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Current Perspective

Transcriptional Factors in the Cochlea Within the Inner Ear

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Abstract. Differential regulation of gene expression by transcription factors is widely viewed as one of the principal mechanisms guiding development. Although numerous DNA binding proteins have been identified in various tissues, the role of individual transcription factors in the differentiation of specific cell groups, such as those populating the inner ear, is just beginning to be elucidated. It is known that transcription factors are induced in response to many signals that lead to cell growth, differentiation, inflammatory responses, the regulation of apoptosis, and neoplastic transformation. There are various transcription factors in the cochlea of the inner ear. These include activator protein-1 and nuclear factor-kappa B, glucocorticoid receptor, and so on. Based on recent reports and our investigation, in this article we review possible functions and expression of these transcription factors.

Keywords: transcription factor, cochlea, inner ear, hearing loss

Introduction

In eukaryotic cells, the de novo synthesis of proteins is mainly controlled at the level of transcription of genomic genes by RNA polymerase II, which is responsible for the formation of mRNA from DNA in the nucleus. Gene transcription involves molecular mechanisms associated with protein-DNA and protein-protein interactions, in addition to topological alterations of genomic DNA. Transcription factors are nuclear proteins that specifically recognize particular core nucleotide sequences at promoter/enhancer elements on target genes and thereby elicit quantitative control of mRNA formed from genomic DNA as a result of modulation of the activity of RNA polymerase II in the nucleus. Thus, specific recognition of a particular core nucleotide sequence and transcriptional modulation are 2 major functions of transcription factors in the nucleus. In addition, transcription factors function to control various processes, such as genesis, differentiation, carcinogenesis, immunities, senescence, and apoptosis through promoting the *de novo* synthesis of functional proteins in mammalian cells (1).

The cochlea within the inner ear contains the cells responsible for the perception of sound. The cochlea is composed of a bony labyrinth, within which is found the cellular structures comprising the membranous labyrinth. The bony labyrinth includes the otic capsule, as the external boundary of the cochlea, and the modiolus, a bony tube that forms the central axis of the cochlea and contains auditory nerve fibers and their ganglion cells (spiral ganglion). In the cochlea, 3 fluid-filled spaces known as scala vestibuli, scala tympani, and scala media are separated from each other by the basilar membrane and Reissner's membrane. The stria vascularis and the spiral ligament lie close to the bone along the lateral wall of the cochlea. The organ of Corti, whose structure contains hair cells (3 outer hair cells and 1 inner hair cell) as sensory cells and supporting cells, spirals on the basilar membrane (Fig. 1).

The most common reason for sensorineural hearing loss is degeneration of cochlear sensory cells, resulting from overstimulation, ototoxic drugs, infections, autoimmune disease, and aging. A considerable large body of evidence suggests that the hearing loss is elicited by overproduction of reactive oxygen species (ROS) formed in the cochlea by the insults, as well as by the overstimulation by sound (2, 3). The formation of ROS is well known to trigger the transcriptional activation

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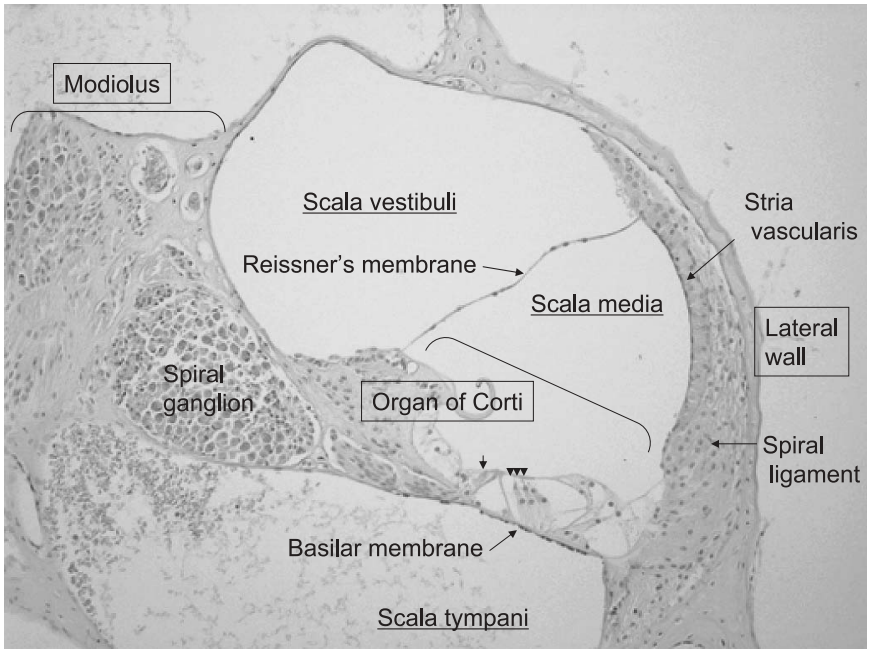

