful as the delivery of small quantities of vectors will have a minimal risk of systemic dissemination of the vector. Due to the blood-cochlea-barrier and the blood-retina-barrier both organs are immune privileged, providing a degree of protection from immune responses against vector antigens. A variety of non-viral and viral vectors have been tested for their ability to transduce the cells affected in USH, namely hair cells in the inner ear and photoreceptor cells and retinal pigment epithelium (RPE) cells in the retina. Therefore, different vector systems, their advantages and disadvantages for the treatment of the USH phenotype in inner ear and retina are described and discussed below.

In USH patients the hearing impairment is congenital and profound, since USH1 and USH2 proteins are necessary for the proper development and maintenance of stereocilia. Molecular strategies to treat hearing loss, namely gene augmentation to target the restoration of cochlear hair cells for USH patients are attractive (reviewed in Hildebrand *et al.*, 2008). A major problem to rescue the developmental phenotype of USH patients is that the interventions have to be applied *in utero*. Such a treatment bears high risk of miscarriage. However, the studies involving prenatal delivery of viral vectors into dogs suggest that *in utero* retinal gene delivery may be feasible (Dejneka *et al.* 2004). In USH patients the postnatal retinal development, together with the later onset of retinal degeneration makes the eye a more amenable therapeutic target for gene augmentation compared to the inner ear. In addition, the retina is a favorable system for the establishment of new therapeutic strategies because it has a highly ordered structure and it is optically and physically accessible. This facilitates the read out and evaluation of therapeutic interventions.

22.2. 4.2. Non-Viral Gene Transfer Using Nanoparticles

Utilizing nanoparticles for disease treatment has become a promising non-viral gene transfer method in recent years (Cai *et al.*, 2008). There exists a great variety of different nanoparticles, but the typical nanoparticle contains either linear or circular DNA or RNA, which is compacted with a polycationic polymer. The usual size of nanoparticles ranges from 10-100 nm in diameter (summarized in Cai *et al.* 2008). One major advantage of nanoparticles is their very large insert capacity of up to 20 kb (Fink *et al.*, 2006). Such an enormous packaging capacity would be sufficient for the largest cDNA of 19.3 kb encoding USH protein GPR98 (McMillan *et al.*, 2002). So far nanoparticles display little to no toxicity and only a modest immune response (Konstan *et al.*, 2004, Farjo *et al.*, 2006, Davis and Cooper 2007), making repetitive administration possible. Furthermore, transgene expression

can last for at least six months; it is dose dependent and not limited to the site of injection of the nanoparticles (Farjo *et al.*, 2006). Polyethylene glycol (PEG) compacted nanoparticles have been shown to efficiently transfect post-mitotic cells *in vitro* and *in vivo* (summarized in Cai *et al.* 2008). Most importantly PEG compacted nanoparticles have high transfection efficiencies in photoreceptor cells (Farjo *et al.*, 2006) and would therefore be useful for USH treatment. These encouraging results offer the opportunity to compact all USH cDNAs even the very large ones into nanoparticles for non-viral gene transfer for the studies involving USH genes.

22.2.4.3. Viral Gene Addition

For the transfer of genes into the inner ear or retina a couple of viral systems are available and most used vectors are lentivirus and recombinant adeno-associated virus (AAV).

22.2.4.3.1 Lentivirus

Lentivirus is derived from the human immunodeficiency virus (HIV) and belongs to the family of retrovirus. Lentiviral vectors are suitable to transduce RPE cells, have the advantage of a relatively large capacity to package molecules of 8 kb in size and mediate high levels of transgene expression. They integrate into the host genome, thereby providing long-term expression of the transgene. Disadvantages of the lentiviral system are high immunogenicity and ototoxicity, as well as the inability to transduce photoreceptor cells.

Hashimoto *et al.*, (2007) delivered cDNA of *MYO7A* to primary RPE cultures by lentivirus vector and via subretinal route to the *shaker1* mice, deficient in *myo7a*. Appropriate levels of myosin VIIa protein were achieved in the RPE. After restoration of the myosin VIIa phagosome expression digestion and melanosome motility were observed in cultured RPE cells. Additionally, melanosome localization and opsin clearance from the connecting cilium *in vivo* was achieved (Hashimoto *et al.* 2007). However, after lentivirus-mediated transfer restoration of myosin VIIa expression in photoreceptors was not detected. This confirmed that lentivirus mediated gene transfer might not be effective for photoreceptor cells (Smith *et al.*, 2009). Since myosin VIIa is the only USH protein expressed in RPE cells, gene transfer by currently used lentiviruses is not a treatment option for other USH subtypes.

