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Targeting vectors for cancer gene therapy

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1. ABSTRACT

Establishment of a system that allows selective gene transfer to a tumor is expected to enable targeted therapy. Using a combination of fiber-modified adenovirus and antibody to a cell surface antigen, we have explored methods to enhance the selectivity of gene transfer. In addition, we aimed to establish a systematic screening method to search for antibody and cell surface target candidates for providing highly selective gene transfer to a variety of malignant tumors.

2. INTRODUCTION

The most important factor in cancer gene therapy is the ability to specifically target cancer cells, i.e., the ability to distinguish tumor cells from adjacent normal cells. Establishment of a new system to locate tumor cells and allow selective gene transfer will enable targeted therapy.

In tumor-targeting gene therapy, there are several strategies available: (1) Induction of suicide gene expression using a tissue-specific promoter (1, 2) Use of

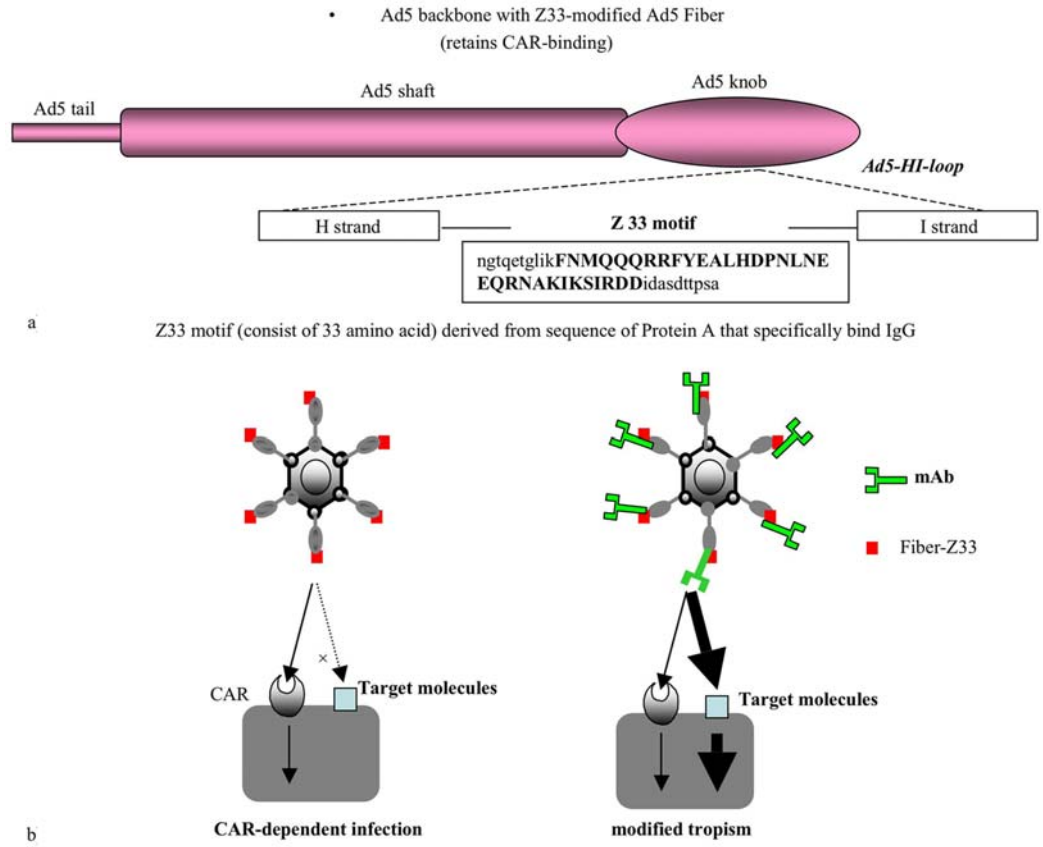

Figure 1. Ad5 backbone with Z33-modified Ad5 Fiber. (a)Scheme for FZ33 fiber-modified adenovirus.A synthetic 33-amino acid IgG-binding domain (Z33), derived from staphylococcal protein A, was inserted into the HI loop of knob protein. (b) Scheme for targeting with FZ33 fiber-modified adenovirus.This modified fiber retained the ability to assemble into trimers, it bound IgG with high affinity, and was incorporated into viral particles. Adv-FZ33 binds immunoglobulins and allows an antibody to redirect the vector to a new target molecule on the cell surface. Our Adv-FZ33 had intact CAR-binding structure and retained CAR-binding ability.

