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Research Article

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Stem Cell Differentiation Stage Factors (SCDSFs) Taken from Zebrafish Embryo during Organogenesis and their Role as Epigenetics Regulators able to Reverse Neurosensory Hearing Loss

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Introduction

Hearing loss, the most common form of human sensory deficit, is the partial or total inability to hear sound in one or both ears. It may be a sudden or a progressive impairment that gradually gets worse over time. Depending on the cause, it can be mild or severe, temporary or permanent. It may be a bilateral loss occurring in both ears or unilateral. Hearing loss may be fluctuating, that is, varying over improving at times and getting worse at other times. In other cases, hearing loss is stable, not changing at all with time. Hearing loss is caused by many factors, including genetics, age, exposure to noise, illness, chemicals, and physical trauma. Hearing loss may affect all ages, delaying speech and learning in children, and causing social and vocational problems for adults. [1]

Hearing dysfunctions can be classified by type, degree, configuration, time of onset, etiology, and finally, consequences on speech development. They can be divided into conductive, mixed, central types and neuro sensory hearing loss [2]. Conductive hearing loss results from interference with the mechanical transmission of sound through the external and middle ear; it can be congenital, as a consequence of anatomic abnormalities, but it can commonly be acquired following middle ear inflammatory pathologies. Neuro sensory hearing loss results from failure to transduce vibrations to neural impulses in the cochlea and usually is a consequence of an irreversible damage to the differentiated cells which make up the organ of

hearing and the acoustic paths at various levels. [3] Mixed hearing loss involves a combination of these two types in the same ear [1]

Clearly in the etiology of the neuro sensory hearing loss are relevant many chemical toxic factors, like many pharmacological substances or physic agents like noise which induce degenerative or apoptotic damages, which however manifest themselves in a monotonous way with the loss not only of the cytological structure but also of the dedicated function: in this case the acoustic ability. In the binomial structure-function, as it is reasonable to expect a neuro sensory hearing loss in the case of lesion, it is equally reasonable to expect that an auditory recovery demonstrated with a tonal audiometric examination is supported by cellular regeneration. Such correspondence is considered specific both in Legal and Occupational Medicine, where there are tables dedicated to the interpretation of the indemnity.

In the present work we record an observational study on neuro sensory hearing loss using stem cell growth and differentiation factors (SCDSFs) which have demonstrated that these factors collected at the early developmental stages of Zebrafish embryo – just at the beginning of stem cell differentiation – are able to regenerate human adipose-derived stem cells (hASCs) [4][1].

These researches demonstrated that SCDSFs are significant in activating important genes, which counteract human cells senescence. Indeed, these factors represent very effective tools to increase stem cell expression of multipotency, reducing the expression of the beta-galactosidase marker and enhancing the stemness genes Oct-4, Sox-2 and c-Myc.[5]

Furthermore, it was possible to activate the gene expression of TERT, the catalytic subunit of telomerase, and the transcription of Bmi-1, [6-7] which plays a role in counteracting senescence, as a key repressor of telomerase-independent aging. [8-9].

Based on researches on stem cell rejuvenation and differentiation, we have also conducted studies on the prevention of cell degeneration and tissue regeneration without stem cell transplantation.[10] Studies in this field have shown that the prevention of cell degeneration is only possible if all the factors taken at the different stages of stem cells' multiplication and differentiation are administered together. We have demonstrated this in a recent study on the ability of SCDSFs to prevent neurodegeneration in hippocampal cells of the CA1 zone in mice [11]. This experiment demonstrates that the degeneration of the cells of a tissue can be avoided only by administrating all the differentiating factors able to regenerate and differentiate the stem cells of that tissue, that is to say when the information is complete and redundant. This study confirms previous findings demonstrating that early development of zebrafish embryo extracts could act as a modulator of senescence in human mesenchymal stem cells (hMSC) isolated from many adult tissues [7-12-13,14]. These findings have open a promising way for the approaches promoting the rejuvenation and regeneration of different tissues, by-passing stem cell transplantation.

In the present clinical trial we have used SCDSFs to study the possible reversion of neurosensory hearing loss, until now considered an irreversible condition.