Nevertheless, restoration of myosin VIIa expression in RPE cells can have beneficial effects on the visual capacity in USH1B patients. Thus, for USH1B lentiviral based gene-addition appears promising. With the objective of advancing to the Phase I/II clinical trials in 2011 Sanofi-Aventis and Oxford BioMedica are currently collaborating to develop a gene based therapy for USH1B patients (www.oxfordbiomedica.co.uk).

22.2.4.3.2. Adeno-Associated Viruses (AAV)

Gene augmentation using recombinant AAV is the most promising therapeutic approach for the treatment of USH. Over the past few years a number of laboratories started to evaluate the potential of this non-pathogenic virus as a vehicle for gene delivery to cells. Robust transgene expression was detectable for six months in the inner ear (Lalwani *et al.*, 1998) and several years in the retina (Acland *et al.*, 2005, Le Meur *et al.*, 2005). Thus, the risk of inflammatory and/or immune responses, which could occur following repeated treatments with virus, is reduced. Another issue that has been discussed for the AAVs is a potential random integration of the virus into the hosts' genome, thereby generating the risk of

unwanted side effects in the cells after transduction. On the contrary recent publications state an episomal localization of the AAVs (discussed in Schultz and Chamberlain 2008). Whichever the case, AAVs lead to a sustained expression of the transgene, which is an important point for USH therapy, since a life long expression of the USH genes, is necessary for maintenance of the photoreceptor cells.

The diversity of existing and designed serotypes of AAVs offers the possibility to target different cell types specifically. To restrict the transduction to a special, desired cell type, common AAV2 vector has been engineered with capsids of other serotypes. Depending on the species to be treated, different AAV serotypes show efficient application for the inner ear (as for example Luebke *et al.*, 2009). AAV2/1 was found to be the optimal vector for *in utero* cochlear gene transfer in mice (Bedrosian *et al.*, 2006). In the retina, AAV2/5 or AAV2/8 efficiently transduced photoreceptor cells, whereas AAV2/1 and AAV2/4 were more effective in transducing RPE cells (Surace and Auricchio 2008). So far AAVs are the only viral vectors, which efficiently transduce RPE, rod and cone photoreceptors (Smith *et al.*, 2009). Consequently, AAVs are the most commonly used vectors for retinal gene therapy. Recently three clinical trials of AAV-mediated gene therapy for patients with Leber congenital amaurosis (LCA) 5 showed improvement in vision of the treated LCA5 patients (Hauswirth *et al.*, 2008, Bainbridge *et al.*, 2008, Maguire *et al.*, 2008).

For the USH genes, Zou and colleagues (Zou, J. M., Luo, L., Chiodo, V. A., Ambati, B., Hauswirth, W. W., Yang, J. 2010, AAV-mediated gene replacement therapy in a mouse model of Usher syndrome type II lacking whirlin. *Invest Ophthalmol Vis Sci*, **51**: E-Abstract 3102) report successful delivery of the cDNA encoding for whirlin (USH2D) into photoreceptors of a whirlin knock-out mouse by application of AAV vectors. The transduced whirlin was correctly localized in the retina and restored the localization of the two USH proteins USH2A and GPR98, known to interact with whirlin (van Wijk *et al.* 2006).

One limitation for the application of AAV in treating USH is its lower packaging capacity of 5 kb (Grieger and Samulski 2005). However, a recent publication revealed the possibility of creating recombinant viral genomes containing at least 9 kb constructs, which were successfully cloned and transferred into cells (Allocca *et al.*, 2008). Large size packaging has already been tested with *myo7a* and successfully evaluated in cultured RPE cells. Unfortunately, most of the USH cDNAs, namely USH2A isoform b, and VLGR1/GPR98 (Kelley *et al.*, 1997, Levy *et al.*, 1997, McMillan *et al.*, 2002, Liu *et al.*, 2007), are far larger than this (Table 22.1). One conceivable possibility is to employ a *trans*-splicing strategy for cDNAs of larger proteins, meaning that fragments of cDNA should be incorporated into separate AAVs, which are then used as a mixture for the therapeutic application. The target cells namely photoreceptors produce a fusion protein encoded by all fragments of the large cDNA (Atkinson and Chalmers 2010).

An obstacle in using gene augmentation for USH gene therapy is the existence of different isoforms for most of the USH genes products (Table 22.1). For example, the *USH1C* gene consists of 28 coding exons of which eleven are differentially spliced, therefore generating a variety of alternatively spliced transcripts (Verpy *et al.*, 2000, Becker *et al.*, unpublished data). So far the isoforms necessary for the proper development or survival of inner ear hair cells and photoreceptor cells are unknown. The presence of multiple protein isoforms complicates the choice of cDNA to be used for gene augmentation. It appears that treatment of patients with several splice variants might be required. Nevertheless, due to the promising results of the clinical trials with LCA patients, who suffer like USH patients from

retinitis pigmentosa, AAVs provide a valuable tool for USH gene augmentation (Table 22.1). We hope that further clinical trials will be initiated for USH patients within the next few years.

