Expression of nestin-associated genes in the inner ear of newborn rats following injury and hypoxia

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Received: January 27, 2015
Published online: March 22, 2015

We used organotypic cultures of the stria vascularis (SV), the organ of Corti (OC) and the modiolus (MOD) of newborn rats to analyze the differential expression levels and responses of cytoskeletal, myelin- and neural growth factor-associated genes following preparatory injury and hypoxia. The transcript mRNA levels of the neurofilaments Nefl, Nefm, the microtubule-associated proteins Mapt, Map1a, Map2, and of the myelin basic protein Mbp decrease during 24 h in a coordinated way across the MOD and OC regions. The increased cell death rate in the MOD region is associated with an increased expression of Nes, the transcript encoding the intermediate filament nestin, and of the neural growth factor receptor Ngfr, the growth-associated protein 43 Gap43 and the Purkinje cell protein Pcp4. The correlation analysis revealed close associations between Nes expression and genes involved in apoptotic and necrotic cell death (caspase Casp3, calpains Capn1, Capn2, Capns1), the glutamate pathway (glutamate receptor NMDA associated protein 1 Grina, glial high affinity glutamate transporter Slc1a3), cytoskeletal genes (Nefm, Map2, Map1a, Mbp), transcription factors (hypoxia inducible factor Hif-1a, jun proto-oncogene Jun, brain expressed myelocytomatosis B-myc, HES family bHLH transcription factor 1 Hes-1, forkhead-box D3 Foxd3) and neural growth factors (Ngfr, Gap43 and Pcp4). The unique response and the composition of the Nes cluster made us conclude that the expression of cell-death-associated genes and the regeneration-associated genes takes place in a coordinated way, and differs between the MOD and the OC/SV regions.

Keywords: nestin; inner ear; modiolus; spiral ganglion; organ of Corti; stria vascularis; organotypic culture; cytoskeleton; growth factors; microarray


Introduction

The cochlea consists of three main complex structures, each serving a specific function: the stria vascularis (SV), the organ of Corti (OC) and the modiolus (MOD)\cite{1}. Each region is composed of diverse cell types with distinct functions (Fig. 1A). The SV contains different epithelial cells that maintain the specific ion composition of the endolymph. The neuro-sensory outer and inner hair cells (HCs) that transform the mechanical signal into an electrical one are located in the OC. This region also contains the non-neural supporting cells that are regarded to be highly specialized forms of the glia cells. The MOD region mainly comprises the bipolar spiral ganglion neurons (SGN) and the Schwann cells (SCs). The SGNs provide the contact between the hair cells and the cochlear nucleus in the brainstem. The Schwann cells myelinate the neurites of the SGNs. The peripheral processes innervate the hair cells in the organ of Corti and the central processes terminate in the cochlear nucleus\cite{1,2}.
Previously, we had introduced organotypic cultures of the SV, the OC and the MOD of newborn rats as an experimental model to study the differential response of neurobiological genes to injury stress and hypoxia\(^3\). Analysis of gene expression showed that two basic pathogenetic mechanisms are involved in this experimental model: mechanically induced inflammation and hypoxia\(^4\). To compare the expression changes with the cell death rate, we in parallel determined the proportion of dead cells immediately after tissue preparation and after 24 h in culture\(^5\). After 24 h, the cell death rate increased to about 25-30 % in the MOD, but did not change significantly in the OC and SV regions (Fig. 1B). Remarkably, these cell death patterns are associated with two gene coexpression clusters, a cluster around $\text{Hif-1a}$ (hypoxia inducible factor - 1 alpha), the key transcription factor that regulates adaptation to hypoxia\(^6\), and a cluster around several cell-death-associated transcripts that include, among other genes, the ROS-associated molecules $\text{Sod3}$, the inflammatory chemokine $\text{Ccl20}$, the glutamate associated NMDA receptor-associated complex protein $\text{NMDARA1}$ $\text{Grina}$ and the glutamate transporter $\text{Slc1a3}$\(^4,7\).

