Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9747–9751, October 1995
Medical Sciences

Ligand-directed retroviral targeting of human breast cancer cells

(ligand–receptor interaction/heregulin/HER-2/neu/ERBB2/HER-4/ERBB4)

XIAOLIANG HAN*†, NORIYUKI KASAHARA*, AND YUET WAI KAN*†‡

*Department of Laboratory Medicine and †Howard Hughes Medical Institute, University of California, U426, San Francisco, CA 94143-0724

Contributed by Yuet Wai Kan, June 23, 1995

ABSTRACT **We explored the feasibility of designing retroviral vectors that can target human breast cancer cells with characteristic receptors via ligand–receptor interaction. The ecotropic Moloney murine leukemia virus envelope was modified by insertion of sequences encoding human heregulin. Ecotropic virus, which normally does not infect human cells, when pseudotyped with the modified envelope protein now crosses species to infect human breast cancer cell lines that overexpress HER-2 (human epidermal growth factor receptor; also called ERBB2) and HER-4 (also called ERBB4), while human breast cancer cell lines expressing low levels of these receptors remain resistant to infection. Since about 20% of human breast cancers overexpress HER-2 and some of breast cancer cell lines overexpress both HER-2 and HER-4, cell-specific targeting of retroviral vectors may provide a different approach for *in vivo* gene therapy of this type of breast cancer.**

As gene delivery vehicles, retroviral vectors have been used extensively in clinical trials, including those for gene therapy of cancer. Retroviral vectors commonly used for gene transfer are derived from Moloney murine leukemia virus (Mo-MLV) and are classified as ecotropic or amphotropic according to their species specificity. The former only infects mouse cells, while the latter infects most mammalian cells, including human cells (1). The ecotropic virus attaches to mouse cells via a binding interaction between the ecotropic envelope and a ubiquitous cationic amino acid transporter on the mouse cell surface. It does not infect human cells (2) presumably because the sequence of this amino acid transporter is divergent between murine and nonmurine species. The amphotropic virus enters mammalian cells by interacting with a phosphate transporter on the host cell surface that is also ubiquitous (3, 4) and highly conserved across different species, and hence it can infect mammalian cells of many species. Although these vectors thus show species specificity, within a given species neither vector exhibits tissue specificity because of the widespread distribution of amino acid transporters and phosphate transporters among various tissue types. Recently, Kasahara *et al.* (5) devised a strategy that alters retroviral host range by introducing modifications into the envelope of an ecotropic retrovirus. A peptide ligand was engineered into the envelope, and viruses pseudotyped with this modified envelope showed enhanced infection of murine cells expressing the receptor for that ligand. Furthermore, the ecotropic retrovirus bearing the human ligand could cross species and infect human cells bearing the appropriate receptors. Other studies have also shown that modifications of the retrovirus envelope could alter viral binding activity or tropism (6–8). In this paper we describe the application of this strategy to the targeting of human breast cancer cells via the peptide ligand heregulin.

About 20% of breast cancer cells overexpress HER-2 [human epidermal growth factor (EGF) receptor; also called ERBB2] (9). Two other members of the human epidermal growth factor receptor family, HER-3 and HER-4 (also called ERBB3 and ERBB4), have also been identified (10, 11). A ligand for these receptors, heregulin, was first identified by Holmes *et al.* in 1992 (12). Heregulin exists in mature form as a 45-kDa protein with 240 amino acid residues and contains an immunoglobulin-like domain as well as an EGF-like domain. The mature circulating form is derived by proteolytic cleavage from a membrane-bound form with 645 amino acid residues. Through alternate splicing, heregulin can further exist in four different isoforms, α, β1, β2, and β3 (12). These are found in normal tissues such as breast, prostate, brain, skeletal muscle, ovary, testis, and small intestine. Heregulin is also expressed in many tissues as other isoforms, such as glial growth factors and acetylcholine receptor-inducing activity (13, 14). The neu differentiation factor is the rat homologue of human heregulin (15). The physiologic functions of heregulin are not understood and may be complicated and cellular context-dependent. However, it can induce proliferation of human breast cancer cell lines that express HER-2 (12, 15). It is believed that binding of heregulin may depend on the coexpression of HER-3 or HER-4 with HER-2 in breast cancer cells (16, 17). These interactions are probably mediated through the EGF-like domain; however, heregulin shows no cross-reactivity with the EGF receptor itself (12).