22.2.4.4 Gene Repair by Homologous Recombination Mediated by Zinc Finger Nucleases

As mentioned above, a major problem in the application of gene augmentation therapies to USH patients is the expression of different isoforms for some USH proteins. This further complicates the choice of cDNA to be transferred. An excellent way to circumvent this problem in gene augmentation is gene repair by homologous recombination mediated by zinc finger nucleases (ZFN) (Figure 22.1D).

Homologous recombination is a cell autonomous repair mechanism activated by the introduction of double strand breaks in the DNA by e.g. x-ray irradiation. The limitation of double strand breaks for application in therapeutic strategies is that they occur at a very low frequency (10^{-6}) in somatic cells. To increase this efficiency, double strand breaks are introduced close to the mutated genomic DNA sequence by using ZFNs. ZFNs are hybrid proteins composed of an engineered DNA binding domain, designed to bind to a specific target sequence within the genome and the nonspecific cleavage domain of the FokI enzyme. Subsequent to the ZFN mediated DNA cleavage an exogenously introduced rescue plasmid, coding for the healthy gene, replaces the endogenous mutated segment of the mutated genomic DNA by homologous recombination. The repair of a gene at the site of the mutation ensures sustained and tissue specific expression of the gene product because it remains under control of its endogenous promoter.

So far the ZFN technique has been applied successfully for targeted mutagenesis in cell culture, zebra fish, *Caenorhabditis elegans* and rats (as for example Morton *et al.*, 2006, Foley *et al.*, 2009, Mashimo *et al.*, 2010) summarized in Remy *et al.*, (2009). Some laboratories have already evaluated the potential of ZFN for therapy: Urnov and colleagues (Urnov *et al.* 2005) corrected a mutated GFP and the IL2Rγ gene in cultured cells and impressively demonstrated the potential of this approach for gene based therapy. The development of specially designed ZFN for specific mutations in different genes affected in USH could be used as personalized therapy for individual USH patients. This is independent of the size of the gene, presence of splice variants or the type of mutation. In our laboratory we are currently generating and testing ZFN designed for mutations in the *USH1C* gene with promising preliminary results.

Taken together, application of ZFN offers an opportunity for the treatment of USH by adopting a repair mechanism namely homologous recombination which is endogenous to the cell. This technique to repair the defective gene in its endogenous context would be the most effective and elegant way of therapy for USH. Nevertheless, this therapy strategy is still far from being applied in patients and considerable effort has to be made to proceed in the direction of clinical trials.

22.2.4.5. Translational Read-Through

In-frame nonsense mutations synonymously called premature-termination-codon (PTC) account for approximately 11% of all USH cases (Baux *et al.*, 2008). The translation of mRNAs containing PTCs often results in truncated, mostly nonfunctional polypeptides

(Figure 22.1E). More than two decades ago, aminoglycosides were found to facilitate read-through of PTCs in eukaryotic cells and thereby stimulate the expression of full length functional proteins (Figure 22.1E). Although, the amino acid inserted at the position of the PTC is not necessarily the one present in the wild type protein, the resulting protein is still fully or at least partially functional (Zingman *et al.*, 2007, Linde and Kerem 2008). In comparison to gene augmentation the translational read-through inducing drugs have the advantage that the targeted gene remains under normal endogenous control. Therefore, tissue specific expression, timing, duration of expression and alternative splicing are not altered. These findings in basic research have raised hope for the usage of aminoglycosides as a pharmacogenetic therapy for PTCs in various genes responsible for a variety of diseases (reviewed in Linde and Kerem 2008). Indeed, encouraging results have paved the way to clinical trials on patients suffering from Duchenne muscular dystrophy and cystic fibrosis (Politano *et al.*, 2003, Wilschanski *et al.* 2003).

The most critical factor that limits the potential of aminoglycosides for "read-through therapy" is their insufficient biocompatibility namely nephrotoxicity and ototoxicity (Mingeot-Leclercq and Tulkens 1999, Forge and Schacht 2000). Two major efforts were undertaken to identify new compounds with better biocompatibility while still having a sustained read-through activity namely modification of the structure of aminoglycosides (Nudelman *et al.*, 2006, Nudelman *et al.*, 2009) and screening for novel read-through agents (Welch *et al.*, 2007).

