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Chapter VI

Morphometrical and Molecular Biological Analyses of Facial Nerves in Healthy Adults and Patients with Facial Nerve Palsy

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Abstract

Details of the aging process in healthy adults and molecular biological aspects in patients with facial nerve palsy have not been available in textbooks. I morphometrically and genetically analyzed peripheral nerves and clarified these points. The materials used to study the aging process were obtained from Japanese cadavers. I performed morphometric analyses of facial nerve fibers and estimated the total number of myelinated axons (TN) in the facial nerve to be 6,245 ± 860 (mean ± SD), and the average transverse area of myelinated axons (ATA)

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to be 6.31 ± 0.81 μm^2 (mean ± SD). The facial nerve showed a significant decrease in TN with increasing age ($r = -0.77$; $p < 0.01$), but showed no significant changes in ATA with age ($r = -0.01$; $p = 0.96$). I assumed that the TN decrease with age was a factor in the delayed recovery from Bell's palsy seen among the elderly. I also performed microarray analysis of gene expression in materials from Japanese patients with Bell's palsy. There were a total of 174 types of fluctuations in genes in patients with moderate dysfunction, and 763 types in patients with severe dysfunction. The total number of genes that matched between the two patient groups included 25 types, while total number of genes that matched in terms of the direction of fluctuation included only 13 types. These numbers indicated that the moderate dysfunction condition differed from that of severe dysfunction. Therefore, I conclude that the gene expression in Bell's palsy changes with the degree of nerve palsy.

Introduction

Details of the aging process in healthy adults and molecular biological aspects in patients with facial nerve palsy have not been available in textbooks. I morphometrically and genetically analyzed facial nerves and clarified these points.

Materials and Methods

Human facial nerves in a narrow sense (special visceral efferent nerves) were resected in the vicinity of the brainstem. The materials were obtained from 20 Japanese cadavers (20 males) aged 24–92 years (average: 64.7). The materials used to study the aging process were all obtained from Japanese cadavers. All the cadavers were donated with the individual's consent. We proceeded to perform this research in accordance with the law concerning autopsy and preservation of corpses, and concerning donations for medical and dental education. In no case was there a history of facial nerve disorders such as Bell's palsy or facial schwannoma, or of treatment with toxic agents or irradiation therapy to the head. The cause of death did not directly or indirectly influence the nervous system, so the facial nerves were considered to be normal. I used right side specimens from males to avoid any interaction between the effects of sex and side.

The methods for section preparation were described in our previous report [1].

Morphometry

I observed the fascicles at low power. I covered the entire area of the distributed myelinated axons in the facial nerve by moving the eyepiece grid vertically and horizontally and confirmed that I could distinguish myelinated structures from vessels in the tissue with a computer or grouped unmyelinated axons with the naked eye in each grid. I counted the myelinated axons and measured the transverse area of the myelinated axons in a square eyepiece grid at high power. To avoid duplicate counts, I counted and measured all axons on the side of the grid that did not come into contact with the other grids. In the case of grids adjacent to other grids, I counted and measured only the axons on the lower right side of the grid, but not those on the upper left side. I used a microscope in transmitted light mode (BX50, Olympus, Tokyo, Japan) equipped with a high-resolution digital camera (ColorView12, Soft Imaging System, Münster, Germany), a motorized XYZ stage (Märzhäuser, Wetzlar-Steindorf, Germany), a stage controller (Märzhäuser, Wetzlar-Steindorf, Germany), and a computer (Precision 530, Dell, Austin, TX, USA) with analyzing system software (analySIS 3.0, Soft Imaging System, Münster, Germany) to store data on-line, do calculations, and perform statistical analyses.

Statistical Analyses

All statistical analyses were performed using JMP statistical software version 9.0.3 (SAS Institute Inc. Cary, NC, USA) on a Macintosh personal computer.

Researchers have studied shrinkage of embedding materials, and found that celloidin and plastination embedding exhibit less shrinkage (around 10%) than paraffin and other embeddings [2]. Therefore, although I measured every myelinated axon, I calculated the average transverse area of myelinated axons after excluding data far from the median (15%) due to shrinkage.

The specimens used were sampled randomly. Two variables (X: age and Y: total number or average transverse area of nerve fibers) indicated a bivariate normal distribution. Thus, we calculated the coefficient of correlation

(Pearson's product moment; hereafter abbreviated as ''r'') between the total number, the average transverse area of myelinated axons, and the subject's age. A *p* value of <0.05 was considered to indicate a statistically significant difference.

Microarray Analyses

The materials for microarray analysis were obtained from two Japanese patients with Bell's palsy. One had severe dysfunction (House-Brackmann facial nerve grading system V) and the other moderate dysfunction (House-Brackmann facial nerve grading system III).

The local Ethics Review Board of Showa University Hospital approved the study protocol according to the principles of Declaration of Helsinki. Both patients received detailed information about the study and provided written informed consent prior to their inclusion. They underwent blepharoplasty of the superior eyelid for blepharoptosis on both sides. Blepharoptosis on the healthy side was caused by the aging process. I used materials from the neuromuscular junction between the facial nerve and palpebral part of the orbicularis oculi.