Fig. 1. A mid-modiolar section of the mouse cochlea. The 3 fluid-filled spaces of scala vestibuli, scala media, and scala tympani are separated from each other by Reissner's membrane and basilar membrane. The stria vascularis and spiral ligament lie close to the bone alone the lateral wall of the cochlea. The organ of Corti can be seen resting on the basilar membrane and contains supporting cells, the inner hair cells (arrow), and outer hair cells (arrow heads). Auditory nerve fibers and spiral ganglion are in the modiolus.

of genes through several distinct transcription factors, such as antioxidant-response element binding proteins, activator protein-1 (AP-1), and nuclear factor-kappaB (NF-κB). Both AP-1 and NF-κB are redox-sensitive transcription factors that participate in stress-induced apoptotic pathways in neuronal cells, as well as in normal development. In addition to AP-1 and NF-κB, the glucocorticoid receptor also is a transcription factor existing in the inner ear. This receptor participates in clinical effects of steroids on acute sensorineural hearing loss. However, very little is known about the functions of the glucocorticoid receptor. In this article, we focus on the expression of AP-1 and NF-κB in the cochlea after noise exposure, as well as on the pharmacological effects of glucocorticoid on the inner ear.

AP-1

The generation of ROS and the ensuing changes in the redox state of the cell may trigger a variety of intracellular signaling cascades leading to the activation of transcription factors and culminating in the modulation of gene expression. The genes and their respective gene products can be associated with homeostatic mechanisms controlling cell death. The redox-sensitive transcription factor AP-1 is coupled to changes in the levels of intracellular biological signal molecules, such as ROS, cAMP, and Ca^{2+}, which are produced by extracellular signals (4 – 6). The AP-1 transcription factor is composed of a mixture of homo- and heterodimers formed between Jun (c-Jun, JunB, and JunD), Fos (c-Fos, FosB, Fra1, and Fra2), and ATF families. AP-1 regulates the expression of a myriad of genes in a variety of tissues and cell types. AP-1 factors are a paradigm for transcription factors that are involved in several cellular functions such as apoptosis, differentiation, proliferation, and transformation. Furthermore, AP-1 is linked to the pathophysiology resulting from stress, including trauma, ischemia, and seizure (7 – 11).

Exposure to intense noise can lead to permanent damage to the sensorineural epithelium of the cochlea, whose damage is based primarily on the loss of the sensory cells (the inner and outer hair cells). Previously we reported that intense noise exposure leads to an increase in DNA binding of AP-1 in the guinea-pig cochlea (12, 13). By means of an electrophoretic mobility assay using a radiolabeled oligonucleotide having the consensus sequence for AP-1, DNA binding of AP-1 in nuclear extracts of the organ of Corti, lateral wall, and modiolus (spiral ganglion) was determined at various time points up to 48 h after a 5-h exposure of guinea pigs to noise (115 dB SPL octave band noise centered at 4 kHz). A clear biphasic elevation of the AP-1 DNA binding was observed in the organ of Corti; that is, the binding increased almost 5-fold immediately after the noise exposure, returning to near the baseline level 5 h later and then increased again to a maximum at 15 h after the noise exposure. In the lateral wall tissues, the binding was significantly elevated to approximately 4 times the control value immediately after the 5-h noise exposure, but there was no second peak. In contrast, there was no significant increase in AP-1 binding in the modiolus (spiral ganglion) following the noise exposure. In order to evaluate the participation of Fos/Jun family