We created ecotropic Mo-MLV-based vectors expressing the α or β1 form of human heregulin in the viral envelope glycoprotein gp70 as a model system for ligand-directed retroviral gene transfer to specific breast cancer cells. Here we report that, in contrast to wild-type ecotropic vectors, which are noninfectious for human cells, these modified viruses can cross species to specifically target human breast cancer cell lines that overexpress HER-2 and HER-4, while human breast cancer cell lines that show low or baseline levels of these receptors remain resistant to infection. The development of retroviral vectors that can specifically target malignant cells that overexpress certain types of cell surface proteins would have important implications for gene therapy of cancer.

MATERIALS AND METHODS

Plasmids and Cell Lines. Plasmids used include pEnv, which encodes the ecotropic Mo-MLV *env* gene (generously provided by A. Bank, Columbia University, New York); pCRIP-SVlac and pBAG, which contain retroviral vector constructs carrying the neomycin-resistance (neo^R) gene and β-galactosidase (β-gal) gene as markers (provided by R. Scharfmann, Salk Institute, La Jolla, CA); and pGEM-T (Promega). Cell lines used in these studies are the ψ-2 ecotropic packaging cell line; ψ-2/BAG, an ecotropic virus producer cell line derived from ψ-2, which generates ecotropic BAG-containing vectors [American Type Culture Collection (ATCC), CRL 9560]; the PA317 amphotropic packaging cell line (ATCC, CRL 9078);

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EGF, epidermal growth factor; HER, human EGF receptor; Mo-MLV, Moloney murine leukemia virus; RT, reverse transcription; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; β-gal, β-galactosidase; cfu, colony-forming unit(s).

‡To whom reprint requests should be addressed.

NIH 3T3 mouse fibroblasts (ATCC, CRL 1658); and the following human breast cancer cell lines: MDA-MB-453 (ATCC, HTB 131), MDA-MB-361 (ATCC, HTB 27), and BT-474 (ATCC, HTB 20), which overexpress HER-2 and HER-4, and MDA-MB-231 (ATCC, HTB 26) and MCF-7 cells (ATCC, HTB 22), which express low or undetectable levels of these receptors (11, 18). All cell lines were cultured according to ATCC instruction.

Cloning of Heregulin cDNA and Construction of Envelope Expression Vectors. Cytoplasmic RNA was isolated from MDA-MB-231 cells for cloning of heregulin cDNA. Reverse transcription (RT) coupled with polymerase chain reaction amplification (RT–PCR) was used to isolate heregulin cDNA from RNA derived from MDA-MB-231 cells. The RT reaction was carried out as follows: 8 μg of RNA and 1.4 μg of random primer in 4 μl of H_2O was hybridized at 65°C for 2 min, and, after addition of 4 μl of 5× RT buffer (GIBCO/BRL) containing 200 units of Mo-MLV reverse transcriptase (GIBCO/BRL) and 200 units of RNase inhibitor (Promega), 2 μl of 0.1 M dithiothreitol, and 8 μl of 2 mM dNTPs, the mixture was brought to a final volume of 20 μl and incubated at 37°C for 1 hr. The PCR reaction was then performed as follows: to 5 μl of the above RT mixture was added 5 μl of 10× PCR buffer (Perkin–Elmer) containing 50 pM 5′ primer and 50 pM 3′ primer, 5 μl of 2 mM dNTPs, 5 units of *Taq* DNA polymerase (Perkin–Elmer), and H_2O to a final volume of 50 μl; this PCR mixture was heated at 94°C for 2 min, followed by 30 cycles of heating at 94°C for 1 min, annealing at 66°C for 1 min, and elongation at 72°C for 2 min. For PCR of both α and β1 isoforms of heregulin, including amino acid residues between 35 and 239 (which contains both the immunoglobulin-like and EGF-like domains), 5′-GAGGTAACCTTGCCTCCCCAATTGAAAGAGATGAAAAGCCAG-3′ was used as a common 5′ primer in combination with isoform-specific 3′ primers. For α heregulin, the sequence of the specific 3′ primer used was 5′-TCGGATCCCGTACAGCTGCTCCGCCTTTTCTTGGTTTTGGAC-3′, and for β1 heregulin, the sequence of the specific 3′ primer used was 5′-TCGGATCCCCTCCATAAATTCAATCCCAAGATGCTTGTAGAA-3′ (the underlined nucleotides denote sequences added to generate restriction sites for cloning). Heregulin–gp70 chimeric envelope gene expression vectors were constructed by substitution of the Mo-MLV *env* gene between unique restriction sites *Bst*EII (position 5923) and *Bam*HI (position 6537) with α or β1 isoforms of heregulin cDNA. As mentioned above, *Bst*EII and *Bam*HI sites were generated by PCR-mediated site-directed mutagenesis on the 5′ and 3′ ends of the 615-bp α or β1 heregulin cDNA fragments encoding amino acid residues between 35 and 239, which contain both the immunoglobulin-like and EGF-like domains.

