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Clinical Report

A View of the Therapy for Bell's Palsy Based on Molecular Biological Analyses of Facial Muscles

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OBJECTIVE: Details regarding the molecular biological features of Bell's palsy have not been widely reported in textbooks. We genetically analyzed facial muscles and clarified these points.

MATERIALS and METHODS: We performed genetic analysis of facial muscle specimens from Japanese patients with severe (House-Brackmann facial nerve grading system V) and moderate (House-Brackmann facial nerve grading system III) dysfunction due to Bell's palsy. Microarray analysis of gene expression was performed using specimens from the healthy and affected sides, and gene expression was compared. Changes in gene expression were defined as an affected side/healthy side ratio of >1.5 or <0.5.

RESULTS: We observed that the gene expression in Bell's palsy changes with the degree of facial nerve palsy. Especially, muscle, neuron, and energy category genes tended to fluctuate with the degree of facial nerve palsy.

CONCLUSION: It is expected that this study will aid in the development of new treatments and diagnostic/prognostic markers based on the severity of facial nerve palsy.

KEYWORDS: Bell's palsy, facial muscle, microarray analysis, gene expression

INTRODUCTION

Some patients with Bell's palsy do not completely recover, despite treatment with pharmacotherapy and facial nerve decompression surgery. Additionally, some of them do not wish to undergo plastic and reconstructive surgery. Presently, there is no effective treatment for these patients. We performed genetic analysis of facial muscles in patients with Bell's palsy and assessed the molecular biological aspects to obtaining a key for new treatments.

MATERIALS and METHODS

Materials for microarray analysis were obtained from the orbicularis oculi muscles of six Japanese patients with Bell's palsy. Three patients had severe dysfunction (SD; House-Brackmann facial nerve grading system V, females in their 60s) and the other three had moderate dysfunction (MD; House-Brackmann facial nerve grading system III, females in their 60s). Both groups of patients had previously received pharmacotherapy, but had not undergone surgical decompression of the facial nerve. Plastic and reconstructive procedures were performed at 1.5 years after the onset of facial paralysis. Both groups of patients underwent blepharoplasty of the superior eyelid for blepharoptosis on the affected side and blepharoplasty on the healthy side for esthetic reasons. Biopsy materials were obtained from the palpebral part of the orbicularis oculi on the healthy and affected sides. Microarray analysis of gene expression was performed using the GeneChip Human Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA, USA), and gene expression was compared between the two sides. Changes in gene expression were defined as an affected side/healthy side ratio of >1.5 or <0.5.

All statistical analyses were performed using JMP statistical software version Pro 13.0 (SAS Institute Inc. Cary, NC, USA) on a Macintosh personal computer.

The local Ethics Review Board approved the protocol of this study (No. 149), and the study was conducted according to the Ethical Principles for Medical Research Involving Human Subjects in the Declaration of Helsinki. All patients were provided detailed information about the study and gave written informed consent prior to inclusion in the study.

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RESULTS

Table 1 summarizes data obtained from both groups of patients. Only 13 genes showed similar changes in the two groups of patients, and the overall changes indicated that patients with MD differed from those with SD.

Patients with MD

Totally, 174 genes showed changes in expression, which was a relatively low number. Genes in the neuron category using gene functional classification (The term "category using gene functional classification" will hereinafter be abbreviated "category") tended to be downregulated, while most genes showing changes in the muscle category were upregulated. Genes related to muscle components and genes involved in muscle movement were also upregulated. The expression of only two genes related to energy production was altered, but both were important genes in the glycolysis pathway and both were upregulated. Except for these functional categories, we did not find any large functional clusters. Some genes involved in cell division and cell proliferation were downregulated. However, genes linked to apoptosis and stress markers were not upregulated (Table 1).

Patients with SD

Totally, 763 genes showed changes in expression. Among 59 genes with changes in the neuron category, 39 genes were upregulated and 20 were downregulated. Some genes linked to immunity, inflammation, and stress were also upregulated. In contrast, most genes showing changes in the energy and muscle categories were downregulated (Table 1).

