ORL

ORL 2018;80:326–337
DOI: 10.1159/000493011
Received: May 7, 2018
Accepted after revision: August 19, 2018
Published online: October 25, 2018

Review Article

Combined Growth Factor and Gene Therapy: An Approach for Hair Cell Regeneration and Hearing Recovery

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Keywords
Growth factor · Three-dimensional culture system · Hearing loss · Hair cell regeneration · MicroRNAs

Abstract
Introduction: Fibroblast growth factor, nerve growth factor neurotrophins, and insulin-like growth factor 1 are considered 3 families of growth factors that can be involved in the process of otic neurogenesis. In this respect, otic neurons can also be connected with mechanoreceptors in the ear, the hair cells (HCs), as well as the central nervous system. As a growth factor is combined with gene transfer technology, it can be used for hair cell regeneration. Gene therapy can be similarly employed to introduce genes into a system in order to induce the expression of genes for therapeutic agents, to replace defective genes, or to re-program supporting or surrounding cells to acquire the phenotype of lost or damaged cells in order to repair or regenerate the damaged tissue. ***Objective:*** The purpose of this review article was to investigate the epigenetic and growth factors involved in the differentiation pathway of embryonic stem cells (ESCs) into HCs and auditory neurons (ANs). ***Methods:*** To this end, the databases of Directory of Open Access Journals, Google Scholar, PubMed (NLM), LISTA (EBSCO), as well as Web of Science were searched. ***Results:*** Given the results available in the related literature, the differentiation efficacy of ESCs toward the ANs and the HCs, the important role of growth factors, and 3 different strategies of application of miRNA, epigenetic

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ORL 2018;80:326–337
DOI: 10.1159/000493011

regulation, and preparation of three-dimensional (3D) environments were suggested to be taken into consideration in order to improve these studies in the future. Furthermore, the role of epigenetic mechanisms and miRNA in this differentiation process became quite obvious; hence, the utilization of such procedures in the near future would be significant. ***Conclusion:*** Combining several techniques with a synergic effect (such as growth factor gene therapy and 3D environments) seemed to lead to obtaining the best results as a therapeutic strategy.

Introduction

Hearing loss is recognized as the second most common sensorineural deficit in humans with an incidence rate of approximately 1 out of 500 individuals. Nowadays, a cochlear implant is known as an effective treatment for such patients [1–3]. The prevalence of this deficiency can also reach to 2.7 per 1,000 children during their childhood (before the age of 5) and 3.5 per 1,000 people during their adolescence. Moreover, across the world, about 66% of individuals with hearing loss are living in developing countries [4–6]. Profound hearing loss can affect language learning in children and this can result in problems influencing their quality of life in the future [7, 8]. Also, hearing loss can be divided into pre-lingual (i.e., occurring prior to speech and language learning) and post-lingual deficits (i.e., appearing after the acquisition of speech and language) or, on the basis of the age of onset, into early (i.e., observed in young age) and presbycusis or age-related hearing loss (i.e., reported in old age). Hearing loss can also occur due to an environmental etiology or a genetic and hereditary one (including dominant, recessive, and X-linked types, as well as mitochondrial conditions). Furthermore, hearing loss can be classified on the basis of the involvement of the ear, that is, the deficiency of either the outer and the middle ear or the inner ear sensory neurons, or even both parts [9, 10]. Likewise, it can be based on the position of engagement in the ear that can involve either the outer and the middle ear sensory neurons or the inner ear sensory neurons or even both parts. In the sensorineural hearing loss, the hair cells (HCs) in the cochlea are irreversibly destroyed. In this regard, several factors can be involved in the loss of HCs including infections (cytomegalovirus and rubella), genetic and environmental factors, as well as the use of ototoxic drugs (aminoglycosides and platinum derivatives) [11–13]. HCs in humans and other mammals have no or a very limited capacity for self-regeneration. In contrast, the ear construction in birds is different, since they are endowed with the capacity to regenerate the lost HCs. Growth factors are also expected to induce HC proliferation during the postnatal period because they have a marked capacity for inducing proliferation in treated cells [14]. Several growth factors have been employed in this respect, namely, insulin-like growth factor (IGF), beta (β)-fibroblast growth factor (βFGF), as well as epidermal growth factor [15]. Therefore, the present study aimed at investigating the role of growth factors and genetic manipulation in the regeneration of the HCs and the auditory neurons (ANs).

