Targeting Retroviral Vectors to Specific Cells

N. Kasahara *et al.* (*1*) report that a retroviral vector packaged with a chimeric envelope glycoprotein, consisting of the polypeptide erythropoietin (EPO) fused to part of the *env* protein of ecotropic Moloney murine leukemia virus, infects human cells that express EPO receptors. Retroviruses able to target specific cells would have applications for gene therapy. As noted by Kasahara *et al.* previous chimeric envelope studies that used other ligands fused to *env* had marginal success because the virions were only weakly infectious. Their statement that their EPO-*env* virus is "highly efficient" for infecting human cells that have EPO receptors seems unwarranted.

The virions used by Kasahara *et al.* were hybrids that contained both the chimeric EPO-*env* protein plus the wild-type ecotropic *env* protein that by itself mediates infection of mouse, but not human cells. The titer was 6.1 times higher on mouse fibroblasts with 10,000 EPO receptors per cell than on barren control fibroblasts. Because ecotropic virions adsorb inefficiently onto cultured mouse cells (*2*), a likely interpretation is that the virions were concentrated onto cell surfaces by attaching to EPO receptors and that the membrane fusion step of infection was then mediated by either wild-type *env* protein, EPO-*env* chimeric protein, or mixed oligomers of these virion components. Because control virions lacking wild-type *env* protein were not examined, these alternatives cannot be distinguished.

Consequently, the crucial experiment was the attempt [figure 3B in (*1*)] to infect human cells (HEL leukemia cells, which contained 1000 EPO receptors/cell) with the hybrid-chimeric virions. Careful inspection indicates that these undiluted virions caused only a few foci (roughly 50), in striking contrast to the titer of approximately 10^5 foci per milliliter of these virions on mouse cells that also have EPO receptors. This could not be a result of a post-penetration block in human cells because human cells with recombinant ecotropic receptors are infected 10% as efficiently by ecotropic viruses as are mouse cells (*2*, *3*). The only control for efficiency of infection was a diluted amphotropic virus that also gave approximately 50 foci on the human cells, but this was a different virus used at an unknown and arbitrary concentration.

It is not known why retrovirions adsorb inefficiently onto cultured cells, but studies of human immunodeficiency virus suggest that receptor binding sites in *env* glycoproteins may be in a partially occluded or constrained conformation. This constraint enables receptor attachment to trigger structural rearrangement leading to fusion of cell surface and virion membranes. The report by Kasahara *et al.* (*1*) suggests that virus concentration onto cell surfaces by any means might increase infection and that hybrid retrovirions with multiple envelope components can have weak cell targeting capabilities.

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Response: Kabat objects to our statement that the infection of human erythroleukemic cell line is highly efficient and offers an explanation for the mechanism of enhancement of infection by the virus we constructed.

The basis for Kabat's objection is the relatively low titer of the EPO ligand-bearing ecotropic virus on human cells expressing EPO receptors at a level of about 1000 receptors per cell (approximately 10^2 per milliliter), as compared with its titer on murine cells expressing about 10,000 EPO receptors per cell (approximately 10^5 per milliliter) [compare figures 3, B and A in (*1*)]. Because the EPO ligand was effective in enabling the virus to cross species, its infectivity was more efficient than that of wild-type ecotropic virus, which was unable to infect human cells, and was also more efficient than any previously described targeting strategy. Kabat also objects to our use of amphotropic virus as a control, but because it is not possible to infect human cells with wild-type ecotropic virus, this was the only valid positive control. This experiment was designed to yield a "plus or minus" result, and the purpose of the amphotropic virus was primarily to demonstrate that both human cell lines can be nonspecifically infected with the amphotropic virus, and to contrast this result with the specific infectivity of the EPO ligand-bearing ecotropic virus; the amphotropic control was not intended for comparison of efficiency. Serial dilutions of the amphotropic control virus were made as standard virological procedure, and the 1:10 dilution was shown as the control plate because it gave the closest number of colonies to the experimental plate. We appreciate that the titers currently obtained with this virus are too low to be of practical use for human gene therapy; however, we do not claim any such usefulness and in fact point out the limitations of this particular virus (*1*, p. 1375). The principle of ligand-mediated targeting has been established by these experiments, and increasing the efficiency of the process is an area for further investigation.

As to the mechanism of infusion, the presence of the wild-type *env* protein may be necessary for the recombinant envelope to function properly during target cell entry. We agree that there are several possible explanations as to the mechanism of targeting, and that these can be distinguished by testing with a virus encoated with solely the recombinant EPO ligand-containing envelope. We had not overlooked this possibility, but despite repeated attempts we were unable to generate such a virus. The presence of the wild-type envelope was absolutely necessary for expression of the recombinant ligand-containing *env* protein on the surface of the packaging cells, and virions pseudotyped purely by the recombinant envelope could not be produced (*2*). Only when coexpressed with the wild-type envelope could the recombinant form also be detected on the packaging cell surface. Successful coexpression may result from heterotrimerization with the wild-type envelope, which may rescue the recombinant protein from degradation and which allows it to be cotransported through the endoplasmic reticulum and Golgi to the surface of the packaging cells.

Although the exact role of the wild-type envelope remains to be elucidated, we have demonstrated targeting of retroviral vectors via ligand-receptor interaction to specific cells that would otherwise not have been infected. Many issues remain to be worked out, such as the effects of different types or affinities of ligands, different types of receptors to be targeted and the number of recombinant molecules on the virion surface. Rather than present a perfect vector for clinical therapy, we hope that we have provided a proof of principle that might pave the way for more improvements in vector design.

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