RESEARCH NEWS

Short-order Sindbis vector targeting

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Let's say you were interested in targeting a therapeutic gene to a specific cell type. Wouldn't it be nice if you were able to simply order an antibody from your favorite catalog, mix the antibody with your gene delivery vector, and—voila! Target your vector to the correct cell. Until recently, that was only a dream to those interested in receptor-targeted gene delivery. However, that is just the system reported in this issue by Meruelo and colleagues[1], using a Sindbis viral vector.

They describe the design and production of a Sindbis virus vector comprising a chimeric E2 viral envelope and an optimized IgG binding domain of *Staphylococcus aureus* protein A. The insertion of an optimized protein A sequence into the E2 envelope protein gene blocked binding of the virus to its native high-affinity laminin cellular receptor. The virus was then specifically targeted to different cellular receptors by mixing different receptor-specific IgG monoclonal antibodies with the chimeric Sindbis virus (see Fig. 1). The authors demonstrated the versatility of the system by targeting the virus to transduce cells expressing the cellular receptors CD4, CD33, or human leukocyte antigen (HLA) via antibodies directed toward these molecules.

Targeted gene therapy has been pursued by a number of academic and industrial research groups. An entire Cold Spring Harbor conference[2] was recently devoted to vector targeting strategies for therapeutic gene delivery. In general, groups have attempted to target viral vectors through the genetic incorporation of the targeting moiety into the virus coat[3,4] or by covalently/noncovalently linking the targeting moiety to the viral vector[5,6].

The principal problems encountered in targeting gene delivery have been loss of the viral vectors' original transduction efficiency, ablation of virus binding to its native receptor, and difficulties encountered in incorporating targeting moieties into the virus coat and/or in synthesizing and characterizing exogenously added targeting moieties.

For example, groups trying to target retrovirus have been hampered by the often dramatic reductions in transduction efficiency when new ligands are added to the retrovirus coat proteins[7]. These reductions in efficiency may result from the interference of the targeting ligand with the normal entry mechanism of retrovirus. In targeting adenovirus, transduction efficiency does not appear to be severely affected by the targeting moiety. However, ablation of native adenovirus receptor binding, as well as the ease of synthesizing exogenous targeting moieties, have been somewhat problematic.

The use of bispecific molecules that both neutralize native receptor binding and redirect virus binding to a targeted receptor has been successful[5]. However, production, purification, and characterization of the bispecific molecule, as well as the quantities of ligands that are often required for chemical crosslinking, are cumbersome obstacles to rapidly assessing targeting strategies.

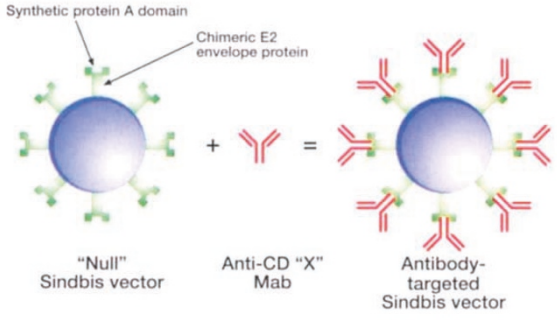

Figure 1. Chimeric Sindbis vectors can be targeted to cells "to order" using monoclonal antibodies (Mabs) against receptors of interest (CD "X").

The approach successfully used for Sindbis virus largely avoids the difficulties above. It is possible to target the vector using small amounts of unmodified, commercially available antibodies by creating a single, "null" vector that lacks native receptor binding and that is capable of binding IgG antibodies. Using this vector, it now should be possible to screen many receptors for their usefulness in targeted gene delivery without significant decreases in transduction efficiency, the presence of native receptor binding, and the need for bispecific targeting moieties.

Despite the ease and simplicity of the Sindbis system, numerous issues remain unresolved. The Sindbis system is rather "off-the-beaten-path" of vectors commonly employed in virus-mediated gene therapy, such as adenovirus, adeno-associated virus, retrovirus, and lentivirus. As such, this vector system is not without its recognized, and as-yet-unrecognized, difficulties. In its current form, the vector is known to cause apoptosis and cell death in transduced cells. However, manipulations of the genome, as well as the development of suitable packaging cell lines, could overcome this limitation.

Another current drawback of the vector is that it is an enveloped RNA virus in which production of viable vectors requires transfection of helper plasmids into packaging cells. There is also a significant, unexplained reduction in viral titers on some of the targeted cell lines. Finally, Meruelo and colleagues only report in vitro data using their system, so the effectiveness of the system in vivo is unclear. For example, it is not known whether the IgG–protein A affinity is high enough to keep the antibodies associated with the virus in vivo. Also, the presence of the Fc regions on the IgG may complicate targeting and activate immune clearance mechanisms through binding to Fc receptors.

Regardless of these hurdles, the greatest advantage of this vector targeting system is its ease and simplicity. As a tool, the system will be quite useful in rapidly assessing different tissue-specific targeting strategies to be used in gene delivery. With further modifications to the system, it should also be possible to genetically incorporate specific targeting domains into the virus. Such targeting domains could include portions of receptor ligands, high affinity peptide-binding motifs, or single chain antibodies. The results of such studies will not only be useful for those working with individual vector targeting systems, but especially for those interested in applying gene targeting technology to the treatment of human disease.

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