Methods

To this end, the databases of Open Access Journals, Google Scholar, PubMed (NLM), LISTA (EBSCO), and Web of Science were searched for the publications about the role of epigenetic and growth factors involved in the differentiation pathway of embryonic stem cells (ESCs) into HCs and ANs from 2000 to 2017 using the EndNote software. The articles retrieved from these databases were analyzed once. Their abstracts were further reviewed based on predefined inclusion and exclusion criteria. If necessary, the full texts were also retrieved to evaluate the study eligibility. The articles without English abstracts and full texts were then

excluded. Only the ones directly addressing the effect of epigenetic and growth factors involved in the differentiation pathway of ESCs into HCs and ANs were selected and analyzed. Finally, 2 and 3 articles were respectively retrieved from the Web of Science and the PubMed. Overall, 5 studies were retrieved from both databases and 100 articles were included in the final analysis. After reviewing the abstracts, 16 articles were crossed out of the analysis. As a whole, 84 articles were found in which the role of epigenetic and growth factors involved in the differentiation pathway of ESCs into HCs and ANs had been investigated.

ESCs

In addition to the unlimited capability of self-renewal, ESCs are known as stem cells that have the ability to differentiate into other cell types and such a capability is called pluripotent [16]. Due to having these 2 important properties, ESCs have several potential applications in basic and clinical studies. Numerous medical researchers also believe that stem cells are endowed with high therapeutic potentials for the treatment of selected human pathologies and can be medically useful. Through facilitating the selection of appropriate therapeutic methods, the ESCs also seem promising for the treatment of incurable diseases [17].

Hair Cell Regeneration

The performance of a cell can be governed by the environment in which it is located. In this respect, cells receive and respond to the signals from surrounding tissues or hormones secreted by other cells or even by themselves. Accordingly, HC death can trigger several different functions in the surrounding cells in order to replace HCs [18]. In humans, the dead HCs can be replaced by the adjacent supporting cells (SCs). There is also a need to keep endolymph and perilymph separated from each other, so that death cells cannot affect the performance of the whole cochlea. In some animals, one of the SCs is changed to a hair-like cell. In birds, one of the SCs can undergo mitosis and consequently produce 2 daughter cells: one of the daughter cells that differentiates into HCs and the other cell that remains as SC [19]. The HCs and SCs have a common ancestor. Considering the subsequent events following the death of the HCs, the remarkable point is the idea of replacing the dead HCs with the stem ones and whether the progenitor cells can differentiate into the HCs [20]. Studies on the regeneration of HCs in the inner ear have shown that the regeneration process of these cells has become possible with the emergence of new techniques and specific gene therapies. An increased expression of Math1 gene in progenitor cells can induce them to differentiate into HCs [21–23]. It has been also demonstrated that stem cells transferred into the inner ear of chicks have the capability to differentiate into HCs [24]; therefore, it can be suggested that ESCs and the progenitor HCs can be used as an external source to transfer them into the inner ear for differentiation into HCs. So, the use of growth factors can increase the rearrangement, differentiation, and restoration of vestibular HCs when vestibular damage occurs in birds and mammals. According to the related literature, vestibular cells damaged by gentamicin were treated with transforming growth factor-α, IGF type 1 (IGF-1), retinoic acid (RA), and brain-derived neurotrophic factor (BDNF). The results of this study also showed that the significant reconstruction of HCs could improve vestibule function of the ear [25]. Considering that there was insufficient information about the precursor of sensory HCs in mammals and the mechanism of action of growth factors involved in differentiation of these cells as well as intracellular communication, further studies seemed to be necessary in order to determine the signaling pathways of these growth factors in the process of differentiation and regeneration of the HCs [25, 26].

