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|  Immune Disease Institute | Office of Technology Development 800 Huntington Avenue Boston, Massachusetts 02115 www.idi.harvard.edu |
| RNAi PORTFOLIO | SMALL INTERFERING RNAs FOR HUMAN THERAPY & PREVENTION OF DISEASES |

Dr. Judy Lieberman, Dr. Premalata Shankar and Dr. Manjunath N. Swamy are pioneers in the area of RNA interference (RNAi) being the first to demonstrate the therapeutic value of small interfering RNAs (siRNAs) *in vivo*. In a landmark experiment, Dr. Lieberman and her colleagues infused a mouse with a solution of siRNAs targeting the *Fas* gene, which protected the animal from fulminant autoimmune hepatitis. Since then, this group of scientists have expanded their understanding of RNAi and further validated the enormous potential of this technology in the development of human therapeutics. Their successes are summarized below.

Human Targets - Therapy

CCR5 (HIV) Studies show that siRNAs targeting the *CCR5* gene inhibit the expression of the corresponding protein and prevent entry of HIV into *CCR5*-expressing, target cells, *e.g.*, macrophages. Additionally, recent work has yielded novel siRNA constructs that have been shown to suppress viral replication in established HIV infection. A sequence that suppresses primary viruses from five clades has been identified. Thus, these novel constructs utilizing post-transcriptional gene silencing, provide means of both preventing and treating HIV infection.

Fas Targeting the *Fas* gene with siRNAs results in the reduction of *Fas* expression in hepatocytes. Recent studies also show that siRNA duplexes targeting renal *Fas* protect mice from acute renal failure after an ischemic episode. Thus, silencing the *Fas* gene may provide a means to prevent or treat myocardial, cerebral or renal infarction resulting from ischemia or reperfusion injury as well as hepatitis, liver cancer and hepatic allograft rejection.

Flavivirus Infection - Therapy

The genus Flavivirus comprises 70 members and is transmitted to humans by ticks and mosquitoes, making the diseases very difficult to eradicate. The important members of this genus are yellow fever virus, dengue fever virus and the Japanese encephalitis virus, all of which cause fatal infections worldwide. The West Nile virus, which has recently appeared in the United States, is also a member of this genus. Studies show that

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siRNAs that target certain conserved regions of the flaviviral genome results in the inhibition of the expression of the corresponding RNA sequences. This in turn suppresses viral replication in established flavivirus infection in cells such as mononuclear phagocytes or megakaryocytes.

Topical Microbicide – STD Prophylaxis

siRNAs that down-regulate viral gene expression or down-regulate the cellular receptors necessary for infection can prevent entry and/or replication of the virus or microbe into target cells such as macrophages and T cells. The preferred method of prophylactically delivering the siRNAs into human subjects consists of applying a topical composition consisting of naked double-stranded siRNAs conjugated to a pharmaceutically acceptable carrier. This composition would then be applied to a mucosal membrane to provide protection against HIV and other sexually transmitted diseases including papilloma, hepatitis B and numerous herpes viruses. Recent studies have shown that siRNA delivery and gene silencing can be achieved in the mouse vagina and can protect against lethal HSV-2 infection in mice.

Cell Specific Delivery

This is a novel approach to targeted delivery of siRNAs into desired cells, both *in vitro* and *in vivo*, avoiding entry into non-targeted cells. This method utilizes a delivery vector comprising a cell-targeting moiety that will bind to a specific molecule on a target cell and an RNA interference-binding moiety that will bind to the RNA of interest. Studies have shown that this vector when combined with a siRNA selectively delivers the siRNA into cells expressing a specific surface antigen. This results in the silencing of gene expression only in those cells that express the surface antigen. The efficacy of this type of targeted delivery was also demonstrated *in vivo* using both subcutaneous and intravenous administration in a tumor model.

Constructs

Multiple constructs have been shown to inhibit the expression of both human and viral genes:

- construct that acts on the invariant regions of all five HIV clades
- constructs that target *Fas* for the treatment of reperfusion injury
- constructs that target *Fas* for the treatment of hepatitis, liver cancer
- multiple constructs that target members of the Flavivirus genus.

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