proteins in AP-1 DNA binding, we tested the effect of the addition of antibodies against c-Fos, FosB, Fra1, Fra2, c-Jun, JunB, and JunD on the DNA binding as a supershift analysis. As a result, JunD was found to be a major protein component of the AP-1 complex in any region of the cochlea in naïve animals. In animals treated with the 5-h noise exposure, Fra2 in addition to Jun D contributed to the enhanced AP-1 DNA binding in the both the organ of Corti and lateral wall tissues. In addition to evaluation using guinea pigs, we have also studied intense noise-induced enhancement of AP-1 DNA binding in the cochlea of mice. Intense noise exposure (125 dB SPL octave band noise centered at 4 kHz for 2 h) was effective in enhancing AP-1 DNA binding 2 to 12 h later, with a peak of the binding being at 5 h after exposure (Fig. 2). Unlike the case of the guinea pig, the noise-induced enhancement of AP-1 binding in the mouse resulted from the increased expression of FosB in all regions of the cochlea. However, the reason for this species difference in terms of the AP-1 component induced by noise exposure remains to be elucidated in the future studies.

The administration of the aminoglycoside antibiotic kanamycin or the loop diuretic ethacrynic acid is known to induce hearing loss in humans. Both drugs have been simultaneously injected into guinea pigs to prepare a drug-induced hearing loss model. When we tested the DNA binding of AP-1 in the cochlea of guinea pigs after treatment of them with kanamycin/ethacrynic acid, the AP-1 DNA binding was significantly enhanced in the organ of Corti, lateral wall, and spiral ganglion 12 h after the treatment; and the enhanced level was sustained at least until 28 days after the treatment. Supershift analysis revealed that enhanced expression of Fos/Jun family proteins, with the exception of FosB, was responsible for the enhanced AP-1 DNA binding in the cochlea of the guinea pigs suffering from the drug-induced hearing loss (unpublished data).

What is the functional significances of AP-1 expressed in the cochlea in response to noise exposure or treatment with kanamycin/ethacrynic acid? In other words, what are the target genes for AP-1 expressed in the cochlea after the treatment? A hint to the answer to this question is that the AP-1 (c-Jun) level is an important determinant of the expression of γ-glutamyl-cysteine synthetase (γ-GCS), which is the rate-limiting enzyme for glutathione (GSH) synthesis (14, 15). γ-GCS is considered to be a candidate of target genes for the AP-1 over-expressed in the cochlea after noise exposure. Indeed, the GSH level is significantly increased in the lateral wall 2 and 4 h after intense noise exposure and returns to normal 6 h post-exposure (16). However, this increase is not seen in the sensory epithelium and modiolus. Furthermore, we have found that the transcript of γ-GCS gene is enhanced in the cochlea after noise exposure (unpublished data). Conditioning noise, which can protect against noise-induced hearing loss, increases the activity of γ-GCS in the cochlea of the chinchilla (17). Thus, AP-1 over-expressed in the cochlea after noise exposure may mediate an increase in γ-GCS expression and thereby enhance GSH synthesis. Glutathione is an important factor in protection against hearing loss after noise exposure. A reduction in the GSH level is known to enhance hearing loss induced

