Gene Therapy for Voice Disorder

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Abstract

Current treatments for unilateral recurrent laryngeal nerve (RLN) paralysis cannot restore movement to the paralyzed vocal fold.

New treatments for RLN paralysis have been developed with the aim of restoring vocal fold movement. In response, we have conducted basic research in gene therapy, in which we transferred gene encoding neurotrophic factor with potent trophic effects on myoneural function into the injured site and promoted regeneration and restoration. We have demonstrated the following:

1. The effect of insulin-like growth factor-I (IGF-1) gene therapy in preventing the atrophy of the laryngeal muscles (thyroarytenoid muscles) and preserving and regenerating motor endplates and nerve fibers.
2. The effects of glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) gene therapy in preventing the loss of motoneurons in the nucleus ambiguus.
3. The effect of GDNF gene therapy in restoring nerve function in an RLN crush model.

These results show that gene therapy for RLN paralysis can at least be utilized to reinforce the effects of current treatment with surgery, and could potentially make surgery unnecessary in some cases.

Key words Gene therapy, Recurrent laryngeal nerve regeneration, Recurrent laryngeal nerve paralysis, IGF-I, GDNF, BDNF

Introduction

Current and future treatment of recurrent laryngeal nerve paralysis

Recurrent laryngeal nerve (RLN) paralysis, one of conditions that causes voice disorders, is caused by direct invasion of thyroid cancer, esophageal cancer, and lung cancer and being after surgery of these cancers, and aortic aneurysm, intratracheal intubation, upper respiratory virus infections, and so on. In the case of unilateral RLN paralysis, impairment of motion in unilateral vocal fold prevents glottal closure and causes hoarseness and aspiration. Occurrence in conjunction with aspiration pneumonia could be life-threatening.

Currently, unilateral RLN paralysis is primarily treated surgically with thyroplasty and intracordal injections. However, these surgeries only statically shift the paralyzed vocal folds internally, and do not restore movement to the paralyzed vocal folds. Surgery to restore innervation is also carried out, but this cannot be expected to restore vocal fold mobility. Accordingly, further research aimed at restoring vocal fold mobility is essential when developing new treatments for RLN paralysis in the future.

This paper discusses the gene therapy for RLN paralysis that we have conducted with the aim of restoring mobility to paralyzed vocal folds.

Neurological problems to be resolved to restore vocal fold mobility

In order to restore vocal fold mobility for RLN paralysis, we must resolve the neurological problems caused after the nerves are damaged, namely (1) motoneuron loss in the nucleus ambiguus, (2) degeneration and poor regeneration of nerve

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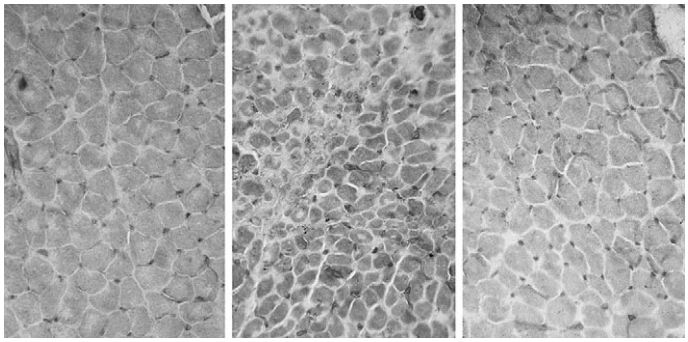
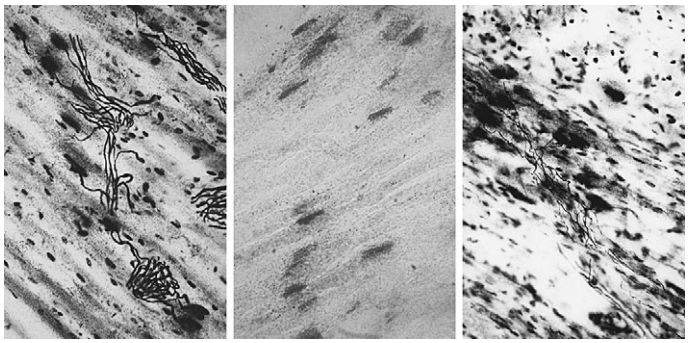


Normal Untreated control IGF-I gene therapy

Fig. 1 Effect of IGF-I gene therapy on muscle atrophy

Left: Cross-section of normal rat thyroarytenoid muscle.
Middle: Rat thyroarytenoid muscle 4 weeks after nerve injury (untreated control group), showing prominent muscle fiber atrophy.
Right: Observation in rat thyroarytenoid muscle 4 weeks after nerve injury immediately followed by IGF-I gene transfer, showing mitigation of atrophy.