For redesigning aminoglycosides, toxic structural motifs were exchanged and basic read-through experiments on a nonsense mutation of *PCDH15* (USH1F) were carried out (Nudelman *et al.*, 2006, Nudelman *et al.*, 2009). In these studies, newly designed compounds, NB30 and NB54, displayed a sustained read-through activity and had excellent biocompatibility. Further investigations revealed NB30 and NB54 mediated read-through of nonsense mutations causing USH1C and USH1F in transfected cells and thereby restored full length functional protein (Rebibo-Sabbah *et al.*, 2007, Goldmann *et al.* 2010). In addition NB30 induced read-through of a nonsense mutation of *USH1C* in retinal explants (Goldmann *et al.* 2010). In contrast to clinically applied aminoglycosides, the new compounds exhibited excellent biocompatibility in cultured cells, cochlear explants, retinal explants and in animals (Rebibo-Sabbah *et al.*, 2007, Nudelman *et al.*, 2009, Goldmann *et al.* 2010).

In a second attempt, a screen of 800.000 small potential molecules was performed for read-through agents with better biocompatibility. In this study, PTC124 was identified as a candidate drug (Welch *et al.*, 2007). The read-through efficiency of PTC124 was successfully demonstrated in animal models of different genetic diseases (Welch *et al.*, 2007, Du *et al.*, 2008). Recent Phase I and IIa trials demonstrated the read-through effectiveness of PTC124 treatment without serious drug related side effects (Hirawat *et al.*, 2007, Kerem *et al.*, 2008) which paved the way for clinical trials in Duchenne muscular dystrophy, hemophilia and cystic fibrosis. The potential of PTC124 as a read-through therapy for a nonsense mutation in USH was recently investigated (Goldmann *et al.* submitted). PTC124 mediated a high read-through efficiency of an USH1C causing nonsense mutation in combination with excellent biocompatibility in murine and human retinal organotypic cultures. These collective data highlight the potential of translational read-through drugs for USH and other ocular diseases to combat nonsense mutation based retinal disorders. Both, modified aminoglycosides and PTC124 are currently considered as therapeutic agents to treat USH.

22.3. CONCLUSION

Cochlear implants currently are the most practicable treatment options for hearing loss in USH. Successful applications of cochlear implants demonstrate the powerful impact of therapy on the quality of life of USH patients. In contrast, retinal electronic implants are still in the developmental stage. The technical maturation to the level of clinical application will certainly take at least a decade and more for development of sophisticated devices as an option for completely blind patients. Even today the molecular treatment opportunities are very promising to slow down the retinal degeneration or cure the retinal phenotype of USH. Recent success in gene-based approaches in other retinal dystrophies and the start of clinical test in USH1B raise hope for future efficient treatment and cure of patients suffering from USH.

22.4. SUMMARY

USH is a monogenetic autosomal recessive disorder. Mutations in twelve USH genes, identified so far, result in the dysfunction or absence of the corresponding USH protein which may lead to disruption of the USH networks causing sensorineuronal degeneration in the inner ear and the retina, the clinical symptoms of USH. Identification of USH genes and deciphering protein networks related to USH have paved the way for the accomplishment of therapeutic strategies. Nevertheless, currently only cochlear implants implemented ameliorate the hearing deficiency symptoms, but no treatment of the sensorineuronal degeneration in the eye exists so far.

In most USH types the hearing impairment of USH patients originates during the development of inner ear hair cells. Therefore, preventative gene replacement strategies would have to be implemented *in uteri*, but such therapy options are currently not practiced. Alternatively, postnatal treatments which may slow down the hearing defect are investigated: stem cell transfer or induction of trans-differentiation of supporting cells following correction of defective genes. However, application of cochlear implants in early childhood successfully benefits hearing deficiency in USH patients.

Conversely, the postnatal development of the retina and the relatively late onset of retinal degeneration make the eye a more approachable therapeutic target. For the retina, several non-gene-based therapeutic options are currently assessed: cell replacement, neuroprotective agents and retinal implants. However, the identification of USH genes allows the deployment of gene-specific treatments by gene augmentation using non-viral or viral vectors. Recent successful progress in reversion of blindness by AAV-mediated gene transfer in humans affected by Leber congenital amaurosis also raises hope for common treatment strategies for USH and other disruptive genetic conditions in the eye. Moreover, the knowledge of specific mutations causing the USH phenotype enables the development of mutation specific therapeutic strategies, namely gene repair by homologous recombination mediated by zinc finger nucleases and treatments with translational read-through drugs, namely modified aminoglycosides and PTC124. Latter compounds target in-frame nonsense mutations which account for ~11% of all USH and other retinal degeneration causing mutations. In particular,

PTC124 yielded promising results in Phase II clinical trials for the treatment of various non-ocular diseases caused by in-frame nonsense mutations.

All discussed gene based treatment strategies restore the expression of functional USH proteins. These adjustments may be sufficient to cure or at least slow down the progression of retinal degeneration which would greatly improve the quality life of USH patients.

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