TaKaRa

RetroNectin™

in Gene Therapy Related Studies

BIBLIOGRAPHY

Characteristics and Properties of RetroNectin™

RetroNectin™ (CH-296) is a chimeric peptide of recombinant human fibronectin fragments. Takara's proprietary expression system constructed in *E. coli* efficiently generates fragment variations as shown in Fig. 1: Fibronectins (FNs) are multi-functional cell adhesive glycoproteins present in extracellular matrix and plasma. RetroNectin™ is comprised of 574 amino acids (63 kDa) and has three functional domains; central cell-binding domain (type III repeat, 8, 9, 10), heparin-binding domain II (type III repeat, 12, 13, 14), and CS-1 site within the alternatively spliced IIICS region.

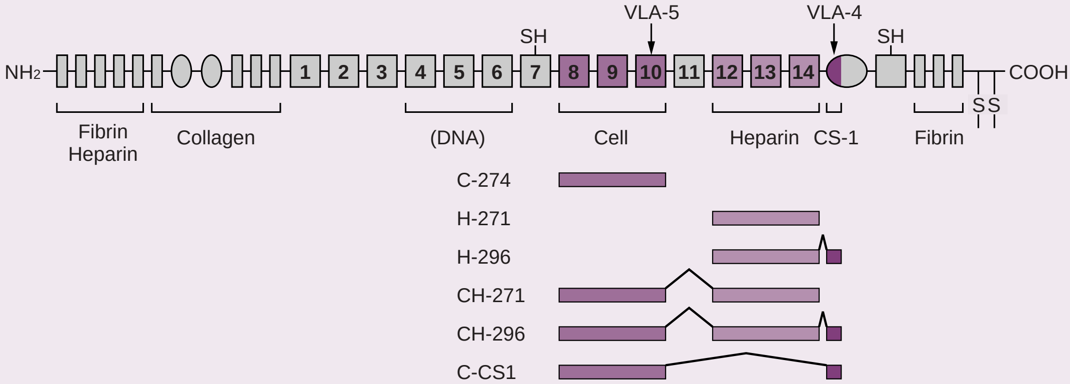

Fig. 1. Structure of FN and Fragments including RetroNectin™

Kimizuka F, et al. Production and Characterization of Functional Domains of Human Fibronectin Expressed in *Escherichia coli*. *J. Biochem.* 110, 284-291 (1991)

When coated on the surface of any containers such as culture dishes, well plates, flasks or bags, RetroNectin™ significantly enhances retrovirus-mediated gene transfer into mammalian cells. This enhancement is hypothetically attributed to the colocalization of retroviral particles and target cells on the molecules of RetroNectin™ (Fig. 2). Virus particles bind to this molecule through the interaction with the heparin-binding domain II. Target cells can be localized on RetroNectin™ mainly through the interaction of the fibronectin CS-1 site with very late antigen 4 (VLA-4) and/or through the RGDS sequence in repeat 10 of fibronectin Cell-binding domain with very late antigen 5 (VLA-5).

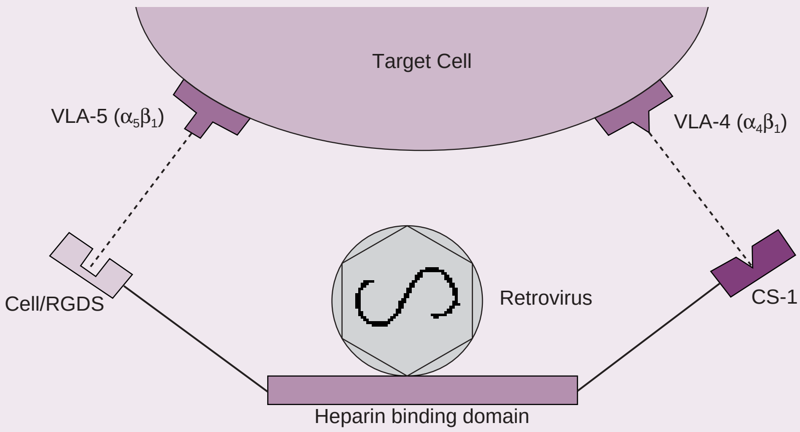

Fig. 2. Hypothetical Mechanism of Transduction Enhancement by RetroNectin™ (Recombinant Human Fibronectin Fragment CH-296)

Hanenberg H, et al. Colocalization of Retrovirus and Target Cells on Specific Fibronectin Fragments Increases Genetic Transduction of Mammalian Cells. *Nature Medicine* 2(8), 876-882 (1996)

Meeting Challenges Toward the Gene Therapy Reality in the 21st Century

TAKARA BIO INC. has contributed greatly to the progress in gene transfer and related fields including hematopoietic cell gene therapy by supplying a recombinant human fibronectin fragment CH-296, or RetroNectin™ for over half a decade. RetroNectin™ is a key component in successful clinical protocols for retroviral-mediated cell transduction in terms of safety and efficiency.

Notes:

*1 FN CH-296 for Clinical Trial Use is produced at GMP facilities in compliance with related regulations, while commercially available RetroNectin™ for Research Use Only is manufactured at completely separate facilities.

*2 RetroNectin™ related patents TAKARA BIO holds:
Patents Issued: US Patents No. 5198423, No. 5686278 & No. 6033907 and European Patent No. 399806
Patents Pending: US Patent Applications No. 09/043,981 & No. 09/394,867 and European Patent Applications No. 95914912.1 & No. 96933209.7

The year 2000 was a watershed in the gene therapy field. In addition to the successful outcomes of the human X-SCID gene therapy in France, there have been conspicuous achievements in clinical trials in several other countries. The latest trials that have been treating congenital diseases were presented at the 5th Annual Meeting of the American Society of Gene Therapy (ASGT) as interim reports of ongoing trials.