apoptotic mechanisms specific for tumors; (3) Immunotherapy targeted at tumor-specific antigens (2, 4) Use of a virus vector that allows the virus to proliferate specifically in the tumor (3, 4, 5) Use of a vector that recognizes antigen specifically expressed at the tumor surface and allows tumor-specific gene transfer (5, 6).

We have attempted to enhance the selectivity of gene transfer by combining a fiber-modified adenovirus and an antibody for cell surface antigen. Furthermore, we have established a new method to systematically screen cell surface antigens, and search for candidates that would enable highly specific gene transfer to malignant tumors such as those of prostatic and pancreatic cancer.

In this review, we introduce the current status of development of a vector we have constructed that allows target antigen-specific gene transfer via an antibody.

3. TUMOR-SPECIFIC GENE TRANSFER SYSTEM

We have developed a new targeting adenovirus vector that has high specificity for tumor cells and allows highly efficient gene transfer and expression. Adenovirus is a markedly useful tool, because it is easy to work with and because findings obtained are directly applicable to other gene transfer and drug delivery systems.

To provide a vector with specificity for tumors and a potential for use in gene transfer, we constructed adenovirus Adv-FZ33 that had the Z33 motif of Protein A-ngtqetglikFNMQQQRRFYEALHDPNLNEEQRNAKIKSI RDDidasdttpsa (7) (upper case letters, the Z33 motif; lower case letters, the amino acid sequence of the HI loop)—that could bind the Fc domain at the HI loop of the Ad5 fiber knob (Figure 1-a).

4. GENE TRANSFER BY ADENOVIRUS FZ33 VIA ANTIBODY

As shown in Figure 1-b, with a combination of this fiber-modified adenovirus and antibody, gene transfer was possible at high efficiency to tumor cells that expressed the corresponding antigen molecule (8~10). Adv-FZ33 to which antibody for surface molecules (such as CD29 and CD54) expressed in Has hamster cells and