ORL 2018;80:326–337
DOI: 10.1159/000493011

Growth Factors Affecting the Differentiation of Neurons and HCs

Three families of growth factors including FGF, nerve growth factor as neurotrophins (NTs), and IGF-1 are involved in the process of otic neurogenesis. In this respect, the otic neurons can be connected with the sensory mechanoreceptors of the ear, the HCs, and the central nervous system [27]. These neurons can be also generated in a chain process. Accordingly, the first step includes the specialization of otic progenitors in the epithelium; the second step is related to the division of epithelial neuroblasts in order to form cochlear-vestibular ganglion (CVG), and the third step is associated with the proliferation of ganglion neuroblasts, their differentiation into neurons, as well as their placement into cochlear-vestibular sensory organs. In this regard, each step can depend on specific signaling pathways and growth factors in order to ultimately form the otic neural structure [28].

Fibroblast Growth Factor

The production of human otic progenitor cells is dependent on the FGFs. During cell culture, this factor can lead to increased expression of markers indicating the differentiation of human ESCs (hESCs) into the inner ear cells [29]. It has been shown that the FGF signaling pathway can repeatedly become active during embryonic organogenesis and also play a vital role in the formation of several tissues. Normally, FGFs can show different functions at each stage of growth of the body; for example, in the embryonic stage, FGFs can have mitogenic and chemotactic properties in mesoderm and ectoderm cells, while they are likely to show a different performance during development and adulthood. FGFs can also play a critical role in inducing neurogenesis, migration, proliferation, as well as cell survival [30, 31]. Besides, FGF2 can be expressed during otic placode and through the stage of otic vesicle formation from the ectoderm [32]. The use of exogenous FGF2 and FGF8 has been similarly reported to increase the transcription of several genes in chicks [33]. In addition, FGF2 and FGF8 can cause the enlargement of the CVG. Also, FGF2 is likely to enhance the migration and differentiation of CVG neurons. Also, FGF2 regulates tropomyosin receptor kinase B and the secretion of BDNF, which is involved in the ANs [34]. FGF3 can be also expressed in the sensorineural epithelium of otic vesicle and cochleo-vestibular ganglion (CVG) in mice [35] and xenopus [36], but it does not express in birds [37]. Besides, the FGF8 can be first expressed in otic vesicle and then at CVG and non-sensory epithelium [38]. The loss of FGF8 function in zebrafish or inactivating FGF3 using antisense morpholinos can also change the induction, while the expression of otic vesicles decreases the size of the auditory ganglion and brings about a defect in sensory organs [39]. FGF10 is expressed in otic placode and otic vesicle, sensory areas of epithelium, and also in CVG neurons. Thus, the number of neurons is reduced in mice lacking this gene, and defects can be observed in the structures of auditory sensory epithelium and vestibule [40]. A null mutation in FGFR2 (IIIB) can also impede the formation of CVGs; however, the exact mechanism is not yet known. Moreover, the disruption of an FGF receptor 1 (FGFR1) signaling pathway in the auditory epithelium can lead to defects in the formation of the organ of Corti, although no reports have so far been released about its effects on CVGs [41]. It can be stated that FGF2, 3, 8, and 10 have a different but not complementary function in the formation process of sensory neurons in the inner ear. However, FGF10 can be more associated with the primary differentiation of neurons, while FGF2 and 8 can be more connected with the final differentiation of neurons and cell migration [42]. Considering the patterns of gene expression along with different experimental manipulations, several members of FGF family including FGF2, FGF3, FGF8, FGF10, and FGF19 can be involved in the differentiation and the formation of the inner ear [43–45]. In the meantime, FGF3 can play a