Materials and Methods

The tonal audiometric test, in its simplicity, is able to provide data on the efficiency of the auditory function in its complexity. That is, it can select the so-called transmissive hearing loss from those of the type neuro-sensory. In the context of the latter, however, it is unable to carry out a topo- diagnosis; such circumstance does not fall within the scope of the present work, as all the cells that make up the cochlear and nervous pathway are to be considered perennial. In this way the tonal audiometric examination was carried out at time T0 and after 2 months of administration of regenerating factors. In this clinical trial we have used the audiometer LEDISO AD629 BY INTERACOUSTICS. The examinations were carried out in a silent cabin and in acoustic rest and from the same operator, a factor often requested by

Table 1. Proteins contained in SCDSFs

Accession	Protein Name	Score	MW (Da)	pI	Coverage
gi|166795887	Vitellogenin 1 precursor	1108	150308	8,68	19
gi|94733730	Vitellogenin 1	1039	149825	8,74	21
gi|94733733	Novel protein similar to vitellogenin 1 (vg1)	913	149828	8,92	19
gi|94733734	Novel protein similar to vitellogenin 1 (vg1)	835	150550	8,83	16
gi|145337918	Vtg1 protein	780	116965	9,07	18
gi|94733731	Novel protein similar to vitellogenin 1 (vg1)	762	149911	8,84	19
gi|94732723	Novel protein similar to vitellogenin 1 (vg1)	745	147826	8,73	17
gi|159155252*	Zgc:136383 protein	720	124413	8,78	17
gi|68448530	Vitellogenin 5	559	149609	8,77	13
gi|92097636	Zgc:136383	402	28924	9,33	36
gi|63100501	Vtg1 protein	345	36580	9,23	28
gi|57864789	Vitellogenin 7	341	24490	8,37	40
gi|57864783	Vitellogenin 4	334	31304	9,48	27
gi|113678458	Vitellogenin 2 isoform 1 precursor	323	181208	8,70	11
gi|125857991	Zgc:136383 protein	171	149328	8,93	9
gi|15209312*	Procollagen type I alpha 2 chain	169	147826	9,35	4
gi|57864779	Vitellogenin 2	122	69906	7,84	8
gi|11118642	Vitellogenin 3 precursor	117	140477	6,92	2
gi|303227889	Vitellogenin 6	73	151677	8,84	4
gi|13242157 *	Egg envelope protein ZP2 variant A	71	48194	6,04	5
gi|6644111 *	Nucleoside diphosphate kinase-Z1	69	17397	7,77	14
gi|18859071*	Nucleoside diphosphate kinase 3	69	19558	7,68	7
gi|126632622*	Novel protein containing a galactose binding Lectin	67	19245	9,33	13
gi|66773080 *	Mitochondrial ATP synthase beta subunit-like	66	55080	5,25	4
gi|38541767*	Ppia protein	60	19745	9,30	13
gi|1865782	HSC70 protein	58	71473	5,18	2
gi|28279108	Heat shock protein 8	58	71382	5,32	4
gi|41152402*	Histone H2B 3	49	13940	10,31	11
gi|41393113*	Collagen, type I, alpha 1b precursor	46	137815	5,39	4
gi|94732492 *	Ras homolog gene family, member F	46	24035	9,00	6
gi|47778620 *	Tryptophan hydroxylase D2	45	55686	6,56	1
gi|68448517 *	Zona pellucida glycoprotein 3.2 precursor	44	47365	4,92	2
gi|326677766 *	PREDICTED: RIMS-binding protein 2-like	41	138659	5,86	0
gi|112419298	Vtg3 protein	40	60622	6,32	2
gi|54400406 *	Glutaredoxin 3	39	36541	5,18	11
gi|41152400*	Peptidylprolyl isomerase A, like	37	17763	8,26	7

List of proteins identified using the nano LC-ESI-Q-TOF with the specification of their NCBI accession number, name, score, molecular weight (MW) in Dalton (Da), isoelectric point (pI) and percentage sequence coverage. Proteins hightlighet with asterisk (*) were not described before in Zebrafish embryo.