The cytoskeleton consists of filaments forming a three-dimensional network; it is the basis of the structure and shape of a cell. In addition, components of the cytoskeleton are involved in cell movement, organelle and protein transport, neurite outgrowth and signal transduction. Components of the cytoskeleton are to a large part involved in the death as well as in the regeneration of cells. In addition, they play an important role in cell signaling by forming a dynamic framework for signal transduction. Three types of cytoskeletal filaments can be distinguished on the basis of their diameters: microfilaments, intermediate filaments, microtubules and microtubule-associated proteins (MAPs)\(^8\). MAPs, mainly MAP2, MAP/tau and MAP1A contribute to the stabilization and dynamic features of the microtubule network \(^9\). Disruption of the cytoskeleton may induce cell death, for example by impairing the function of...
the mitochondria and the formation of reactive oxygen species\textsuperscript{[10]}. It is known that alterations of the myelin sheath and myelin degeneration play a critical role in the degeneration of SGNs in presbyacusis \textsuperscript{[11]}. Axonal degeneration leads to the breakdown of the myelin sheath and dedifferentiation of the Schwann cells \textsuperscript{[12]}.

The aim of the present study is to analyze the expression patterns of cytoskeletal and neural growth genes in functionally different compartments of the inner ear, linked to injury and hypoxia. As the relations between neural and glial cells are close under physiological and pathological conditions, our study included an analysis of several genes associated with myelination \textsuperscript{[13]-[16]}. The selection of the genes was guided by our own microarray study\textsuperscript{[17]}, as it allows to study the basal and stimulated responses of several cytoskeletal, myelin-associated genes and important neurotrophic factors. Selecting biologically relevant genes belonging to a functional pathway and combining this approach with reviewing the corresponding findings from the literature creates new insights into early processes following tissue injury and hypoxia. The use of the RN-U34 neurobiology array offers the possibility of identifying the distribution and features of novel transcripts which have not yet been studied in the inner ear. The essential role of the intermediate filament nestin for regenerative processes following injury and hypoxia encouraged us to search for those genes in our microarray study that are co-expressed across different regions.

\textbf{Materials and methods}

\textbf{Explant cultures}

Details of the preparation and culturing were described previously\textsuperscript{[9]}. Briefly, the membranous cochleae from 3-5 day-old Wistar rats were dissected into the OC, MOD and SV regions and exposed to normoxic and hypoxic conditions for 24 h. For culture, the cochlear parts were incubated in 4-well tissue culture dishes. Three hours after plating, the cultures were exposed to hypoxia in an incubation chamber for 5 h. The number of dead cells was determined in freshly prepared tissue (controls) and after 24 h in culture using the live/dead viability test by propidium iodide (PI) and calcein AM staining\textsuperscript{[9]}. The preparation mode is based on the assumption that the MOD region contains the cell bodies of the SGNs, the Schwann cells and parts of the peripheral and central processes, whereas the OC region contains hair cells, supporting cells and parts of peripheral processes of the SGNs.

\textbf{cDNA microarray analysis}

The cDNA microarray analysis was carried out using the Affymetrix Rat Neurobiology U34 Array (RN-U34; Affymetrix, Santa Clara, USA) which included 1,322 genes. The complete data sets from this study have been deposited to the Gene Expression Omnibus (GEO) database according to the MIAME standard and can be accessed by ID GSE5446\textsuperscript{[17]}. For the microarray study, the total RNA isolated from the OC, MOD and SV regions of six animals were pooled to obtain one RNA sample each. Within one year, three independent series of RNA preparations were analyzed containing samples from freshly prepared tissue (OC1, OC2, MOD, SV) and from cultured samples (two samples for each region from normoxic cultures and two samples for each region from hypoxic cultures). Data from normoxic and hypoxic environments have been combined, because we found no statistical difference in gene expression neither for the numbers of PI-stained nuclei nor for the expression of Hif-1a mRNA or that of other genes. Overlapping gene expression patterns induced by relatively mild hypoxia and mechanical injury might have produced this finding. In our previous work, we directly compared the microarray expression levels and their changes with those determined by quantitative RT-PCR for at least 31 genes. With the exception of one gene (most probably a splice variant), we found a significant correlation to exist \textsuperscript{[3, 5, 18, 19]}. These results as well as those of several studies in the literature confirm the reliability of the microarray data and make it appear justified to analyze and discuss functional subunits of transcripts without confirming each transcript level by RT-PCR.