Rapid Testing of Vectors Expressing Heregulin–gp70 Chimeric Envelope Genes. The α or β1 heregulin–gp70 chimeric envelope genes were first transiently transfected into the ecotropic producer cell line ψ-2/BAG by calcium phosphate precipitation (GIBCO/BRL). The ecotropic retrovirus thus transiently pseudotyped was harvested 48 hr later and tested for infectivity on MDA-MB-453 and MCF-7 cells. Infection of these human breast cancer cells was assayed by 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) staining (see below).

Establishment and Identification of Heregulin–gp70 Packaging Cell Lines. Vectors expressing the α or β1 heregulin–gp70 chimeric envelope genes and the hygromycin-resistance gene were cotransfected into ψ-2 packaging cells by using the calcium phosphate precipitation method. The medium was changed 24 hr later, and 3 days after transfection, the selection was begun with Dulbecco's modified Eagle's medium (DMEM) containing 100 μg of hygromycin per ml; the medium was changed every 3 days thereafter. After selection for 20 days, hygromycin-resistant cell colonies formed and were picked for expansion and screening of cell lines that express the heregulin–gp70 chimeric envelope gene products. For identification of positive cell lines, cytoplasmic RNA was isolated from hygromycin-resistant colonies, and RT–PCR was performed as described above, using the same common heregulin 5′ primer as that used in cloning and a 3′ primer sequence derived from the Mo-MLV *env* gene, 5′-GAGTGGCTGTTGGTCTGCCAGAAC-3′. The RT mix without addition of reverse transcriptase was used as a negative control. The plasmid DNAs encoding the heregulin–gp70 chimeric envelope genes were used as positive controls.

Production of Replication-Defective Retroviral Vectors and Infection of Target Cells. For transient production of replication-defective retroviral vectors, the plasmids pCRIP-SVlac and pBAG were transfected by calcium phosphate precipitation into packaging cell lines that tested positive for expression of the α or β1 heregulin–gp70 chimeric envelope genes. As the packaging cell lines were selected and maintained in hygromycin, 24 hr after transfection the medium was changed to regular DMEM without hygromycin in preparation for harvesting of virus. Three days after transfection, the supernatant medium was collected, filtered through 0.2-μm syringe-top filters, and immediately added to the culture medium of the target cells for infection. The target cells, consisting of NIH 3T3 cells or various human breast cancer cell lines, were plated out 24 hr in advance at 60% confluency in 25-cm^2 flasks with 5 ml of medium. One millimeter of the same batch of filtered retrovirus-containing medium from each individual packaging cell line was used to infect these target cells simultaneously. Three days after infection, the medium on the target cells was changed to DMEM containing 400 μg of G418 per ml for selection, and the medium was changed every 3 days thereafter. Twenty days later, retroviral infection was evaluated by staining with X-Gal, and titers were determined by staining with Giemsa and counting the number of neomycin-resistant colonies.

Stable virus producer cell lines were created by prior transient transfection of PA317 with pCRIP-SVlac or pBAG by calcium phosphate precipitation, and the transiently produced amphotropic virus was harvested 48 hr later as described above and used for infection of the ecotropic packaging cell lines expressing α or β1 heregulin/gp70 chimeric envelope. Infected packaging cells were selected in medium containing 400 μg of G418 per ml for 14 days, and neomycin-resistant colonies were isolated and grown in individual wells. High titer-producer cells were identified by infection of NIH 3T3 and human breast cancer cells as described above except that 100 μl of overnight culture medium without neomycin or hygromycin was harvested and filtered from the producer cells for infection of each flask of target cells.

X-Gal and Giemsa Staining. For X-Gal staining, the cells were fixed in a solution containing 1% glutaraldehyde, 0.1 M sodium phosphate buffer (pH 7.0), and 1 mM $MgCl_2$ for 15 min and then stained with a solution containing 0.2% X-Gal, 10 mM sodium phosphate buffer (pH 7.0), 150 mM NaCl, 1 mM $MgCl_2$, 3.3 mM $K_4Fe(CN)_6$, and 3.3 mM $K_3Fe(CN)_6$ for 1 hr at 37°C or room temperature overnight. The X-Gal solution was removed, and 70% glycerol was added to the flasks (19). Pictures were taken under inverted light microscope (×100 magnification). For Giemsa staining, flasks were washed with phosphate-buffered saline, cells were fixed in acetone/methanol, 1:1 (vol/vol), for 15 min, and 1 ml of Giemsa solution (BDH) was added for 2 min, followed by addition of 5 ml of H_2O, mixing, and incubation for 2 min, after which the flasks were washed with H_2O and dried and the number of colonies were counted.