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Candotti F, Podsakoff GM, Schurman SH, et al. Evaluation of Adenosine Deaminase (ADA) Gene Transfer by Retroviral Vectors for the Treatment of SCID. *Mol Ther* 5(5), Abstract 888, p. S289 (2002)

Aiuti A, Slavin S, Aker M, et al. Correction of ADA-SCID by Stem Cell Gene Therapy Combined with a Non-Myeloablative Conditioning. *Mol Ther* 5(5), Abstract 931, p. S304 (2002)

There have been several comprehensive review articles published that summarize the past progress and recent advances in strategies for efficient and optimized gene transfer into hematopoietic cells. Several also foresee a wide range of applications to be realized as the choice of therapy in the 21st century. However, there will be many problems to be addressed and overcome.

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Thomas AK, June CH. The Promise of T-Lymphocyte Immunotherapy for the Treatment of Malignant Disease. *Cancer J* 7, Suppl 2, S67-S75 (2001) [Review]

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Dick JE. Gene Therapy Turns the Corner. *Nature Medicine* 6(6), 624-626 (2000) [Comment]

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Improved transduction protocols and successful translation of preclinical methods, which have been developed and evaluated *in vivo* with animals from mice to canines to non-human primates, have enhanced human trial outcomes. Improvement in therapeutic strategies for gene transfer into hematopoietic cells continues to develop in order to modulate immune responses, to protect hematopoietic cells against cytotoxic drugs or viral genes, and to restore congenital or acquired gene deficiencies.

Cell transplantation following cancer treatment such as chemotherapy has been expanded to utilize gene therapy in order to introduce drug-resistance genes for protecting hematopoietic cells and the HSV-tk suicide gene for controlling GVHD in allogeneic transplantation that are widely practiced for the treatment of malignant diseases. A further use envisioned is immunogene therapy to increase the immunogenicity of tumor cells or cytotoxicity of specific cells to kill tumors. A variety of adoptive cellular immunotherapy strategies using *ex vivo* gene transfer have aimed at boosting the immune system; these *ex vivo* strategies include gene delivery into cellular components of the immune system, such as cytotoxic T cells, NK cells, macrophages and dendric cells.

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A report by French physician-scientists suggests a successful application of gene transfer methods in the treatment of two children with severe combined immunodeficiency (SCID) due to defective interleukin 2 receptor common gamma chain. The protocol used in this clinical trial was derived from a number of preclinical and basic studies leading to improved transduction of Hematopoietic stem and primitive progenitor cells using retrovirus vectors. These improvements have also been shown to impact transduction of a long-lived progenitor cell in a chemotherapy protocol in cancer patients. The improved results of these human trials come during a period of increased scrutiny and criticism of human gene therapy trials, due, in part, to significant toxicities in some trials using adenovirus-based vectors. The potential efficacy versus toxicity of phase I trials of human gene therapy is also under question. After many years of research, however, there appears to be real evidence that genetic diseases may be successfully treated by gene transfer techniques. Future clinical studies should be based on continued progress in the understanding of the toxicology of gene delivery systems, vector technology, and target cell manipulation.

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As the ultimate goal is the achievement of successful gene therapy, it is essential to transfer specific genes into target cells at high efficiency, and to maintain long-term stable expression by optimizing transduction conditions. Ongoing optimization efforts are focused on establishing better transfer systems by modifying oncoretroviral vector designs and pseudotyping with alternative envelope proteins, and by incorporating safety-modified HIV-1 based lentiviral vectors, together with further understanding of the biology of hematopoietic cells. The development of simple and clinically applicable fibronectin (FN CH-296)-assisted protocols that obviate the need for co-cultivation and extended *ex vivo* manipulations with multiple exposures to viruses has been improving gene therapy trials and will make them more successful.

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Gene transfer into haematopoietic stem cells has become an important strategy to tackle a number of inherited disorders of blood cell and immune system development. It can also be used in the treatment of leukaemia and other cancers if drug resistance genes are inserted into non-malignant stem cells before the tumour cells are attacked with chemotherapeuticals (Abonour et al., 2000).

High-rate virus production in specific cell lines and pseudotyping these viruses with foreign proteins, such as Gibbon ape leukaemia virus and RD114 feline retrovirus envelope, significantly increase the ability of these vectors to specifically transfect their target cells. Researchers have modified retroviral vectors to enhance expression and avoid silencing of the gene after its insertion in the target cell's genome. Another important finding is that fibronectin and CH-296, a recombinant fragment of this protein, can significantly improve the efficiency of retroviral gene transfer. In addition, a better understanding of how growth factors regulate the development of blood and immune cells enables researchers to direct their vectors to the right stem cells. For instance, it has recently been shown that a combined use of various cytokines can induce the proliferation of human CD34 (+) CD38 (-) cells. The pretreatment makes these primitive haematopoietic cells permissive for integration of onco-retroviral provirus into their genome without inducing further differentiation. Stem cells with the therapeutic gene do not differentiate and thus get lost, but produce transduced daughter cells for a long time.

Although they come with limitations, these findings have provided sufficient results in gene therapy research to make clinical applications possible in selected settings.

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Note : Shown above are a limited number of citations among those that have been published since 1996 reporting results obtained from studies using FN CH-296 or RetroNectin™. Most articles published in the 1990's are not included, except those of seminal nature, due to space constraints. *SYP, Aug 2002*

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