\textbf{Statistical analysis}

In total, 22 genes were evaluated. Other genes, belonging to the target groups of this study, but with no statistically significant expression levels, were not included in the analysis. In our microarray study, the cut-off value for the statistically significant expression level has been 300 relative units. Intensity of expression was classified on the basis of the histogram of normalized log2 signals which resulted in a normal distribution (data not shown). In this study, the mean coefficient of the variation (CV) of expression intensity was 7.9 ± 7.6 (mean ± SD, n = 22) and that of fold change 19.9 ± 9.7 (n = 52). We analyzed the features of the genes from the expression data by the following procedures: (i) The fold change of the expression levels induced by injury and hypoxia was calculated as the ratio between the expression intensity of the 24 h cultures and that of freshly prepared tissue. The significance of the fold changes was tested by the paired t-test or the Wilcoxon paired test. (ii) We used the Pearson’s correlation analysis with the Bonferroni post hoc test to identify co-expression changes among selected transcripts across the three regions.
Coexpression analysis of transcripts related to nestin

The role of nestin in regeneration and the unique response of Nes to injury and hypoxia encouraged us to search for transcripts significantly associated with its expression. Fig. 3 summarizes the multiple significant correlations between the response of Nes and the transcripts characterized within the context of our microarray study[4, 17]. (i) The co-expression changes of Sod3, Ccl20, Grina and Scl1a3 may be indicative of the roles of increased production of oxygen radicals, secretion of chemokines and glutamate toxicity in the inflammation process[43]. The reactive oxygen species (ROS) pathway is also involved in the death of hair cells and SGNs.
Expression of cytoskeleton- and myelination-related genes in the modiolus, the organ of Corti and the stria vasculare

<table>
<thead>
<tr>
<th>Gene</th>
<th>MOD</th>
<th>Expression</th>
<th>OC</th>
<th>SV</th>
<th>Fold change</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vim (X62952)</td>
<td>155465</td>
<td>80683</td>
<td>77876</td>
<td></td>
<td>0.9</td>
<td>Vimentin</td>
</tr>
<tr>
<td>Nes (M434384 )</td>
<td>6706</td>
<td>4681</td>
<td>4585</td>
<td></td>
<td>2.2</td>
<td>Nestin</td>
</tr>
<tr>
<td>Nefl (AF031880)*</td>
<td>18611</td>
<td>16976</td>
<td>A</td>
<td>0.1</td>
<td>0.3</td>
<td>NF, light polyp.</td>
</tr>
<tr>
<td>Nefm (Z12152)*</td>
<td>20637</td>
<td>14432</td>
<td>A</td>
<td>0.1</td>
<td>0.5</td>
<td>NF, medium polyp.</td>
</tr>
<tr>
<td>Nefh (X13804)*</td>
<td>1646</td>
<td>2627</td>
<td>A</td>
<td>0.3</td>
<td>0.4</td>
<td>NF, heavy polyp.</td>
</tr>
<tr>
<td>Tubb5 (AB011679)*</td>
<td>10396</td>
<td>9029</td>
<td>11832</td>
<td>0.6</td>
<td>0.7</td>
<td>Tubulin, beta 5, class I</td>
</tr>
<tr>
<td>Tubg1(A0B15946)*</td>
<td>4113</td>
<td>3231</td>
<td>3206</td>
<td>0.7</td>
<td>1.0</td>
<td>Tubulin, gammal</td>
</tr>
<tr>
<td>Map1a (M33196)*</td>
<td>4106</td>
<td>4951</td>
<td>3272</td>
<td>0.8</td>
<td>0.5</td>
<td>MT-assoc. protein 1a</td>
</tr>
<tr>
<td>Map1lc3b (U05784)*</td>
<td>15209</td>
<td>18788</td>
<td>16303</td>
<td>0.8</td>
<td>0.6</td>
<td>MT-assoc. protein 1lc3b</td>
</tr>
<tr>
<td>Map2 (U30938)*</td>
<td>4052</td>
<td>3967</td>
<td>1592</td>
<td>0.2</td>
<td>0.8</td>
<td>MT-assoc. protein 2</td>
</tr>
<tr>
<td>Mapt (X79321)*</td>
<td>5281</td>
<td>6340</td>
<td>A</td>
<td>0.3</td>
<td>0.5</td>
<td>MT-assoc. protein tau</td>
</tr>
<tr>
<td>Mbp (K00512)*</td>
<td>29677</td>
<td>6767</td>
<td>A</td>
<td>0.1</td>
<td>0.2</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>Mpz (K03242)*</td>
<td>36172</td>
<td>9695</td>
<td>A</td>
<td>0.2</td>
<td>0.2</td>
<td>Myelin protein zero</td>
</tr>
<tr>
<td>Mag (M22357)*</td>
<td>4875</td>
<td>2318</td>
<td>1833</td>
<td>0.7</td>
<td>0.7</td>
<td>Myelin-assoc. glycopr.</td>
</tr>
<tr>
<td>Gap43 (165532)*</td>
<td>26938</td>
<td>7754</td>
<td>7756</td>
<td>0.4</td>
<td>0.4</td>
<td>2',3'-cyclic nucleotide 3' phosphodiesterase (also known as Cnp1).</td>
</tr>
</tbody>
</table>