neurosurgeons to check with patient treated with radiotherapy against acoustic neurinoma. The comparison of the graphs obtained at time T0 and T1 (after 2 months of therapy) was carried out evaluating each single frequency recorded on the abscissa (250,500,1000,2000,4000,8000 Hz) crossing the intensity plotted in ordinate in dB scale. Gain values equal to or greater than 10 dB were considered significant. The timing of administration at 2 months, for the audiological follow-up, was obtained following pilot cases suffering from chronic atrophic rhinitis, through nasal cytology. Precisely, serial nasal cytologies performed at 10 days of interval had shown the first sign of regeneration of the ciliary apparatus, of which they are provided cells the nasal epithelium, at 2 months. Therefore the administration of 2 months was chosen as a useful period to evaluate a tissue regeneration. Regarding the evaluation of the effectiveness of the perceptive ability or at least the possibility of support a correlation between changes in tonal audiometric thresholds and epigenetic factors, some pilot cases with neurosensory hearing loss had been preliminarily investigated also with vocal audiometry and evoked potentials examination. This methodological premise allowed us to believe that the tonal audiometry alone could be sufficient for the present research. Two products containing the SCDSFs where prepared: one product, named Cell Integrity Brain, contains tablets to dissolve in the mouth which in addition to the SCDSFs are consisting of some anti-oxidant substances, like L-Glutathion, some vitamins like vitamin A, B2, B6, C, D, E, substances like zinc, extracts of Curcuma longa and of Bacopa Monnieri. This product was conceived to protect the cognitive function (Bacopa Monnieri), to preserve the cells from oxidative stress (Vit. C, E, curcuma longa) and to preserve the immune system (Vit A, B6, C, D. Zinc). Another product, named Cell Integrity Age contains tablets to dissolve in the mouth which in addition to SCDSFs are consisting of many other anti-oxidants substances like Resveratrol, Coenzyme Q10, some vitamins like vitamin A, B1, B6, B12, C, D, E, folic acid, and some different substances like Rhodiola Rosea, N-Acetylcarnitine Hydrochloride, N-acetylcysteine, Creatine, and some extracts like Curcuma longa and Blakcurrent. This last integrative product was conceived first of all with the scope to prevent aging, to support the body energy and to reduce the fatigue. It should be emphasize that in any case all the substances added to the 2 products have never shown the ability to regenerate tissues: at experimental level, only SCDSFs have demonstrated the ability to regenerate different tissues in several experimental studies as already reported. The two nutraceutical products were used in this way: three daily administration of Cell Integrity Brain (at 8,13 and 17 hours) and three administration of Cell Integrity Age (at 11, 15 and 20 hours) during all the clinical research. These tablets contained SCDSFs were dissolved in the mouth so that the low molecular weight proteins contained in SCDSFs could be absorbed directly in the mouth, as already reported [11, 12, 13, 14, 15]. The proteins which are present in SCDSFs extracted from the earliest Zebrafish developmental stage (50% epiboly) were identified by using a liquid chromatography mass spectrometry (LC-MS/MS) analysis, after the in-gel digestion procedure. We listed in Table 1 the identified proteins with the correspondent NCBI accession number, the score, their isoelectric point (pI). Identified proteins include multiple form of yolk protein vitellogenin, heat shock protein (e.g. HSP8 and HSP70) and other proteins that have not been described before (indicated in Table 1 with an asterisk) [40, 41]. These proteins are implicated in many pathways as in signalling, cell cycle regulation, protein trafficking, chaperoning, protein synthesis and degradation, as already published [4]

The audiograms at times T0 and T1 were evaluated. A gain of 10 decibels or higher and a recovery of the sensitivity previous absent in certain frequencies were considered a positive response to our treatment. In this clinical trial 26 women and 15 men were recruited.

Results

The number of responsive patients was 37. The number of unresponsive patients was 4. The age of the