RESULTS

Construction of the Chimeric Heregulin–gp70 *env* Gene. Sequences encoding α and β1 heregulin were cloned from human breast cancer cell line MDA-MB-231 by RT–PCR. By

using specific primer pairs, cDNA sequences corresponding to 205 amino acid residues of heregulin were amplified that contained both immunoglobulin-like and EGF-like domains, with a 20-amino acid difference at the carboxyl ends of the two mature α and β1 isoforms. Both isoforms were isolated and cloned in frame between unique *Bst*EII and *Bam*HI sites in the Mo-MLV gp70 envelope protein, thus largely replacing the amino-terminal portion of gp70, including both variable regions VR-A and VR-B (20). The junction sites in the α and β1 heregulin–gp70 fusion constructs were all sequenced to confirm that the reading frames had not been shifted during cloning. These chimeric envelope genes are driven by the 5′ long terminal repeat (LTR) and processed with splicing donor and acceptor signals from Mo-MLV. In a pilot experiment done with a transient expression system, we tested retrovirus with the heregulin–gp70 chimeric envelope proteins on human breast cancer cell line MDA-MB-453 and confirmed cross-species infectivity before packaging cell lines stably expressing the chimeric envelope protein were created (data not shown).

Creation of Packaging Cell Lines Expressing Heregulin–gp70 Chimeric Envelopes. The α or β1 heregulin–gp70 chimeric envelope constructs and the hygromycin-resistance gene were cotransfected into the ψ-2 packaging cell line, which expresses the *gag*, *pol*, and *env* gene products of ecotropic Mo-MLV but lacks the ψ packaging signal. After selection in hygromycin, 86 hygromycin-resistant colonies were obtained. Of these, 16 packaging cell lines expressing the heregulin–gp70 chimeric envelope genes were identified by RT–PCR (Fig. 1): 10 packaging cell lines expressed the α heregulin–gp70 chimeric envelope gene and 6 expressed the β1 heregulin–gp70 chimeric envelope gene. All 16 packaging cell lines thus identified were then transiently transfected with plasmids pBAG and pCRIP-SVlac, which encode replication-defective retroviral vectors containing β-gal and neomycin-resistance genes. Virus-containing medium from the packaging cell lines was collected individually and used to infect NIH 3T3 target cells. The retrovirus titer from transient transfection of these packaging cell lines was determined by counting the number of G418-resistant NIH 3T3 cell colonies obtained with serial dilutions, and it ranged from 1×10^2 to 1×10^4 colony-forming units (cfu)/ml. One packaging cell line expressing α heregulin (designated α-17) and one expressing β1 heregulin (designated β1-38), both of which have titers of 1×10^4 cfu/ml, were chosen to test the infectivity of these retroviruses on human breast cancer cell lines.

Cell-Specific, Cross-Species Infection by Viruses Bearing Chimeric Heregulin–gp70 Envelope Proteins. CRIP-SVlac or BAG-containing replication-defective retroviruses produced by transient transfection of the two ψ-2-based cell lines expressing the α or β1 heregulin–gp70 envelope chimeras were used to infect the human breast cancer cell line MDA-MB-453, which overexpresses HER-2 and HER-4 at levels of about 60-fold that of normal human fibroblasts and human mammary epithelial cells (11, 18), and the human breast cancer cell line MDA-MB-231, which shows undetectable levels of HER-2 and HER-4. CRIP-SVlac or BAG-containing retroviruses produced by wild-type ψ-2 cells were used as negative control viruses. All virus preparations were also titered on NIH 3T3 cells, both as a positive control for infection and to ensure that an adequate transfection efficiency had been achieved in each experiment. The infected cells were selected in medium with G418 at 400 μg/ml for 3 weeks, and the surviving colonies were stained for β-gal activity. As expected, the ecotropic virus-based vectors produced from all three packaging cell lines infected NIH 3T3 mouse fibroblasts at titers ranging from 10^4 to 10^5 per ml. Also as expected, the wild-type ecotropic virus vector produced by the wild-type ψ-2 packaging cell controls could not infect either human breast cancer cell line. However, the ecotropic-based virus vectors produced by packaging cells expressing the heregulin–gp70 chimeric envelopes were able to cross species and to infect the human breast cancer cell line MDA-MB-453, which expresses high levels of both HER-2 and HER-4. In contrast, the human breast cancer cell line MDA-MB-231, which does not express either receptor at detectable levels, remained resistant to infection by these vectors and showed no surviving colonies after G418 selection despite an adequate viral titer as determined by NIH 3T3 cell infections (Fig. 2). The same results were obtained whether the virus vectors were produced by packaging cells expressing the α or β1 heregulin–gp70 chimeric envelope. The titer of these viruses, produced by transient transfection of packaging cell lines α-17 or β1-38, was on the order of 1×10^3 cfu/ml when they were used to infect MDA-MB-453 cells, ≈1 log below the titer when assayed on NIH 3T3 cells. These results suggest that viruses pseudotyped by heregulin–gp70 chimeric envelopes show specific infectivity for certain human breast cancer cell lines that express high levels of HER-2 and HER-4.

