Center for Gene & Cell Therapy

Division of Molecular and Medical Genetics
分子遺伝医学分野

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To promote the clinical development of gene therapy in Japan, we have been developing facilities and fundamental technologies for the manufacture of viral vectors such as adeno-associated virus (AAV) vectors and lentivirus vectors under Good Manufacturing Practices (GMP) grade. We are also developing treatments for intractable rare diseases using AAV, next-generation AAV vaccines, new cancer gene therapies, and treatments for Duchenne muscular dystrophy (DMD) using mesenchymal stromal cells (MSC).

Virus vector-related technology development

Parvovirus-based vectors, including adeno-associated virus (AAV) vectors, are regarded as one of the most promising modalities for gene therapy and vaccine development, as they enable efficient delivery of therapeutic genes to a wide range of tissues while minimizing adverse effects. However, gene therapy products based on AAV vectors that have been approved in recent years have reached record-high prices, raising concerns about the financial burden on healthcare insurance systems and posing a significant societal challenge. This issue largely stems from the complexity and labor-intensive nature of AAV vector manufacturing processes.

The production of AAV vectors can be broadly divided into three stages: (i) upstream processes, including cell culture and AAV production; (ii) downstream processes, involving purification and separation of crude viral preparations; and (iii) quality control (QC) processes, which determine the final quality of the vectors. To address these challenges, the Okada Laboratory has been developing a range of efficient and high-quality AAV vector manufacturing and quality control technologies at each stage of the production pipeline, as outlined below.

(i) Upstream processes: development of original, novel AAV vector–producing cell lines; development of novel plasmids that enhance AAV vector yield and increase the proportion of full capsids; and development of novel nonwoven scaffold materials for cell culture.

(ii) Downstream processes: development of a novel ultracentrifugation-based technology that enables rapid purification of large quantities of AAV vectors (zonal ultracentrifugation); and development of a novel AAV vector purification method using surfactants and hollow-fiber systems.

(iii) QC processes: development of a novel method for discriminating full AAV particles using solid-state nanopores.

In addition, the limited packaging capacity of AAV vectors (~4.8 kb) represents a major bottleneck for the delivery of recently developed genome-editing tools. To overcome this limitation, we are developing human bocavirus (HBoV) vectors capable of packaging larger genetic cargos of up to 6.1 kb. HBoV, like AAV, belongs to the family Parvoviridae and is

primarily known as a respiratory virus. HBoV vectors are expected to enable the delivery of genome-editing tools such as base editors and prime editors, which are difficult to accommodate within AAV vectors.

Development of next-generation AAV vaccines

We are applying our proprietary AAV vector-related technology as a "next-generation AAV vaccine modality" to implement safe and effective vaccines for various infectious diseases and neurodegenerative diseases. AAV vectors are expected to be an excellent vaccine modality due to their stability and high immunogenicity. Using COVID-19 as a model disease, we have established standards for an AAV vector vaccine that effectively induces immunity with a small dose, and in animal experiments, we have confirmed that a single dose of the vaccine provides protection equivalent to two doses of an existing mRNA vaccine (mRNA-1273). We are currently promoting the development of efficient gene expression methods that escape neutralizing antibodies, new modalities through conjugation with exosomes, and a new nucleic acid vaccine targeting rabies. We have established a small-scale GMP manufacturing facility capable of producing viral vector vaccines for clinical trials, which supports gene therapy research and production under normal conditions, as well as rapid vaccine manufacturing in response to infectious disease outbreaks.

Novel treatment for DMD with MSCs

Mesenchymal stromal cells (MSCs) have emerged as a promising novel treatment for Duchenne muscular dystrophy (DMD). MSCs offer several potential benefits for patients with DMD, including muscle regeneration, anti-inflammatory effects, and paracrine signaling. MSCs can differentiate into muscle cells, potentially replacing damaged dystrophin-expressing cells and improving muscle function. In addition, MSCs secrete anti-inflammatory cytokines that can modulate immune responses, thereby reducing inflammation and protecting muscle tissue from further damage. MSCs also secrete bioactive molecules that promote tissue repair and regeneration, enhancing the survival and function of existing muscle cells. Several clinical trials are currently evaluating the safety and efficacy of MSC-based therapies for DMD. To date, we have developed a human amnion-derived mesenchymal stromal cell (hAMSC) therapy for the treatment of DMD in collaboration with Kaneka Corporation, which has recently initiated a clinical trial. While MSC therapy holds significant promise for the treatment of DMD, the remaining challenges include elucidating the underlying mechanisms of action and developing combination approaches with gene therapy aimed at achieving a fundamental cure. Ongoing research is directed toward addressing these challenges to further enhance the therapeutic potential of MSCs for the effective treatment of DMD.

Development of gene therapy using lentiviral vectors

In recent years, *ex vivo* gene therapy, chimeric antigen receptor (CAR) T-cell therapy, has been approved for the treatment of hematological malignancies, including leukemia and malignant lymphoma, and its use is increasing due to its high efficacy. CAR-T cells are genetically modified T cells that bind to and kill tumor cells via the transduced CAR and are mainly produced using lentiviral vectors. We are working to develop next-generation CAR immune cell therapy by overcoming the problems of current autologous CAR-T cell therapy, such as insufficient persistence *in vivo*, long production time, and low efficacy against solid tumors. In the context of *in vivo* gene therapy for genetic diseases, lentiviral vectors that enable long-term stable gene expression are also attracting attention. Our laboratory is utilizing our expertise in optimizing AAV vector production to develop methods for producing lentiviral vectors suitable for *in vivo* administration.

Publications

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