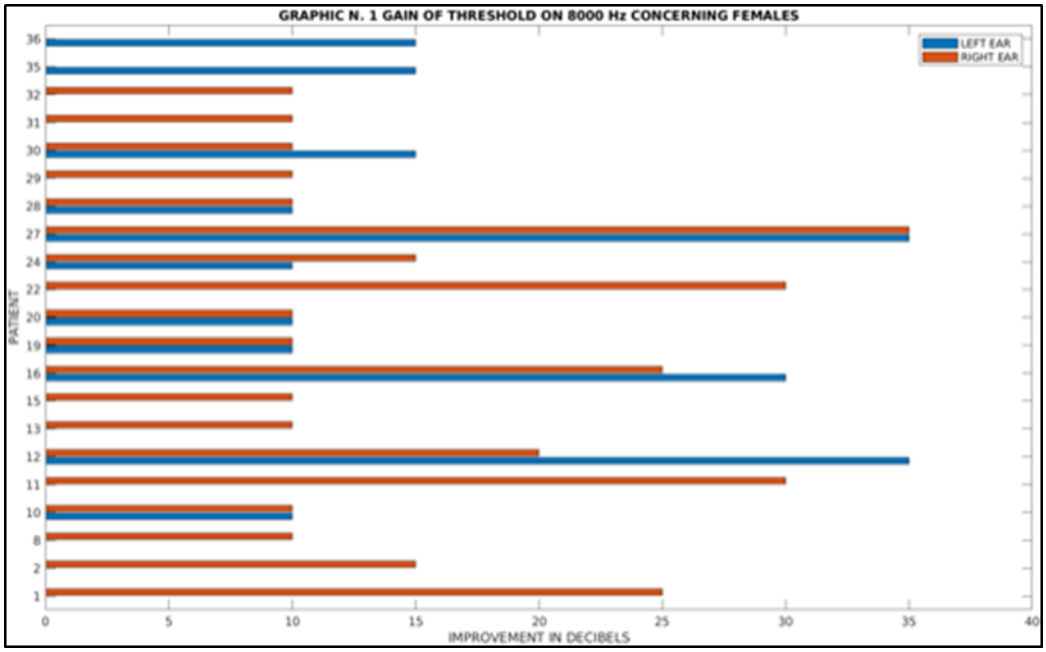

Figure 1. Gain of Threshold on 8000 Hz concerning female

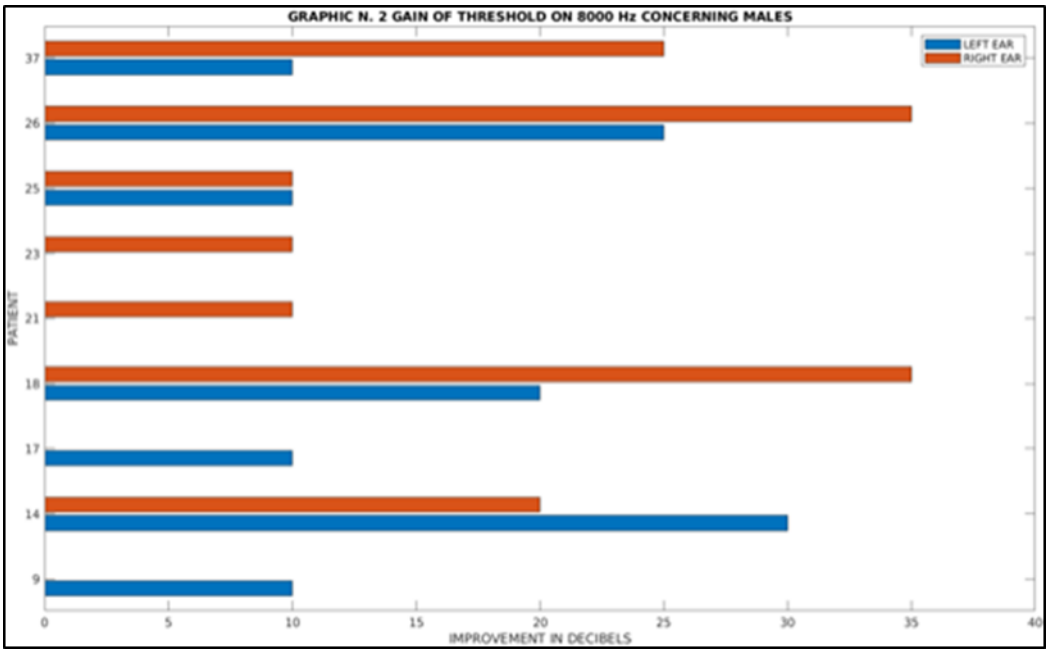

Figure 2. Gain of Threshold on 8000 Hz concerning male

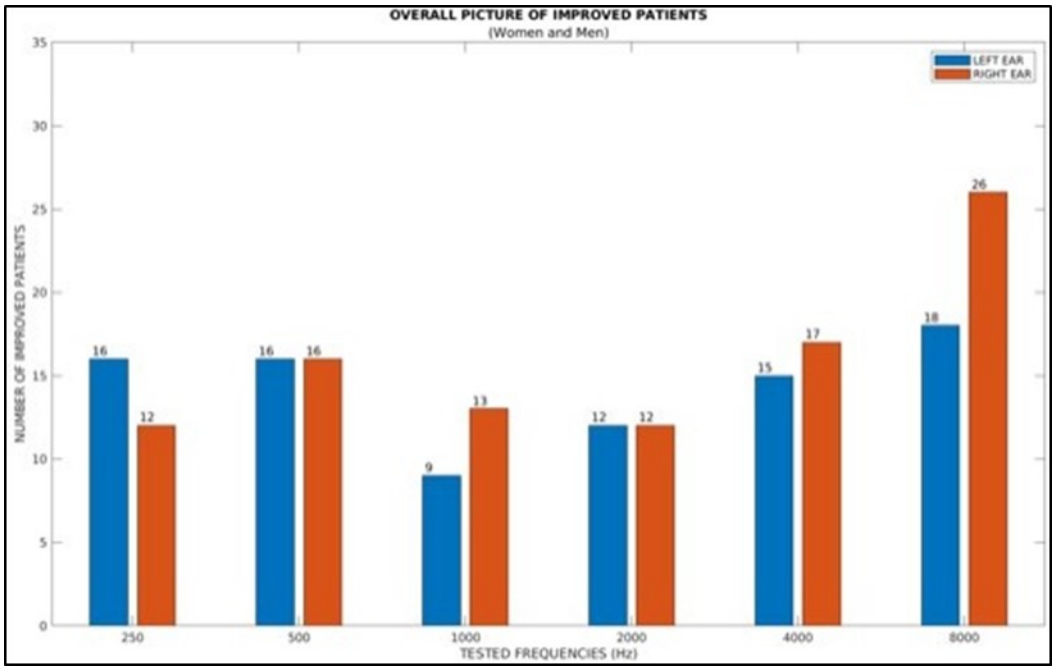

Figure 3. Number of the improvements of the treated patients

patients ranges from 32 to 89 years, the average age was 69. We present here some figures which show the increases in db at the different frequencies respectively in males and females for the single frequencies. A concluding figure of this study demonstrates an overall picture of patients improving on individual frequencies. Specifically, it should be emphasized that the improvements obtained in ten cases on the 8000Hz frequency represent an important recovery where previously this possibility was completely abolished. It is believed that these cases may represent a sure epiphenomenon of cell regeneration. These considerations are also relevant in 2 cases who were affected by sudden hearing loss: they, even following a protocol characterized by the administration of cortisone, vasoactive agents and multiple hyperbaric cycles showed a residual neurosensory damage; the complete recovery of the auditory function in these patients occurred only after the administration of the epigenetic factors. All the patients do not have any side effects; on the contrary they demonstrated an improvement of the performance status and of the quality of life. Last, but not least it should be emphasized that the finding of hearing improvements persists also after many times of expiration of the treatment with the epigenetic factors: it can be considered one indirect evidence of the stability of the molecular mechanisms underlying neuro-regeneration, once started. The figures 1-2 show the results obtained as gain in terms of auditory function at the frequency of 8000 Hz concerning male and female patients. A total number of all improvements obtained in the cohort of patients examined is recorded in figure 3