role as the first candidate in the early development of the inner ear. Initially, the pattern of its expression can be detected in the hindbrain and then in the formation of placode and the inner ear vesicle; hence, it can play a major role in inducing the inner ear development [46]. Thus, FGF signaling is necessary to induce a number of early markers in the area of otic placode. In addition, recent data have suggested the existence of reciprocal signaling loops between the function of different FGFs during morphogenesis, for example, during the limb bud formation [47]. FGF3 can also play a role in the early development of the inner ear of mammals via regulating the formation of endolymphatic duct [48]. Accordingly, a prerequisite for understanding the function of FGF3 in the inner ear is to evaluate its receptors and compensatory signals of FGF that can form semi-penetrant phenotypes. Among 4 FGFR, FGFR-3 can be expressed in the sensory epithelial of the cochlea in the late stage of embryogenesis and after birth [49, 50]. Gene expression studies have also shown that cochlear neurons derived from FGF1 and the inner HCs derived from FGF8 are ligands for the FGFR-3 receptor in the late embryonic period and the postnatal period. In addition, exogenous FGF2 can stimulate the migration and differentiation of nerve cells into the inner ear of early chick embryos [34]. Null and hypomorphic mutations in FGFR2 can also cause early embryonic mortality and even death in the middle of pregnancy respectively. Recently, mice deficient for FGFR2 (IIIb) have been found to be able to survive albeit they can have some developmental disorders in different organs such as the inner ear [51].

Insulin-Like Growth Factor 1

The cellular function of IGF-1 is mediated by binding it to its membrane receptor tyrosine kinase. This factor can have 2 types of receptors on the membrane, that is, the insulin receptor and IGF 1 receptor (IGF-1R). Both receptors are also hetero-tetramer consisting of 2 extracellular subunits (for binding) and 2 membrane subunits (for activation of tyrosine kinase signal). The expression of IGF-1 and IGF-1R in otic epithelium and CVG is observed in the early stages of the inner ear growth of chick embryo [52, 53]. During the puberty of reptiles, IGF-1 is expressed in mature HCs and the auditory system [54]. After birth, from day 5 to 20, the inner ear organ is growing and the expression of IGF1 can be observed in the organ of Corti and the cochlear ganglion [55]. Studies conducted on primary cell cultures and genetic modifications in animal models have also shown that IGF-1 can be an essential factor for the normal development and the function of the nervous system in vertebrates. The function of IGF-1 in the nervous system can include control of cell size, stimulation of cell proliferation and survival, increase in nerve cell differentiation, axonogenesis, myelination, and ultimately facilitation of synaptogenesis, as well as transmission of neurotransmitters [27, 52]. Furthermore, IGF-1 can also play a key role in the development of the ear. In fact, any defect in this gene is likely to cause a sensorineural hearing loss in humans [56]. Lack of IGF-1 expression can also influence the differentiation and maturation of cochlear ganglion cells in mice and lead to abnormal stimulations of the sensory cells in the organ of Corti [57]. It has been demonstrated that 20-day mice lacking IGF-1 are likely to exhibit a decrease in the size of the cochlea, the cochlear ganglia, and the average size of the cochlear ganglion sensory neurons. In addition to the aforementioned problems, a developmental retardation can be observed in these mice. During the ear development of mice in the postnatal period until maturation (from birth to day 20), the cochlear ganglion neurons are highly dependent on IGF-1 and a decrease in this factor can result in reduced cell survival and also failure in the function of the auditory system [57]. In fact, about 3,500 neuronal cells in the cochlea are destroyed in mice lacking the IGF-1 compared to healthy ones and this process occurs as a result of cell apoptosis. Individually or in combination with other NTs, IGF-1 and insulin can

have a protective effect on otic cells against ototoxic drugs and also cause the regeneration of HCs. Thus, the IGF-1 potential in the treatment of accompanied neurological diseases as well as the positive effects during the development of the inner ear can strengthen the hypothesis concerning the role of the IGF-1 in the reconstruction of the inner ear HCs.

Brain-Derived Neurotrophic Factor

BDNF can increase the expression of potassium voltage-gated channel subfamily Q member 4 (KCNQ4) and it is necessary for the differentiation of neurons derived from ESCs into Spiral Ganglion Neuron (SGN) [58]. In a study, mouse ESCs (mESCs) were treated with the BDNF and glial cell line-derived neurotrophic factor after transient expression of Neurog 1 and 75% of neuronal cells derived from mESCs exhibited the glutamatergic phenotype after a 5-day culture in vitro. Moreover, the mESCs were placed into the cochlea of deaf guinea pigs and expression of Neurog1 was induced, while 2 factors including BDNF and glial cell line-derived neurotrophic factor were also injected. The results showed that 50–75% of the mESCs could express the primary markers of neurons and majority of these cells could exhibit glutamatergic phenotype [59].