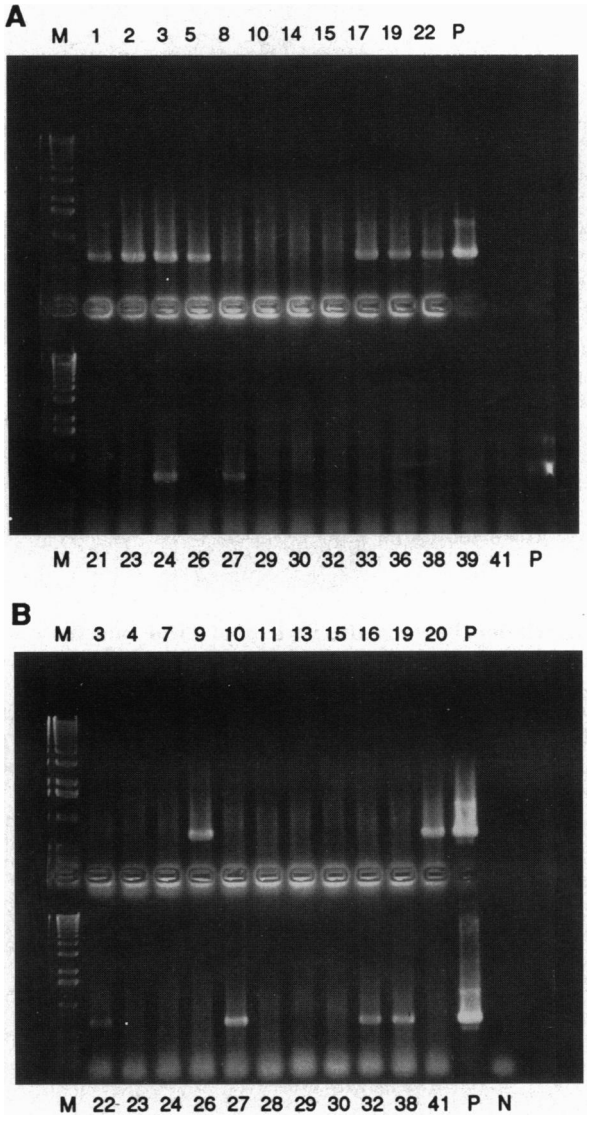

FIG. 1. Screening packaging cell lines expressing the heregulin–gp70 chimeric gene by RT–PCR. (*A*) Packaging cell lines transfected with the α heregulin–gp70 chimeric gene construct. (*B*) Packaging cell lines transfected with the β1 heregulin–gp70 chimeric gene construct. The 5′ primer was the same for α and β1 heregulins and was located at the amino-terminal end of heregulin. The 3′ primer was from the Mo-MLV *env* gene. Sequences of both primers are shown in *Materials and Methods*. Lanes: M, 1-kb DNA marker; P, positive control (heregulin–gp70 chimeric gene plasmid used for transfection of packaging cell lines); N, negative control (PCR without RT). Numbers refer to the packaging cell lines tested.