(Extracted from Shiotani A, et al.[1])



Normal Untreated control IGF-I gene therapy

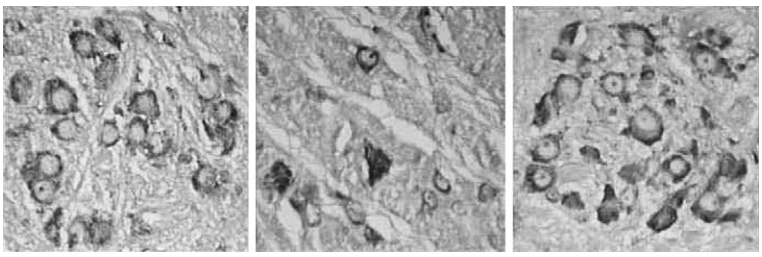
Fig. 2 Effect of IGF-I gene therapy to prevent motor endplate degeneration and promote nerve fiber regeneration

Left: Motor endplates in normal rat thyroarytenoid muscle.
Middle: Motor endplates in rat thyroarytenoid muscle 4 weeks after nerve injury (untreated control group), showing disappearance of nerve fibers and elongated major axes of endplates, which indicates denervation changes.
Right: Observation 4 weeks after nerve injury immediately followed by IGF-I gene transfer, showing endplates keeping contact with regenerating nerve fibers.

(Extracted from Shiotani A, et al.[1])

fibers and motor endplates, and (3) atrophy of the laryngeal muscles. Moreover, even if the RLN are successfully regenerated, nonselective nerve regeneration may result in (4) faulty innervation, meaning that the wrong neurons innervate other laryngeal muscles, and proper motor function is not restored. This problem must also be resolved.

These problems are well known, but there are no effective treatments. However, recent developments in neuroscience have contributed to the discovery of a range of neurotrophic factors, which have been shown to be effective in promoting regeneration due to their potent trophic effect on myoneural function (motoneurons, motor nerve fibers, motor endplates,



Normal Untreated control GDNF gene therapy

Fig. 3 Effect of GDNF gene therapy to prevent loss of motoneurons
Left: Motoneurons in normal nucleus ambiguus.
Middle: Motoneurons in nucleus ambiguus 4 weeks after nerve injury (untreated control group), showing remarkable loss of motoneurons.
Right: Observation 4 weeks after denervation immediately followed by GDNF gene transfer, showing reduced loss of motoneurons.
(Extracted from Saito K, et al.[6])

muscle tissue, etc.) and in protecting it from damage. These neurotrophic factors include brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and insulin-like growth factor-I (IGF-I), and are expected to be applied in treating RLN paralysis.

Applications of gene therapy to RLN paralysis

Why is gene therapy necessary?

In the clinical application of neurotrophic factors, the simplest administration is to directly inject neurotrophic factor proteins into sites such as the atrophied laryngeal muscle and the injured RLN fibers. However, to sustain the effect of these neurotrophic factors over time, injections must be administered several times a day over a long period of time. Systemic continuous administration is another option, but can result in systemic side effects. This is why a method for gene transfer (gene therapy) is recommended for use in this context.

Instead of injecting the neurotrophic factor, using a gene carrier or vector, the gene (DNA) encoding the neurotrophic factor is conveyed to human cells. Thus, the neutrophic factor can be created in the cells. With one shot of vector, the neurotrophic factor is expressed in high concentration at this site for a few weeks and up to a month. Local gene expression has a smaller risk of adverse reactions compared to systemic administration.

Effects of gene therapy

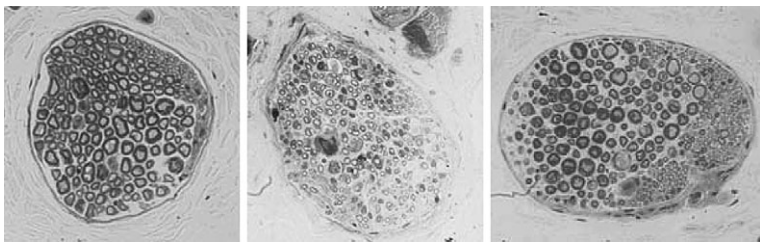
As described above, there are neurological problems that must be solved to restore vocal fold mobility. Our research demonstrates that gene therapy has the following effects.