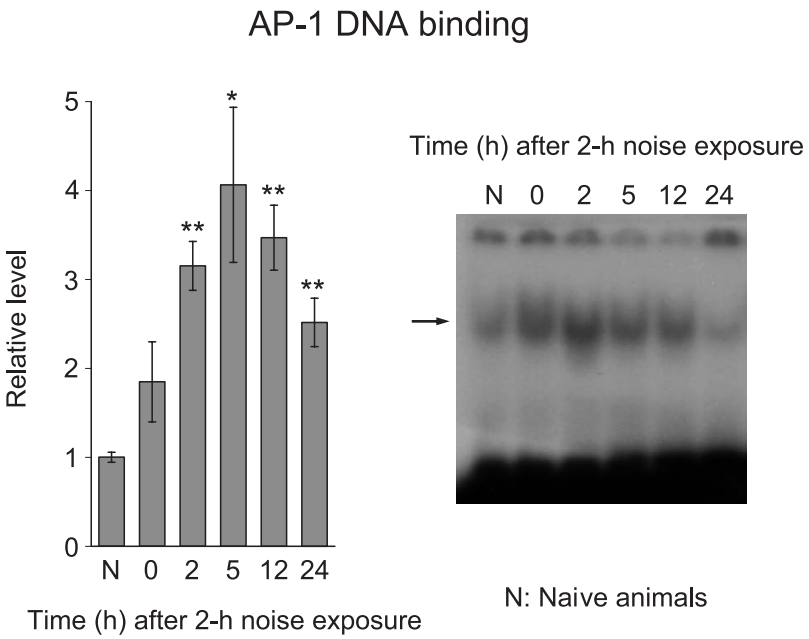

Fig. 2. Enhancement of AP-1 DNA binding after noise exposure. Mice were exposed to noise (125 dB SPL octave band noise centered at 4 kHz) for 2 h and then decapitated at the various time points indicated after noise exposure for preparation of nuclear extracts from the cochlea. An aliquot (6 μg of protein) of nuclear extracts was incubated with a ^{32}P-labeled AP-1 probe and then subjected to the electrophoretic mobility shift assay. The right panel shows typical autoradiograms where each lane corresponds to a sample from 1 animal. Quantitative data are shown in the right graph, where each value is the mean ± S.E.M. from 4 separate animals. *P<0.05, **P<0.01, significantly different from the control value obtained for naive animals.

by the combination of aminoglycosides and ethacrynic acid (18). In addition, it has been demonstrated that noise-induced threshold shifts and hair cell loss are aggravated under conditions where cochlear GSH is lowered. Such aggravations are reduced by restoration of the GSH level to normal by dietary supplementation (19). Therefore, noise-induced expression of AP-1 may be involved in a host defense system for preventing hearing loss through upregulation of GSH, which is utilized in scavenging reactive oxygen species.

NF-κB

The transcription factor Rel/NF-κB is well known to be induced in response to many signals that lead to cell growth, differentiation, inflammatory responses, the regulation of apoptosis, and neoplastic transformation (20). NF-κB is composed of homo- and heterodimers of Rel proteins (21). The κB protein complexes are composed of RelA (p65), RelB, c-Rel, NF-kB1 (p105/p50), and NF-κB2 (p100/p52). The dimer complex is sequestered in the cytoplasm in an inactive state by IκB. Diverse stimulants lead to degradation of the NF-κB inhibitory protein followed by activation of NF-κB. The activated NF-κB dimer can then be translocated into the nucleus where it activates target genes by binding with high affinity to κB elements in their promoters. Major inducers of NF-κB include proapoptotic and necrosis-induced stimuli, such as oxygen-free radicals and UV irradiation.

There is increasing evidence to suggest that expression of many molecules in the lateral wall of the cochlea plays important roles in noise-induced stress responses. Therefore, we investigated the activation of NF-κB in the cochlea of mice exposed to intense noise (125 dB SPL octave band noise centered at 4 kHz for 2 h). NF-κB DNA binding was significantly enhanced in nuclear extracts of the cochlea 2 – 5 h after the noise exposure, with a return to the basal level 12 h later (Fig. 3). Supershift analysis using antibodies against p65 and p50 proteins demonstrated that enhancement of NF-κB DNA binding was at least in part due to nuclear translocation of p65. An immunohistochemical study also showed that nuclear translocation of both p65 and p50 occurred in the lateral wall after noise exposure. These results suggest that NF-κB may be involved in the expression of genes in response to acoustic overstimulation in the cochlea of mice.