Expression: Intensity (relative units) indicates the normalized signals. Fold changes: *p < 0.01 using data of two or three regions (n = 8 or 12, Wilcoxon paired test). #p<0.002 versus OC and SV; #2p<0.001 vs OC and SV; n = 4, using the paired t-test. Official name according to the rat Genome Database (RGD). Abbr.: A – absent, NF – neurofilament, polyp. – polypeptide, MT-assoc. – microtubule associated, glycopr. – glycoprotein, Map1lc3b, microtubule-associated protein1, light chain 3 beta. Cnp, 2',3'-cyclic nucleotide 3' phosphodiesterase (also known as Cnp1). |

Expression of neuron-specific transcripts in the modiolus and the organ of Corti

<table>
<thead>
<tr>
<th>Gene</th>
<th>MOD</th>
<th>Expression</th>
<th>OC</th>
<th>Fold change</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bdnf (S76758)</td>
<td>1401</td>
<td>1378</td>
<td>1.3</td>
<td>1.1</td>
<td>Brain-der. neurotrophic factor</td>
</tr>
<tr>
<td>Nefl (M434643)</td>
<td>1786</td>
<td>5777</td>
<td>1.0</td>
<td>0.7</td>
<td>Neurotrophin 3</td>
</tr>
<tr>
<td>Nefm (M55291)*</td>
<td>3168</td>
<td>3713</td>
<td>0.2</td>
<td>0.2</td>
<td>Neurot. tyr. kinase , type 2</td>
</tr>
<tr>
<td>Ngrf (X05137)</td>
<td>8881</td>
<td>6302</td>
<td>1.8#1</td>
<td>0.5</td>
<td>Nerve growth factor receptor</td>
</tr>
<tr>
<td>Gap43 (L21192)</td>
<td>12858</td>
<td>7284</td>
<td>1.8#2</td>
<td>0.3</td>
<td>Growth associated protein 43</td>
</tr>
<tr>
<td>Pcp4 (M24852)</td>
<td>551</td>
<td>2667</td>
<td>2.3#3</td>
<td>0.7</td>
<td>Parkinjc cell protein 4</td>
</tr>
<tr>
<td>Nptx2 (S82649)</td>
<td>A</td>
<td>1689</td>
<td>A</td>
<td>2.5#*</td>
<td>Neuronal pentraxin 2</td>
</tr>
</tbody>
</table>

**Ntrk2 is also known as Trk-B; Ngrf is also known as p75NTR; Bdnf show a low, but significant level of expression in the SV (1256 RU). #p<0.007, #2p<0.000, #3p<0.03 vs OC.; #4p<0.000 vs control (n=4, paired t-test). Abbr.: Brain-der.- brain derived, Neurotr. tyr.- neurotrophic tyrosine.**

during aging[20], (ii) Nes expression correlates significantly with Casp3 and various Capn subunits, transcripts closely associated with cell death[41]. (iii) The co-expression changes of Nefm, Map2, Map1a and Mbp indicate associations with the neural cytoskeletal and myelin-associated genes. (iv) Nes expression correlates closely with the expression of the transcription factors Hif-1a, Jun, Bmyc, Hes-1 and Foxd3, key transcription factors involved in the response to injury and hypoxia[41]. (v) The concomitant co-expression of Gap43 and Ngrf indicates processes associated with regeneration, activation of cochlear progenitor cells and outgrowth of new fibers.

Discussion

**Expression of cytoskeletal and myelination-associated transcripts**

The data show clear differences in the response of the non-neuronal IF Vim, the stem cell IF Nes and the neuronal IFs Nefl, Nefm and Nefh. Vimentin is a major component of the cytoskeleton in many of the inner ear’s cells derived from mesenchymal tissue and is important for tissue repair and regeneration[21]. Its extremely high expression level and marginal decrease following injury and hypoxia underlines this function. Nestin is an intermediate filament expressed in neural progenitor cells, but also in proliferating vascular endothelial cells; it is used as a stem cell marker[22, 23]. In the developing inner ear, nestin was shown to be expressed in sensory epithelial cells in the rat organ of Corti[24]. Isolation of sphere-forming stem cells from the early postnatal organ of Corti and from the spiral ganglion gives rise to features of hair cells, to neurons and glial cell types[25]. The differential response of Nes in the present study made us conclude that stem cells are activated in the MOD region, but not in the OC and SV regions.