Immediately after removal of materials, all samples were preserved in RNAlater TissueProtect Tubes® (Qiagen) for stability and the protection of RNA. Affymetrix Human Gene 1.0 ST arrays were used to analyze gene expression levels. I performed microarray analysis of gene expression in both the healthy and the palsy side, and compared gene expression in both sides.

Results

Aging Process

I estimated the total number of myelinated axons (TN) in the facial nerve to be 6,245 ± 860 (mean ± SD), and the average transverse area of myelinated axons (ATA) to be 6.31 ± 0.81 μm^2 (mean ± SD). The facial nerve showed a significant decrease in TN with increasing age ($r = -0.74$; $p < 0.01$; Figure 1), but showed no significant changes in ATA with age ($r = -0.09$; $p = 0.70$; Figure 2).

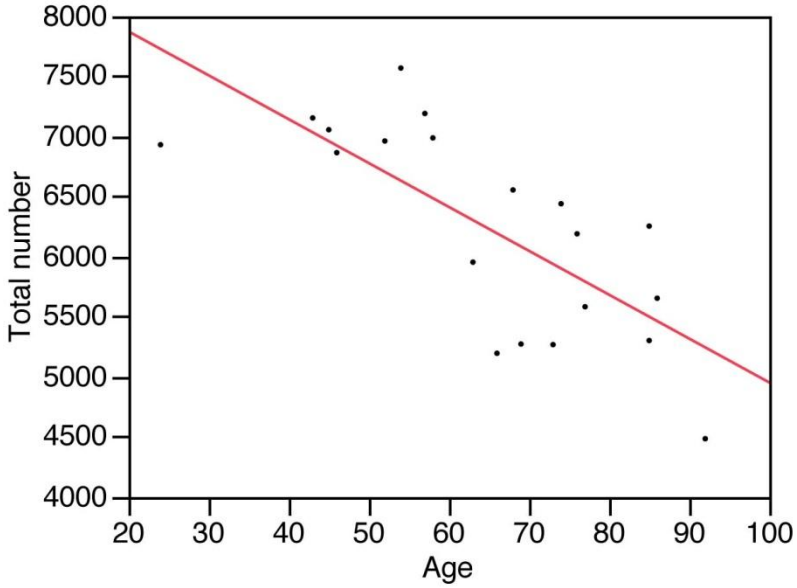

Figure 1. Scatter diagram of the human facial nerve showing a regression analysis of the total number of myelinated axons and age ($r = -0.74$; $p < 0.01$).

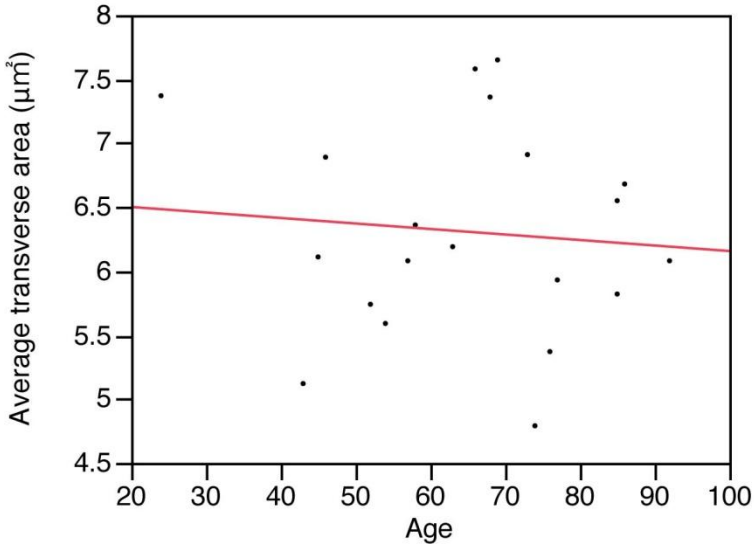

Figure 2. Scatter diagram of the human facial nerve showing a regression analysis of the average transverse area of myelinated axons and age ($r = -0.09$; $p = 0.70$).

Microarray Analyses

Fluctuations in genes were defined as a palsy/healthy side ratio of genes > 1.5 or < 0.5 in this study.

(1) Patient with moderate dysfunction (House-Brackmann facial nerve grading system III)

The total number of fluctuations in genes included 174 types, and this was relatively low. Thus, specific genes seemed to fluctuate. In 100 genes in the epidermis category, 93 were downregulated and three were upregulated. Most of the genes were downregulated. Especially, genes from cells that structure the skin and genes that control and differentiate materials in skin composition were downregulated. With regard to neuron category, six genes were downregulated and one gene was upregulated. Genes in the neuron category also tended to be downregulated. Meanwhile, in 24 muscle category genes, 20 genes were upregulated and four were downregulated. Most genes were upregulated. Genes related to material composition in muscle and genes involved in muscle movement were upregulated. Only two genes fluctuated in the energy category, but both of them were upregulated, and they were important genes in the glycolysis pathway. Except for the above-mentioned functional categories, I could not find any large functional clusters. Some genes involved in cell division and multiplication of cells were downregulated. However, genes linked to apoptosis and stress markers that affected cells were not upregulated.