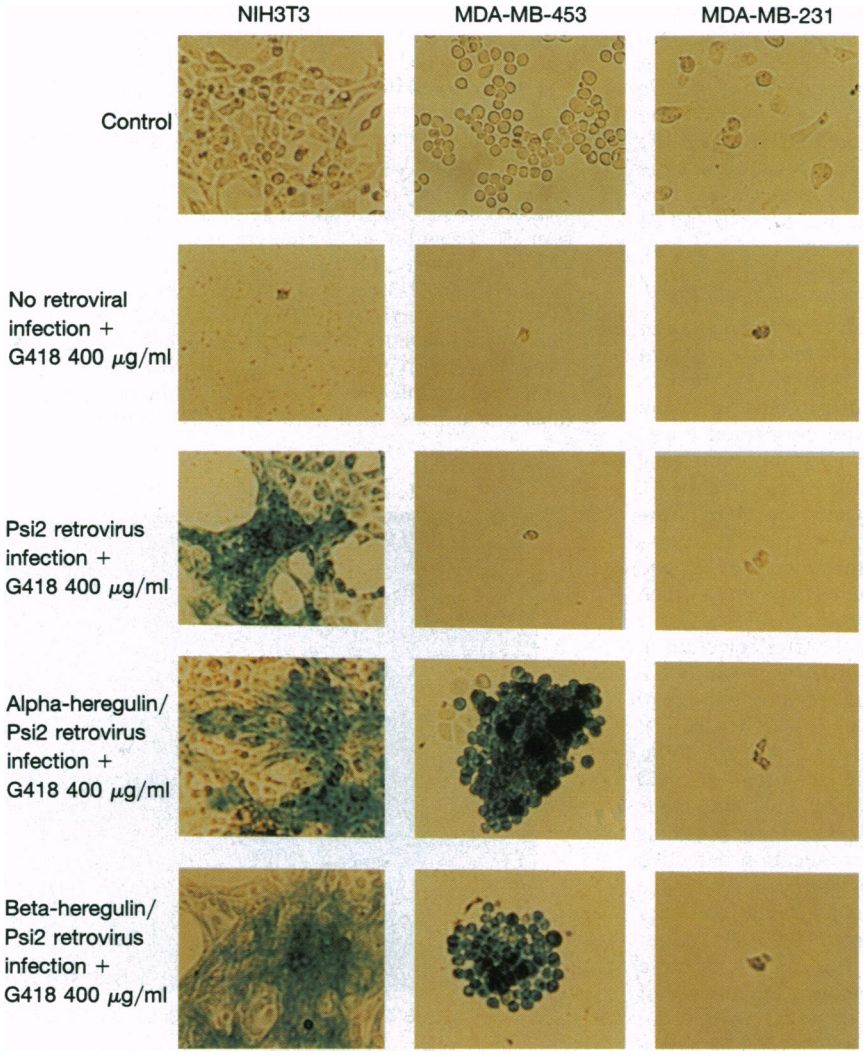

FIG. 2. Infectivity of pseudotyped retroviruses on NIH 3T3, MDA-MB-453, and MDA-MB-231 cell lines. Control shows unselected NIH 3T3, MDA-MB-453, and MDA-MB-231 cell lines stained by X-Gal. No retroviral infection with G418 at 400 μg/ml indicated that the three cell lines were selectable with G418 at 400 μg/ml. Retrovirus produced by wild-type ψ-2 packaging cell line only infected the NIH 3T3 cell line, as indicated by G418 selection and X-Gal staining of the surviving cells. Pseudotyped retrovirus produced by α or β1 heregulin–gp70 packaging cell line could infect human breast cancer cell line MDA-MB-453, which overexpresses HER-2 receptor, but not the MDA-MB-231 cell line, which does not overexpress HER-2 receptors. Both pseudotyped retroviruses, as expected, infect NIH 3T3 cells.

Correlation Between the Pattern of Viral Infectivity with Receptor Expression in Human Breast Cancer Target Cells. Packaging cell lines α-17 or β1-38, expressing the α or β1 heregulin–gp70 chimeric envelopes, respectively, were infected with replication-defective amphotropic retrovirus carrying the CRIP-SVlac or BAG vectors for generation of stable producer cell lines. These producer lines were screened for titer by infection of NIH 3T3 and MDA-MB-453 cells, and two lines (producer α-17-46 and producer β1-38-8) were further tested on other human breast cancer cell lines that express varying levels of HER-2 and/or HER-4. Amphotropic virus produced by the PA317 cell line was used as a positive control for infection of human cell lines. As before, cross-species infection by virus from the heregulin–gp70-expressing producer cells was observed only when the target cells expressed high levels of both HER-2 and HER-4 and, in this case, was achieved at titers that were comparable to those seen on NIH 3T3 cell infection as well as the titer obtained on infection

Table 1. Titer of pseudotyped retrovirus

Infected cell line	Virus titer, cfu × 10^{-3}/ml					Overexpression	
	No virus	ψ-2 virus	α-17-46	β-38-8	PA317	HER-2	HER-4
NIH 3T3	0	8.7	9.4	8.0	6.0	—	—
MDA-MB-231	0	0	0	0	5.0	—	—
MDA-MB-453	0	0	8.8	8.3	6.0	+	++
MDA-MB-361	0	0	3.8	3.0	2.6	+	+
BT-474	0	0	2.2	1.8	2.4	++	+
MCF-7	0	0	0	0	2.7	—	+