Gene therapy for atrophied laryngeal muscles (thyroarytenoid muscles)

In order to prevent muscle atrophy resulting from nerve injury, we carried out experiments in which formulated plasmids were used to transfer IGF-I genes with potent trophic effects to the muscle cells and nerve cells of a rat's thyroarytenoid muscles immediately after the RLN is severed.[1–3] In four weeks after the gene transfer, the gene therapy group demonstrated significant increase in muscle fiber diameter and mitigation of muscle atrophy compared to the untreated control group (**Fig. 1**). In addition, the gene therapy group showed a significant improvement in peripheral nerve regeneration and histologic protection of motor endplates (**Fig. 2**). These findings demonstrated that IGF-I gene therapy was effective in preventing laryngeal muscle atrophy and promoting peripheral nerve regeneration. The effects of this kind were recognized in a chronic model one month after nerve transaction.[4,5]

Gene therapy for motoneuron loss in nucleus ambiguus

In order to control motoneuron loss resulting from nerve injury, we resected the vagus nerve at the level of the jugular foramen to create a model of induced motoneuron loss in the rat



Normal Untreated control GDNF gene therapy

Fig. 4 Effect of GDNF gene therapy to promote axon regeneration

Left: Cross-section of a normal RLN fiber, showing many myelinated axons.
Middle: Cross-section of a RLN fiber 4 weeks after nerve injury (untreated control group), showing demyelination and atrophy of many axons.
Right: Observation 4 weeks after nerve injury immediately followed by GDNF gene transfer, showing mitigation of axonal atrophy and preservation of many myelinated axons.

(Extracted from Araki K, et al.[8])

nucleus ambiguus. An adenovirus vector was used through the jugular foramen to selectively transfer the GDNF gene to nucleus ambiguus motoneurons.[6] Four weeks after gene transfer, compared to the untreated control group, the GDNF gene therapy group showed a significantly lower loss of nucleus ambiguus motoneurons (motoneuron survival rate at four weeks: 71.3 ± 3.2% in the GDNF gene therapy group versus 55.1 ± 4.0% in the untreated control group) (**Fig. 3**).

Moreover, concurrent transfer of the GDNF gene and the BDNF gene resulted in a significantly lower rate of loss of nucleus ambiguus motoneurons (motoneuron survival rate at four weeks: 84.7 ± 2.2% in the GDNF and BDNF gene therapy group).[7]

From these findings, it can be demonstrated that GDNF gene therapy and BDNF gene therapy can suppress the loss of motoneurons in the nucleus ambiguus stemming from nerve transection and can facilitate nerve regeneration as well.

Gene therapy to restore nerve function

The research results described above confirmed the morphological effects of gene therapy, but we also used the RLN crush model to evaluate the extent to which nerve functions can be restored.[8,9] Accordingly, we crushed the RLN in rats using hemostatic forceps, after confirming vocal fold fixation, we injected an adenovirus vector carrying the GDNF gene into the nerve bundle at the crush site. Two and four weeks after the gene transfer, we evaluated RLN conduction velocity and vocal fold mobility. This revealed that the GDNF gene therapy group demonstrated RLN conduction velocity that was significantly faster than the untreated control group at two weeks and four weeks, and had almost normalized. At two and four weeks, the untreated control group had restored 12.5% and 37.5%, respectively, of its vocal fold mobility, while the GDNF gene therapy group achieved 100% restoration at two and four weeks. This demonstrated that the therapy had a significant effect in restoring vocal fold mobility.

In addition, cross-sections of RLN samples stained with Epon-toluidine blue demonstrated that the axis of the untreated control group had atrophied and the nerve sheath had also thinned, while the axis of the GDNF gene therapy group had become less atrophied and become significantly myelinated (**Fig. 4**). This indicates that myelination achieved in the normalization of the axis plays an extensive role in improving nerve conduction velocity.

Conclusion

The results of our research using rat models thus far have shown that we can expect a recovery in vocal fold capacity by injecting a gene vector directly into the laryngeal muscles and a recovery by injecting the gene vector during surgery and when the laryngeal nerves are damaged in an external injury. Moreover, we believe that gene therapy can be used in conjunction with conventional surgery such as surgery for restoring

innervation to enhance the effect.

Currently, we are examining the extent to which gene therapy can resolve the problem of faulty innervation, and are proceeding with research on the clinical applications of an extremely safe new virus vector[10] as well as research examining endogenous neural stem cells (neural progenitor cells) in the nucleus ambiguus.[11] We hope to continue to dedicate ourselves to this research.

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