Table 1. Comparison of changes in gene expression between patients with moderate dysfunction and those with severe dysfunction

Severity of Bell's palsy	Moderate dysfunction	Severe dysfunction
House-Brackmann facial nerve grading system	**III**	**V**
Gene category		
Muscle	↑20 ↓4	↓↓
Neuron	↓↓	↑39 ↓20
Energy	↑	↓↓
Immunity	→	↑
Inflammation	→	↑
Stress	→	↑
Apoptosis	→	→
Autophagy	→	→
Death	→	→

Changes in gene expression were defined as an affected side/healthy side ratio of >1.5 or <0.5. ↓↓ indicates that most genes in the category were downregulated. ↓ indicates that some genes in the category were downregulated. ↑ indicates that some genes in the category were upregulated. → indicates that expression of genes in the category did not change. Numbers (mean values) on the right side of the arrows indicate the number of genes showing changes in expression.

DISCUSSION

The total number of genes showing changes in expression (mean values for the patients) in patients with MD was 174, while that in patients with SD was 763. The total number of genes that showed changes in expression in both groups of patients was 25, while that of genes that agreed with the direction of the fluctuation included only 13 types. These findings indicated that MD differs from SD in patients with Bell's palsy.

Considering these findings, we can assume that facial nerve conduction is reduced in MD; therefore, the orbicularis oculi muscle cannot function well, and patients present with paresis. To improve this situation, muscle tissue regeneration is promoted. It has been reported that innervation is required for normal energy production in muscles [1]. Accordingly, energy production decreases in muscles with paresis due to denervation, but there was some energy production related to muscle tissue regeneration in patients with MD. Because facial nerve conduction is largely abolished in SD, there was very little energy production in or regeneration of affected muscles. Therefore, muscles innervated by the facial nerve did not show much regeneration, but the neurons showed accelerated regeneration.

On the basis of these molecular biological findings, we suggest that facial nerve neurorrhaphy and nerve grafting are suitable for patients with MD. These procedures will improve facial nerve conduction, leading to increased energy production for muscle tissue regeneration. However, there was very low energy production and little muscle regeneration in patients with SD, although they showed neuronal regeneration acceleration. These findings may explain why facial nerve neurorrhaphy and nerve grafting are unsuccessful if performed more than one year after facial paralysis onset. Authors have reported that recovery is better if the interval between facial nerve injury and nerve repair surgery is short [2-4]. Because improvement in facial nerve conduction is incomplete in patients with SD, we consider that promoting muscle tissue regeneration and energy production is necessary. Regenerative medicine, such as the use of induced pluripotent stem cells, can be used to improve muscle regeneration. However, it will take time for such treatment to become available for clinical use; therefore, we propose the administration of pyruvate or mitochonic acid 5 (MA-5) as a practical means to promote muscle regeneration and energy production. Pyruvate plays a pivotal role in the metabolism of carbohydrates, amino acids, and lipids. It is the end product of glycolysis and can be utilized in any of the three pathways. (1) It is reduced to form lactate and NAD^+; (2) it is oxidized by pyruvate dehydrogenase complex (PDHC) to yield acetyl-CoA; and (3) it is carboxylated by pyruvate carboxylase to form oxaloacetate for the anaplerosis to replenish tricarboxylic acid (TCA) cycle intermediates.

Thus, pyruvate administration restores adenosine 5'-triposphate (ATP) production via the glycolytic pathway. MA-5 targets the mitochondrial protein mitofilin at the crista junction of the inner membrane and can be a novel agent for treating diseases associated with mitochondrial dysfunction [5, 6]. In patients with SD having Bell's palsy, mitochondrial cytopathy may develop with ATP synthesis impairment. As this state is similar to that seen in various mitochondrial diseases, we consider that therapy with pyruvate and MA-5 could be effective [7, 8]. We also propose neurovascular free muscle transfer as an effective surgery for patients with SD having Bell's palsy. Neurovascular free muscle transfer is one of the main reconstruction options for established or long-standing facial paralysis, but it also seems to be suitable for severe Bell's palsy based on our molecular biological findings, and we suggest that neurovascular free muscle

transfer is more effective than facial nerve neurorrhaphy with nerve grafting in patients with SD [9].

CONCLUSION

We conclude that the gene expression in Bell's palsy changes with the degree of facial nerve palsy. It is expected that this study will aid in the development of new treatments and diagnostic/prognostic markers based on the severity of facial nerve palsy.

Ethics Committee Approval: Ethics committee approval was received for this study from the local Ethics Review Board (Approval No: 149).

Informed Consent: Written informed consent was obtained from the patient who participated in this study.

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