Retinoic Acid

RA is known as another growth factor that can affect the reproduction of HCs by suppressing the expression of cyclin-dependent kinase inhibitor 1B ($p27^{kip1}$) and sex-determining region Y-box 2 (sox2) in SCs. RA has the additional capacity to be reproduced in several organs, but its role in the reproduction of HCs has not been fully recognized. This signaling pathway is likely to be a promising method for hearing restoration [60].

Bone Morphogenetic Protein 4

Bone Morphogenetic Protein 4 (BMP4) growth factor is able to induce the generation of sensory neurons from hESCs and even regenerate the sensory epithelium [61]. In a study, the mESCs were treated with a neural induction protocol and then transplanted into the Rosenthal's canal, perilymph, or endolymph of rodent Mongolian gerbils affected by ouabain damage. Small numbers of ESCs inside Rosenthal's canal also showed markers of mature neural or glial cells. Even if ESCs in the perilymph survived in several places, most cells differentiated into glia-like cells and those grafted to endolymph were rarely survived. This experience revealed that a certain period of time was needed to treat damaged ear using ESCs transplantation [61]. In another study, the ESCs treated with BMP4 factor after transplantation were incorporated into damaged regions of epithelial in developing an ear and the incorporated cells were expressed as HC markers. In addition, it should be noted that ESCs can produce hair bundles when placed into cochlea or vestibule under in-vivo conditions [62].

Gene Therapy in Hearing Loss

During the development of the inner ear, a combination of transcription factors and epigenetic changes in a consistent manner can be involved in establishing and maintaining the characteristics of the inner ear. Since the epigenetic changes may play a significant role in

ORL 2018;80:326–337
DOI: 10.1159/000493011

Table 1. The number of candid genes essential for HC development and regeneration [75–77]

Gene	Role	Ref.
Hairy and enhancer of split-1 (Hes1) and Hes5	In the cochlea, Hes1 can have a significant influence on the production of inner HCs, whereas Hes5 can significantly affect the production of outer HCs	[75]
Sox2	Human menopausal gonadotrophin (HMG) domain transcription factor, Sox2, can be a critical gene for the development of cochlear HCs, the receptor cells for hearing. Sox2 is also expressed in the neurosensory domain of the otic placode and it is even necessary and sufficient for HC development	[76]
Math1	Math1 gene, a homolog of the Drosophila atonal gene, can encode the basic helix-loop-helix transcription factor, which is considered a positive regulator of HC differentiation during cochlear development	[77]

hearing loss, hearing protection, and regeneration of the auditory system, it is necessary to allow for the regulatory role of epigenetics in the inner ear and its potential role in the inner ear regeneration in the regeneration of the auditory system from stem cells [63]. Gene therapy also allows the direct manipulation of genes in target cells by inhibiting the expression of inappropriate genes, or via increasing the expression of target ones. Different animal models such as zebrafish, birds, and mammals (e.g., squirrels, rats, mice, and guinea pigs) have been used in the studies of gene transfer for the auditory system. The first step in selecting the appropriate gene for genetic manipulation in the inner ear is the identification of the gene and its signaling pathways. In this regard, transgenic mice technology has been introduced as a useful tool for gene therapy studies. For instance, the transfer of atonal homolog 1 (ATOH1) gene (a critical gene in the differentiation of HCs) to transgenic mice could facilitate the differentiation of progenitor cells into HCs [64]. Among several genes involved in the development of the inner ear, mutations can also cause hearing loss only in a small number of them, and these genes can be selected as the suitable candidate for gene therapy. Some of the candidate genes and their functions are listed in Table 1. However, gene therapy is currently being performed at the level of laboratory research and this technology has not yet been utilized in clinical studies even with the identification of important genes. Gene therapy is based on the change in the expression of a gene. It should be noted that, in some cases, this change can enhance and inhibit or reduce gene expression. For example, in diseases with the dominant negative mutation, the goal of gene therapy is to inhibit the target gene expression. One of the methods for inhibiting gene expression is to inactivate the gene using RNA interference genes [65]. In this respect, 2 types of RNA interference small molecules can prevent the expression of genes, that is, microRNA and small interfering RNA (siRNA) molecules. MicroRNAs (miRNAs) are known as post-transcriptional regulators that can suppress or destroy gene translation through binding to the complementary sequence of messenger RNA [66]. But, the siRNA can interfere with the expression of specific genes via a complementary nucleotide sequence [66]. In the early studies of gene therapy in deafness, the NADPH oxidase 3 (NOX3) siRNA gene (as the main source of active oxygen in the cochlea) was transferred into the inner ear through the tympanic membrane. The result of this study showed that the expression of NOX3 was interrupted, in the long run, and the outer HCs and Spiral Ganglion Neurons could be protected from the side effects of cisplatin produced by the activation of NOX3 gene [67]. Another example was about the application of siRNA in the treatment of mice that had hearing impairment due to a mutation in the connexin 26 [68]. The