Titers of viruses produced by wild-type ψ-2 packaging cells and two producer cell lines, α-17-46 and β1-38-8, derived from ψ-2 cell subclones stably transfected with the α or β1 heregulin–gp70 chimeric envelope construct, after infection of NIH 3T3 mouse fibroblasts and human breast cancer cell lines MDA-MB-231, MDA-MB-453, MDA-MB-361, BT-474, and MCF-7. Amphotropic retrovirus produced by the PA317 packaging cell line was used as a positive control for infection of the human cell lines. The virus-containing supernatant was harvested and filtered through a 0.2-μm filter, and 100 μl of the same batch of virus was added to each target cell plate. Titers are on the order 1×10^3 cfu/ml and are expressed as the average G418-resistant cfu/ml ($n = 4$) of the virus-containing supernatant after selection of the infected target cells in medium containing 400 μg of G418 per ml for 20 days. The overexpressions of HER-2 and HER-4 in breast cancer cell lines are scaled.

by amphotropic virus from PA317 producer cells (on the order of 10^3 per ml). Again, none of the ecotropic-based producer lines produced virus that could infect MDA-MB-231 cells, which do not express HER-2 or HER-4. One human breast cancer cell line, MCF-7, which expresses no HER-2 receptors and low levels of HER-4, also remained resistant to infection, suggesting that, although heregulin is known to bind to HER-4 homodimers, perhaps the affinity of the heregulin-envelope fusion constructs is too low or the HER-4 number on the MCF-7 cells is too low to support ligand-mediated cross-species infection. These results are summarized in Table 1.

DISCUSSION

We report a strategy for producing ecotropic retrovirus-based vectors with an altered host range by expressing chimeric envelope proteins, consisting of the ligand heregulin replacing the amino-terminal variable regions of Mo-MLV gp70, in the packaging cells. Viral vectors produced by these modified packaging cells can target specific human breast cancer cell lines that overexpress both HER-2 and HER-4. As heregulin-specific antisera were not available to us, expression was confirmed at the mRNA level by RT–PCR, and previous experiments using different targeting ligands had demonstrated to us that chimeric envelope proteins of similar design could be successfully expressed on the surface of both packaging cells and virions as long as they were coexpressed with wild-type envelope proteins (5). Packaging cells that expressed either the α or the β1 heregulin–gp70 envelope construct produced virus that was capable of cross-species infection of the same specific human breast cancer cell lines, whereas the control virus produced by the parental wild-type ψ-2 cells was unable to infect any of the human cell lines, as was virus produced by transfected packaging cells that were hygromycin-resistant but RT–PCR-negative for the chimeric envelopes. Therefore, this cross-species retroviral targeting was presumably mediated by ligand–receptor interaction between human heregulin–gp70 envelope proteins on the pseudotyped ecotropic virus and the HER-2 and HER-4 on the surface of the human cell lines.

In fact, the newly acquired host range of the virus vectors produced by the transfected packaging cell lines shows good correlation with the known binding activity of heregulin itself. That is, cross-species infection was observed in human breast cancer cell lines that express high levels of both HER-2 and HER-4 but was not observed in cell lines that show undetectable levels. It is interesting to note that, although heregulin is known to bind to HER-4 homodimers, cross-species infection was not seen with the MCF-7 cell line, which does not express HER-2 but does express HER-4, albeit at very low levels. It is possible either that the binding affinity of the heregulin ligand for HER-4 homodimers is reduced when it is expressed in the context of this fusion protein, or, perhaps as a more likely explanation, that ligand-mediated cross-species infection is dependent on an adequate number of specific receptors being present on the target cell surface, which may not be the case with the MCF-7 cells. The effect of varying ligand affinities, varying the numbers of ligands on the virus surface, and varying the types and numbers of receptors on the target cell surface, as well as other issues pertinent to ligand-mediated targeting of retrovirus vectors, remain to be investigated.

HER-2 is overexpressed in 20% of breast cancers, and these tumors are usually more resistant to chemotherapy and more prone to recur (21). HER-4 is overexpressed in 40% of human breast cancer cell lines and in most cases is coexpressed with HER-2 (11). In normal breast tissue or benign breast diseases, only very weak immunoreactive HER-2 signals are occasionally seen in the cytoplasm of the epithelial, myoepithelial, and vascular smooth muscle cells, in contrast to the intense immunoreactive HER-2 signals seen on the cell surface of breast cancer epithelium (22, 23). With regard to clinical applications, it may be unlikely that this type of vector could be administered systemically by the intravenous route in the near future, largely because of problems such as inadequacy of virus titers and rapid inactivation by serum complement; however, we believe it serves as a useful model system to test the feasibility of targeted gene transfer in cancer therapy, and several potential *in vivo* applications of cell-specific retroviral vectors can be proposed. For example, a cancer-specific virus could be directly injected into tumors for cell-specific delivery of the herpes thymidine kinase gene or cytokine genes to kill the tumor cells with ganciclovir treatment or to induce a cytotoxic T-cell response to the tumor cells. A more practical approach might be to introduce such vectors into the mastectomy site to infect residual tumor cells following surgery. If such procedures are feasible, tumor cell-specific virus vectors may serve as a useful adjunct to radiotherapy and chemotherapy in the treatment of breast cancer.