(2) Patient with severe dysfunction (House-Brackmann facial nerve grading system V)

The total number of fluctuations in genes included 763 types. In 50 genes in the epidermis category, 31 genes were upregulated and 19 were downregulated, while 39 genes were upregulated and 20 were downregulated in the neuron category. Moreover, in the immune and inflammation category, 19 genes were upregulated and 6 were downregulated. Some genes linked to stress markers were also upregulated. Genes in the above-mentioned functional categories tended to be upregulated rather than downregulated. Meanwhile, 88 genes were downregulated and five genes were upregulated in the energy category, while 98 genes were downregulated and 11 were upregulated in the muscle category. Most genes in the energy and muscle categories were downregulated.

Conclusion

Aging Process

As reports by Korczyn [3], Savadi-Oskouei et al. [4], Abraham-Inpijn et al. [5], and Brundage [6] showed that DM, hypertension and climate influence the incidence of BP, I excluded cadavers that had past histories of DM and hypertension, or lived outside of the Tokyo metropolitan area. I initially examined the relationship between my data and the aging process. In general, an important factor in recovery from BP is the age of the patient. For example, Devriese and co-workers [7] reported that increased age was associated with a greater loss of function (paresis). In this study, the TN showed a significant decrease with age ($r = -0.74$; $p < 0.01$; Figure 3), but the ATA showed no significant changes with age ($r = -0.09$; $p = 0.70$; Figure 4). As axonal degeneration on the facial nerve fibers may be one of the significant etiologies of BP, these results indicated that elderly facial nerves have weaker conduction of nerve impulses than younger facial nerves under the same nerve damage conditions. I assume that the TN decrease with increasing age is a factor in the delayed recovery from Bell's palsy among the elderly.

Microarray Analyses

The total number of fluctuations in genes of patients with moderate dysfunction included 174 types, and there were 763 types in patients with severe dysfunction. The total number of genes that agreed between both patients included 25 types, and the total number of genes that agreed with the direction of fluctuation included only 13 types. These numbers indicated that the condition of moderate dysfunction differed from that of severe dysfunction. Most genes in severe dysfunction in the energy category were downregulated. Meanwhile, no genes were downregulated, but rather two genes were upregulated in moderate dysfunction in the energy category. Thus, severe facial nerve neuropathy caused a disorder in the energy production system, but moderate facial nerve neuropathy did not extend to that level. There were 20 upregulated genes and four downregulated genes in moderate dysfunction in the muscle category, but most of the genes in severe dysfunction in the muscle category were downregulated. This showed that there was regeneration of the muscle tissue against moderate dysfunction of

the orbicularis oculi muscle tissue, but there was inhibition of muscle tissue regeneration in severe dysfunction. With regard to neuron category, genes in moderate dysfunction tended to be downregulated, but in the neuron category of severe dysfunction, 39 genes were upregulated and 20 genes were downregulated.

Thus, moderate facial nerve neuropathy tended to the inhibit neuron regeneration, but severe facial nerve neuropathy showed acceleration of neuron regeneration rather than inhibition. In the epidermis category of moderate dysfunction, most genes were downregulated, but in the epidermis category of severe dysfunction, 31 genes were upregulated and 19 genes were downregulated. Therefore, moderate facial nerve neuropathy inhibited the epidermis regeneration markedly, but severe facial nerve neuropathy showed an acceleration of epidermis regeneration rather than the inhibition. Except for the above-mentioned functional categories, I could not find any large functional clusters in moderate dysfunction. However, with regard to severe dysfunction, genes related to immune function and inflammation were upregulated, and some of genes in the stress category were also upregulated. However, genes linked to apoptosis, autophagy and death were not upregulated. Thus, the situation in severe dysfunction barely maintained homeostasis compared with that of complete paralysis.

Considering the circumstances mentioned above, in moderate dysfunction, as some of the facial nerve conduction is cut off, the orbicularis oculi could not move completely and presented with paresis.

To improve this situation, the damaged muscle promoted the regeneration of the muscle tissue. Researchers reported that there was nerve innervation for the energy production system [8]. Thus, in case of denervation, the energy production decreased in the muscle presenting with paresis, but there was some energy production for the regeneration of the muscle tissue in moderate dysfunction. While a large amount of facial nerve conduction was cut off in severe dysfunction, there was very little energy production for the regeneration of the muscle tissue. Thus, regions innervated by the facial nerve did not show much tissue regeneration, but the neuron per se showed acceleration of regeneration.

Therefore, I conclude that the gene expression in Bell’s palsy changes with the degree of nerve palsy.

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