ORL 2018;80:326–337
DOI: 10.1159/000493011

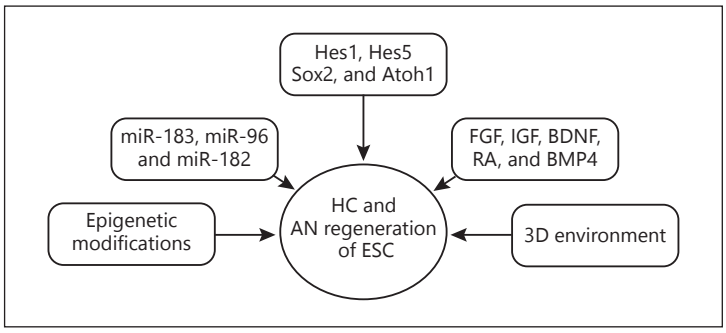

Fig. 1. Transcription factors, miR-183 family, epigenetic modifications, 3D environments, and growth factors playing different roles in the regeneration of HCs and ANs of ESCs.

miRNAs can be also involved in the molecular regulation of stem cell gene expression. Also, it has been determined that some of the stem cell transcription factors such as Nanog, Sox2, and octamer-binding transcription factor 4 (Oct4) involved in self-renewal could be associated with some of the miRNAs. According to the results of these studies, it was concluded that miRNAs could be the primary regulator to maintain stemness and differentiation status and to function before other known factors associated with cell stemness including OCT4/NANOG [69]. Moreover, miRNAs could play an important role in the function of stem cells, cell differentiation, as well as the creation of new features in cells. Genetic manipulation of ESCs is also considered a useful way to perform genetic studies on differentiation processes of these cells. In this regard, it is necessary to develop genetic manipulation techniques and assess the functionality of elements and relevant tools in ESCs. One of the gene manipulation techniques is the use of miRNAs that can easily pass through the cell membrane and enter the cell to affect epigenetic functions such as DNA methylation, acetylation, and methylation of histones [70]. It should be noted that miRNAs can also provide a potential tool for the manipulation of these processes to change cell differentiation and the fate of hearing cells. The necessity of miRNA in the development of the ear has been demonstrated through blocking the miRNA biosynthesis by genetic engineering strategies during the early stages of the ear development and also HC differentiation. Since hundreds of transcripts are simultaneously regulated by miRNAs, miRNAs can be considered potential therapeutic agents for the repair or the regeneration of HCs in animal models [71]. A large part of human transcriptome is regulated by multiple miRNAs and considering the potential impact of these molecules on gene expression, and therefore, they can be used in diagnosis, prognosis, as well as in the development of drugs. Trans-differentiation of SCs into HCs that does not happen under normal conditions may be also achieved with the help of miRNAs, especially the miR-183 family in adult mammals [72–74].