Discussion

The improvements here described have demonstrated a direct relationship between the administration of epigenetic factors and the defined pathology like neuro sensory hearing loss; this observation constitutes a fact in a specialized field such as otolaryngology, which confirms the breaking of a dogma about the impossibility of tissue regeneration without stem cell transplantation. Indeed the treatments here described have demonstrated a precise correlation between the administration of epigenetic factors and improvement of defined pathology like neuro sensory hearing loss; this observation constitutes a fact in a specialized field such as otolaryngology, which confirms the breaking of a dogma about the impossibility of tissue regeneration without stem cell transplantation.

Furthermore, the overcoming of a reductionist vision is outlined of medicine which opens a new vision towards the medicine of complexity and omics sciences. Indeed a defined holistic approach leaves the theorical contexts and actually enters the context of medical praxis as information medicine. The present observational work carried out, while presenting some limits, as its essentiality does not allow to evaluate the action of the epigenetic factors at the single levels of the auditory function from the organ of the Corti to the cerebral cortex, is fully inserted as a therapy worthy of attention in the context of fragility associated with secretory phenotype (SAPS). In the future observational researches, it will be our concern to submit each patient affected by neurosensory hearing loss on examination with evoked potentials and speech audiometry. It is reasonable to hope that the simple approach, which leverages functional aspects of diagnostics, can become a model of investigation in individual specialist branches to validate the use of cell regeneration factors in degenerative pathologies, still lacking in therapy.

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