Is the NF-κB pathway involved in cell survival or death in the cochlea damaged by various insults? Opposing findings have been described in recent reports. One report demonstrated that the NF-κB pathway plays a role in hair cell survival, but is not involved in hair cell death, in aminoglycoside anitibiotics-induced outer hair cell death, which is known to be mediated by ROS, in adult CBA mice (22). When kanamycin (700 mg/kg) was subcutaneously injected twice per day into adult CBA mice, the hair cells were progressively destroyed, but after 7 days of treatment, the auditory function and morphology had not yet been affected significantly, permitting investigations of early events in drug-induced cell death. NF-κB composed of p50 and p65 proteins was increased at 3 h, 3 days, and 7 days of treatment. Immunoreactivity for p50 was present in the nuclei of inner hair cells and supporting cells that survived the drug treatment. In contrast, nuclei of outer hair cells were devoid of immunoreactivity. Concomitant injections of antioxidants, however, such as 2,3-dihydroxy-

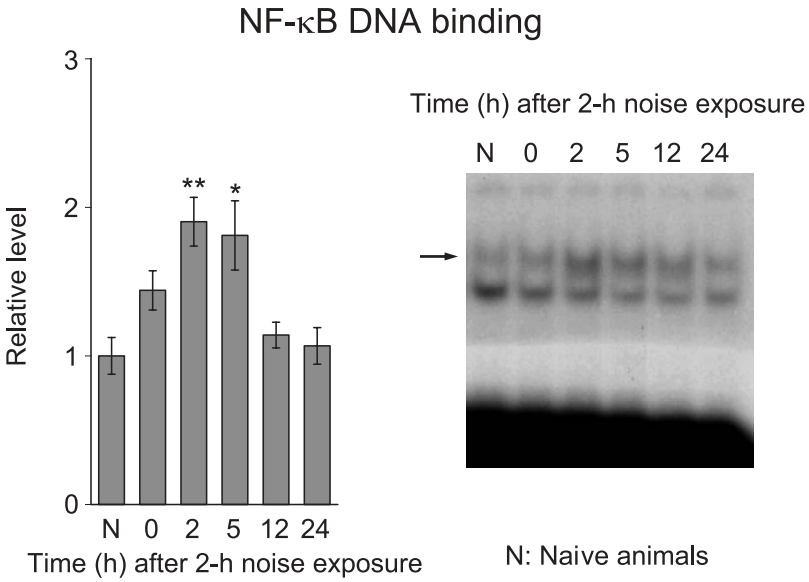

Fig. 3. Enhancement of NF-κB DNA binding after noise exposure. Mice were exposed to noise (125 dB SPL octave band noise centered at 4 kHz) for 2 h and then decapitated at the various time points indicated after noise exposure for preparation of nuclear extracts from the cochlea. An aliquot (6 μg of protein) of nuclear extracts was incubated with a ^{32}P-labeled NF-κB probe and then subjected to the electrophoretic mobility shift assay. The right panel shows typical autoradiograms where each lane corresponds to a sample from 1 animal. Quantitative data are shown in the right graph, where each value is the mean ± S.E.M. from 4 separate animals. *P<0.05, **P<0.01, significantly different from the control value obtained for naive animals.

benzoic acid or salicylate (which prevents cell death induced by kanamycin), promoted the translocation of NF-κB into the nuclei of outer hair cells. These results suggest that changes in the redox state of the cochlea stimulate the activation of NF-κB and that this activation is cell protective at least in kanamycin-induced hair cell death. Contrary to this proposition, another report demonstrated that in cisplatin-induced damage of the cochlear lateral wall including the stria vascularis and stria ligament, NF-κB mediated hair cell death in the cochlea through NO generation by expression of inducible nitric oxide synthase in both stria vascularis and stria ligament (23). So far, however, very little has been elucidated with respect to survival or death of the hair cells following intense noise exposure.