Three-Dimensional Culture System

Recently, three-dimensional (3D) cell culture models have received much attention because of improving the level of cell differentiation and tissue organization in such a way that is not possible in the conventional two-dimensional culture system. In addition, 3D systems can provide the opportunity to evaluate the feasibility for the generation of mechanosensitive HCs from ESCs. In 3D systems, a considerable number of functional sensory cells can also be produced. These cells can be employed to examine the mechanism of the development of the inner ear, the inner ear diseases, as well as regenerative mechanisms [78]. Previous attempts to produce HCs in vitro using two-dimensional culture have also led to low productivity, lack of homogeneity, and even impaired cell phenotype. The 3D culture mediums can also allow researchers to produce a tissue under in vitro conditions that is similar to the

structures and the organs of in vivo ones. These 3D models can similarly have the potential application in tissue engineering, drug screening, disease modeling, as well as evolutionary studies [78]. In an in vitro study, neural precursors derived from hESCs were cultured along with cochlea in the 3D culture medium and it was observed that the precursor neurons were electrically active and they could differentiate into the sensory HC alongside the implanted cochlea. These results showed that neural precursors derived from hESCs might be useful in developing treatments that could directly replace the ANs [79].

Epigenetics and Hearing Loss

Considering epigenetic changes in the inner ear, it should be noted that there is insufficient information in this respect even though a large volume of data has been provided in the domain of epigenetics for other tissues [80]. Epigenetics can lead to an understanding of how hearing loss can influence gene regulatory networks and how such epigenetic modifications can be manipulated and consequently prevail over the use of epigenetic therapeutic strategies [80]. Moreover, the NuRD cofactors including LSD1 can be observed in most of the organ of Corti from E18.5 to P4. They can also be completely absent by P7 and even detectable once again from P8 via P21 [81]. EZH2 as the enzymatic subunit of the PRC2 is similarly abundantly present from E18.5 to P0 in the mouse organ of Corti, absent between P2 and P4, and evident once again in the organ of Corti by P6 and consequently persistent through P21 [81]. In addition, DNMT3A and DNMT3B are reported to have a remarkable rise in expression in the mouse organ of Corti following the first postnatal week [81, 82]. Epigenetic changes of ATOH1 regulation can also be an underlying factor for HC differentiation and the consequent maturation. The H3K4me3/H3K27me3 bivalent chromatin structure found in progenitors can also persist at the ATOH1 locus in perinatal SCs, accounting for the latent capability of the given cells to transdifferentiate into HCs, and draw attention to their potential as therapeutic targets within HC regeneration [83]. Developing evidence in this regard has also shown that more than 100 miRNAs can be regulated by epigenetic mechanisms, and about 50% of them can be modulated by DNA methylation [84].

Conclusion

To differentiate ESCs into ANs and HCs according to the previous studies, it is necessary to employ 3 strategies including the use of miRNA, epigenetic regulation, and provision of a 3D environment along with growth factors (FGF, IGF, BDNF, RA, BMP4) (Fig. 1). Differentiation of pluripotent stem cells into committed and specialized cells also requires making thorough changes in the pattern of gene expression in stem cells. In this regard, epigenetic mechanisms are the most well-known changes including DNA methylation, changes at the level of histones, as well as non-coding RNA-dependent regulations, which are interpreted as the main factors controlling gene expression during cell growth. Given the information related to genome sequencing, the investigation of epigenetic changes can also provide a good understanding of the possibility of stemness and targeted differentiation of stem cells. Currently, various epigenetic mechanisms and their importance in the control of gene expression have been recognized particularly during changes in cell conditions, for example, all through different stages of embryonic development and cell differentiation. Therefore, a better understanding of the relationship between these mechanisms and their roles in the emergence of structural and functional changes in the genome can provide considerable information regarding the molecular pattern of the differentiation process of ESCs. Such an understanding can also pave

the way for establishing targeted cell differentiation conditions. Nowadays, following extensive advances in generating stem cell lines, it is of high necessity to differentiate these cells into specialized target cells and tissues for the treatment of some diseases such as hearing loss. Furthermore, the role of epigenetic mechanisms and miRNA in stem cell differentiation can become obvious and their use can be of utmost importance in the future studies.

Acknowledgment

This work was supported by Hamadan University of Medical Sciences, Hamadan, Iran and Shahrekord University of Medical Sciences, Shahrekord, Iran (grant No. 1396-08-74-3606).

Disclosure Statement

The authors declare that there are no conflicts of interest to disclose.

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