The strong decrease of the members of the cytoskeletal clusters (Nefm/Nefl/Map1a/Map2/Mbp) appears to be the result of the preparatory deaffentation of the SGNs from the hair cells, associated with nerve-fiber and SGN degeneration [26]. The transcripts are synthesized in the soma of SGN or Schwann cells and are transported to the
peripheral and central processes. Our data are in line with the general observation that the loss of mRNAs of neural cytoskeletal NFs and of myelin-associated genes is a sensitive precursor of cell damage and quantitatively higher than the cell death rate.

Points of interest are the different responses of Map2 and Map1a resulting in a negative correlation of the expression changes. The basic property of the different neuronal MAPs (MAP1A, MAP2, MAP-tau) is their ability to stabilize the microtubules. MAP1A expresses different microtubule-binding sequences and is considered a candidate to be expressed in different cell compartments and at different times during development. MAP1A is not only important for neuronal morphology, but also for mitotic spindle formation and dendritic remodeling. The positive relation between Map2 and Mbp may have its basis in the cooperative response of Schwann cells and SGNs as previously reported.

The specific roles of MAP-tau, Mpz, and Cnp in the inner ear are unknown. The significant correlations between Nefl, Nefm, and Map2 suggest a functional association of MAP-tau with the neurofilaments. The close correlation between Mpz and Cnp, both of which are myelin-related molecules synthesized in Schwann cells, indicates co-expression changes of these transcripts in the Schwann cells. It is interesting to note that the response of two other transcripts associated with myelin, 2’, 3’-cyclic nucleotide phosphodiesterase (Cnp) and myelin-associated glycoprotein (Mag), differs from that of Mpz or Mbp. The differential expression patterns of Cnp and Mag may indicate different localizations and additional functions of their gene products apart from myelination.

Expression of neuronal growth-associated genes

The neurotrophins BDNF and NT3 have an important role to play in normal ear development and promote SGN survival during and after inner ear damage. The receptors NTRKB and NGFR (p75NTR) can bind neurotrophins and thus mediate responses to BDNF and NT3. It is remarkable that in the present study the neurotrophins Bdnf and Nt3 do not increase their expression levels, but Ntrk decreases strongly in the MOD and OC regions, while Ngfr increases in the MOD and decreases in the OC region. This response may be associated with the functional roles of the corresponding proteins. BDNF and NT3 affect SGN survival on the basis of long-term effects. Signaling through TRKB is generally considered to be hypertrophic and increasing neuronal metabolism, whereas p75NTR signaling is generally considered to be atrophic, promoting apoptosis and inhibiting neurite growth. The response of the receptors appears plausible and efficient in terms of cells’ energy balance during injury and hypoxia. Tan and Shepherd (2006) observed a similar pattern of expression changes in a study on aminoglycoside-induced degeneration of adult rat SGNs and concluded that an antagonistic interplay of p75NTR and TRKB receptor signaling mediates SGN death. Remarkably, the functional role of p75 NTR is contradictory having both anti- and pro-apoptotic effects. p75NTR expression in spiral ganglion and Schwann cells correlates with cell proliferation and has a remarkable influence on spiral ganglion neurite growth behavior. We conclude that the down-regulation of TrkB combined with the up-regulation of Ngfr is involved in the survival/death decision of SGNs.

The expression profiles of Gap43, Pcp4 and Nptx indicate that these growth-associated transcripts are of neural origin. The up-regulation of growth-associated genes is a characteristic event occurring in nerve regeneration. Whereas GAP43 expression was previously observed in the inner ear functioning of a marker of axonal outgrowth, synaptogenesis and regeneration, the expression of Pcp4 and Nptx had previously not been identified in the inner ear. Pcp4 encodes for the small brain-specific protein Purkinje cell protein 4. We assume that the early response of Gap43...
and Pcp4 and their corresponding protein products in their roles as early-acting factors may be important for protecting and regenerating SGNs. The coexpression changes of Gap43 and Pcp4 may couple the synthetic events that accompany the regeneration of SGNs[49]. Both may also have a role to play in calmodulin and calcium availability[50]. Nptx2 (Narp) encodes for the neuron-specific protein pentraxin (NPTX2, NP2); it was found to be increased following hypoxia/ischemia in neonatal brain[51]. The specific increase in the OC region may indicate a role Nptx2 has to play in preventing HC death und in forming new synapses following injury of the neural connections between SGNs and hair cells[52].