This work was partially supported by National Institutes of Health Grants AM16666 and HL 20985. Y.W.K. is an investigator of the Howard Hughes Medical Institute.

1. Miller, A. D. (1990) *Hum. Gene Ther.* **1,** 5–14.
2. Albritton, L. M., Tseng, L., Scadden, D. & Cunningham, J. M. (1989) *Cell* **57,** 659–666.
3. Miller, D. G., Edward, R. H. & Miller, A. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 78–82.
4. van Zeijl, M., Johann, S. V., Closs, E., Cunningham, J., Eddy, R., Shows, T. B. & O'Hara, B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1168–1172.
5. Kasahara, N., Dozy, A. M. & Kan, Y. W. (1994) *Science* **266,** 1373–1376.
6. Russell, S. J., Hawkins, R. E. & Winter, G. (1993) *Nucleic Acids Res.* **21,** 1081–1085.
7. Chu, T.-H. T., Martinez, I., Sheay, W. C. & Dornburg, R. (1994) *Gene Ther.* **1,** 292–299.
8. Valsesia-Wittmann, S., Drynda, A., Deleage, G., Aumailley, M., Heard, J. M., Danos, O., Verdier, G. & Cosset, F. L. (1994) *J. Virol.* **68,** 4609–4619.
9. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. & McGuire, W. L. (1987) *Science* **235,** 177–182.
10. Kraus, M. H., Issing, W., Miki, T., Popescu, N. C. & Aaronson, S. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9193–9197.
11. Plowman, G. D., Culouscou, J.-M., Whitney, G. S., Green, J. M., Carlton, G. W., Foy, L., Neubauer, M. G. & Shoyab, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1746–1750.
12. Holmes, W. E., Sliwkowski, M. X., Akita, R. W., Henzel, W. J., Lee, J., Park, J. W., Yansura, D., Abadi, N., Raab, H., Lewis, G. D., Shepard, H. M., Kuang, W.-J., Wood, W. I., Goeddel, D. V. & Vandlen, R. L. (1992) *Science* **256,** 1205–1210.
13. Marohionni, M. A., Goodearl, A. D. J., Chen, M. S., Bermingham-McDonogh, O., Kirk, C., *et al.* (1993) *Nature (London)* **362,** 312–318.
14. Falls, D. L., Rosen, K. M., Corfas, G., Lane, W. S. & Fischbach, G. D. (1993) *Cell* **72,** 801–815.
15. Wen, D., Peles, E., Cupples, R., Suggs, S. V., Bacus, S. S., Lou, Y., Trail, G., Hu, S., Silbiger, S. M., Levy, R. B., Koski, R. A., Lu, H. S. & Yarden, Y. (1992) *Cell* **69,** 559–572.
16. Sliwkowski, M. X., Schaefer, G., Akita, R. W., Lofgren, J. A., Fitzpatrick, V. D., Nuijens, A., Fendly, B. M., Cerione, R. A., Vandlen, R. L. & Carraway, K. L., III (1994) *J. Biol. Chem.* **269,** 14661–14665.
17. Plowman, G. D., Green, J. M., Culouscou, J.-M., Carlton, G. W., Rothwell, V. M. & Buckley, S. (1994) *Nature (London)* **366,** 473–475.
18. Kraus, M. H., Popescu, N. C., Amsbaugh, S. C. & King, C. R. (1987) *EMBO J.* **6,** 605–610.
19. Lim, K. & Chae, C.-B. (1989) *BioTechniques* **7,** 576–579.
20. Battini, J. L., Danos, O. & Heard, J. M. (1995) *J. Virol.* **69,** 713–719.
21. Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A. & Press, M. F. (1989) *Science* **244,** 707–712.
22. Gusterson, B. A., Machin, L. G., Gullick, W. J., Gibbs, N. M., Powles, T. J., Elliott, C., Ashley, S., Monaghan, P. & Harrison, S. (1988) *Br. J. Cancer* **58,** 453–457.
23. Hanna, W., Kahn, H. J., Andrulis, I. & Pawson, T. (1990) *Mod. Pathol.* **3,** 455–461.