Glucocorticoid receptor

Glucocorticoid activates the cytosolic glucocorticoid receptor, which is then translocated to the nucleus to regulate gene expression. Glucocorticoid receptor and other members of the steroid/thyroid/retinoid receptor superfamily are ligand-dependent transcription factors known collectively as nuclear receptors. The prototypic mode of glucocorticoid receptor action following ligand activation is the formation of its homodimers that bind a specific DNA sequence termed the glucocorticoid response element in their promoter regions of genes. The regulation of gene expression accounts for many, and probably most, of the metabolic and endocrine effects of steroids that, in excess, are recognized as Cushing's syndrome.

Glucocorticoid receptors at a high concentration have been found in both cochlea and vestibular tissues by means of in vitro binding studies using radiolabeled dexamethasone (24). Further immunohistochemical studies on glucocorticoid receptor distribution in the murine inner ear reveal that the highest expression of the receptor is seen in the type III fibrocytes of the spiral ligament. Although the immunoreactivity of the cochlear hair cells and of the vestibular sensory epithelium is weak, the neighboring cochlear supporting cells and subepithelial regions of the vestibular sensory epithelium are immunostained to a greater extent. Staining for glucocorticoid receptor is also positive in the spiral ganglia and vestibular ganglia, as well as in the endolymphatic sac (25).

Glucocorticoid has been widely used in therapeutic management as a clinical drug for acute hearing loss in humans. Indeed, increasing evidence indicates that glucocorticoid has the ability to protect against hearing loss in various animal models, such as those generated by intense noise (26), aminoglycoside (27, 28), and salicylate (29) treatment. However, very little is known about the mechanisms underlying the protective effects of glucocorticoid against hearing loss in these models. To elucidate possible mechanisms underlying the therapeutic effect of glucocorticoid on hearing loss, therefore, we evaluated the level of GSH, an antioxidant, in the cochlea after the systemic administration of dexamethasone (2 – 20 mg/kg) into mice. This administration led to a significant increase in the GSH level in the cochlea 2 to 24 h after the injection. Of the discrete structures in the cochlea, the modiolus, but not the lateral wall and the organ of Corti, showed an increase in the GSH level after the treatment [GSH levels (% of naïve animals): the organ of Corti, 99 ± 10; the lateral wall tissues, 102 ± 9; the modiolus, 150 ± 8 ($P<0.05$)]. In addition, significantly elevated mRNA expression of γ-GCS was seen in the cochlea 1 to 16 h after the treatment. These results suggest that glucocorticoid enhances GSH biosynthesis in the cochlea through an activation of γ-GCS transcripts in mice. Thus, the therapeutic effect of glucocorticoid on acute hearing loss could at least in part result from enhanced GSH biosynthesis in the cochlea.

Others

In addition to AP-1, NF-κB, and glucocorticoid receptor, some other transcription factors are found in the cochlea of the inner ear. As development-related factors, the POU domain factor and Islet-1 are found in the cochlea. The POU domain transcription factors Brn3a, Brn3b, and Brn3c are required for the proper development of sensory ganglia, retinal ganglion cells, and inner ear hair cells, respectively. Deletion of Brn3a results in a significant loss of spiral ganglion neurons and defects in their migration (30). Islet-1, which is an LIM/homeodomain protein that acts as a transcriptional regulator in the developing nervous system, is one of the earliest markers of inner ear neural precursors just before they migrate from the otic cup into the anterior medioventral periotic mesenchyme. Islet-1 expression in hair and supporting cells persists until early postnatal stages, when the transcriptional regulator is down-regulated in hair cells (31).

As yet other transcription factors in the cochlea, hypoxia-inducible factor-1 (32), neuregulin-1 (33), and thyroid hormone receptor (34) have been reported. However, the functional significance and regulation of expression of these cochlear transcription factors have not yet been fully evaluated. Further studies on all of the transcription factors mentioned in this review should reveal the developmental regulation of the inner ear and eventually lead to therapy for hearing loss.

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