**Nestin-associated genes**

The identification of the *Nes* gene cluster supports the suggestion that the intermediate filament protein nestin might act as an organizer of survival-determining signaling molecules[53]. In the present model, inflammation is associated with increased production of oxygen radicals, secretion of chemokines and glutamate toxicity; accordingly, the observed nestin network is part of the inflammatory network. Apart from the redox and the glutamate-associated genes this network includes several genes directly associated with cell death. Coexpression changes between *Nes* and Casp3 transcripts were reported previously in traumatic brain injury[54]. Calpain 1 and calpain 2 were found to have distinct roles to play in neural stem cell self-renewal and differentiation[55]. The down-regulation of the calpain subunits may contribute to preserving or repairing the cytoskeleton[56]. We assume that signals from dead cells may activate the expression of *Nes* in stem cells and induce proliferation.

The significant correlations between *Nes* and Nefm/Map2/Map1a and Mbp indicate associations between the neural cytoskeletal and myelin-associated genes. Associations between *Nes* and the transcription factors Hif-1a, Jun, Bmhc, Hes-1 and Foxd3, key transcription factors involved in the response to injury and hypoxia, were observed in different experimental models[4, 55-62].

The positive correlations between *Nes* and Gap43 and Ngfr are in line with the conclusion of Martone et al. ( 2014) that significant up-regulation of nestin required not only the concurrent effects of tissue damage, but also of growth factors[62]. The associations between *Nes* and Gap43 had been shown to exist in the regeneration of cardiac nestin[49] cells[63] and in acute spinal cord injury[64]. We assume that the regulated coexpression changes of *Nes*, Ngfr and Gap43 are important as early-acting factors in regenerating SGNs.

The observed associations around *Nes* are supported by the results of biomedical network databases and pathway tools. For example, the Coremine pathway tool (http://www.coremine.com/medical/#search; PubGene AS, Forskningsveien 2 A, Postbox 37 Vinderen, N-0319 Oslo, NORWAY) indicates many associations between nestin and transcription factors, cytoskeletal/myelin genes and growth factors. In contrast, we could not find associations between nestin and SOD3, CCI20 and GRINA in that database. However, close associations between nestin and several genes belonging to the same functional groups were listed (for example SOD1, CCL3, glutamate receptors GRIN1 and GRIK5). Future studies are warranted to characterize the new associations identified in our work. We are aware that the coexpression changes observed in the present study do not allow conclusions to be made concerning the type or mechanism of interactions. For example, recently it was shown that the high resistance of neural stem/progenitor cells to anoxic conditions in culture, characterized by nestin expression, does not require Hif-1a signaling[65].

**Conclusions**

Although no experimental model completely mimics the condition of disease, the present study may be useful in understanding the differential gene expression changes of different compartments of the inner ear following injury and hypoxia during 24 h, which is the period of the transition from a primarily necrotic pathway (ANCD) to a primarily late apoptotic pathway (SNCD)[49]. These features correspond to the clinical situation of the trauma of the compartments of the inner ear, the SGNs, the OC or the SV as found when performing cochlear implant surgery. The composition of the *Nes* cluster made us conclude that the expression of apoptosis/necrosis-associated genes and the regeneration-associated genes takes place in a coordinated way, and differs between the MOD and the OC/SV regions. In the MOD region, cells that are irreversibly damaged are eliminated and replaced by cells newly formed from progenitor cells. Genes of the *Nes* cluster seem to play a key role in this process. Cells localized in the OC and the SV show a lower vulnerability to injury and hypoxia, their adaptation being characterized by repair processes. Because each culture grows in a compartment of its own, the differential transcriptional response is a reflection of the culture’s endogenous capacity, independent of interactions with other regions.

**Conflicting interests**

The authors declare that they have no conflicting interests.

**Acknowledgments**
We would like to thank the University Hospital Charité for support. It gives us great pleasure to thank Johannes Wendt for his generous help in critically reading and correcting this article.

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