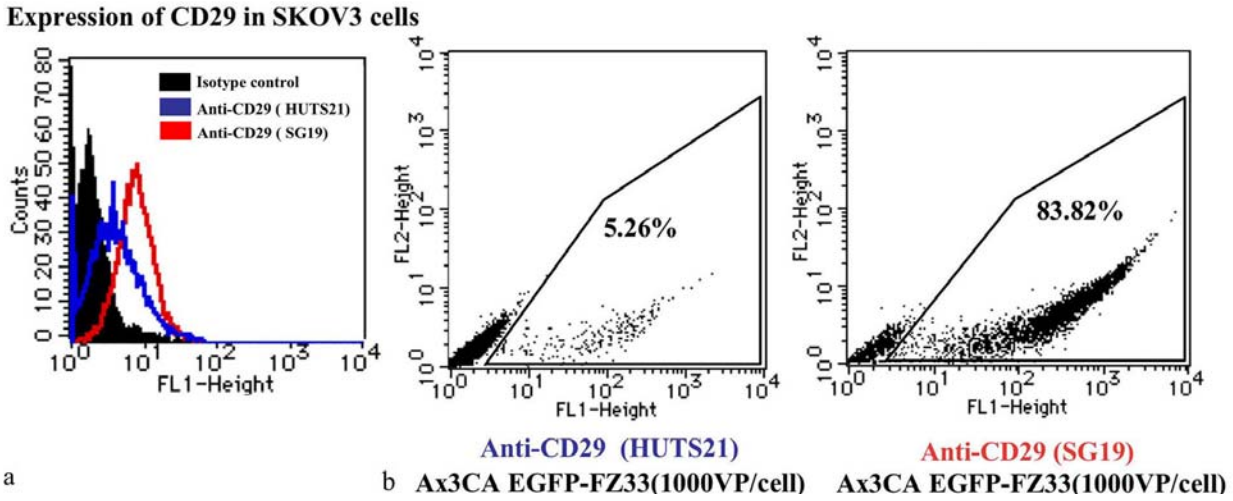

Figure 2. EGFP-expressing FZ33 fiber-modified adenovirus and targeting with antibody for CD29.(a) Expression of CD29 in SKOV3. Analysis of the expression of CD29 on SKOV3 by flow cytometry. Shaded histograms, staining with an isotype control, IgG1k; open histograms-Red, staining with anti-CD29(HUTS21); open histograms-Blue, staining with anti-CD29(SG19). (b) Transduction efficiency in Ax3CAEGFP-Z33-infected SKOV3 was evaluated by flow cytometry. Cells were infected with Ax3CAEGFP-FZ33 at 1,000 VP/cell after incubation with anti-CD29 or isotype control IgG1k. Numbers, percentage of EGFP-positive cells.

A375 human melanoma cells—both of which only expressed a trace of CAR—was attached, provided a tenfold increase in gene transfer and expression compared with the control (Adv-FZ33 without the presence of antibody or combined with isotype control IgG, and wild-type Ad5 fiber Adv-Fwt virus).

Interestingly, as shown in Figure 2, when gene transfer efficiency by FZ33 vector was evaluated with two antibodies to CD29 (integrin β1), the efficiency was low with HUTS21 antibody, but dramatically higher with SG19 antibody. In addition, great variations were often observed in gene transfer efficiencies with different antibodies that recognized the same antigen molecule.

FACS results of EGFP gene transfer and expression by FZ33 adenovirus via several antibodies for CEA are shown in Figure 3. Among the antibodies compared, it was found that the C2-45 antibody constructed by Prof. Kuroki's group, Fukuoka University, Japan, was incomparably excellent in targeting (11). These experimental results have revealed that not only target molecules but also characteristics of antibodies corresponding to the target antigens are important in targeting technology.

5. CANDIDATES FOR TARGET MOLECULES

The coxsackie-adenovirus receptor (CAR) (12~14), an original receptor for human type 5 adenovirus, is distinctly expressed in the liver and a number of normal tissues, but common adenoviruses do not enable tumor-specific gene transfer. On the other hand, CAR is also expressed at low levels in fibroblasts derived from human skin, malignant melanoma, prostatic cancer, bladder cancer, and squamous cell carcinomas in the oral cavity, but adenovirus using CAR as the receptor does not provide sufficient gene transfer efficiency (12). Instead, molecules that have been used as receptors for viruses since prehistoric times, long before the advent of humans, are possible candidates for targeting. The innate cellular specificity that a variety of natural viruses have—such as CD155 (15), a poliovirus receptor (PVR); ICAM (16)/ DAF (17) (CD54/CD55) of coxsackie virus A21; the high-affinity laminin receptor of sindbis virus; and SLAM (18)/ CD46 (19) of measles virus—is applicable for other vectors, including adenovirus, with relative ease. However, these receptor molecules for virus infection are not specific for tumors, and few reports have demonstrated evidence of direct clinical application.

Candidate target molecules present at the tumor surface—growth factor receptors such as ErbB-2 (20) and the EGF (21) receptor, receptors for peptide hormones such as Melanocyte Stimulating Hormone (MSH) (22) and Gastrin Releasing Peptide (GRP) (23), integrins, and glycoproteins such as Melanoma Chondroitin Sulfate Proteoglycan (MCSP) (24)—have been tried.

6. ERBB2-TARGETED SELECTIVE CANCER GENE THERAPY VIA FZ33 FIBER-MODIFIED ADENOVIRAL VECTORS

Among the various candidate molecules previously described, we focused on ErbB-2, which has drawn attention as a potential target molecule for cancer therapy. Since ErbB2 is expressed in breast and ovarian cancer with relative selectivity, and it is an oncogene that plays an essential role for the expression of tumor characteristics, such as proliferation (25), it is one of the most suitable targets for gene therapy. Using fiber-modified adenovirus Adv-FZ33 and ErbB2 as a target molecule, we

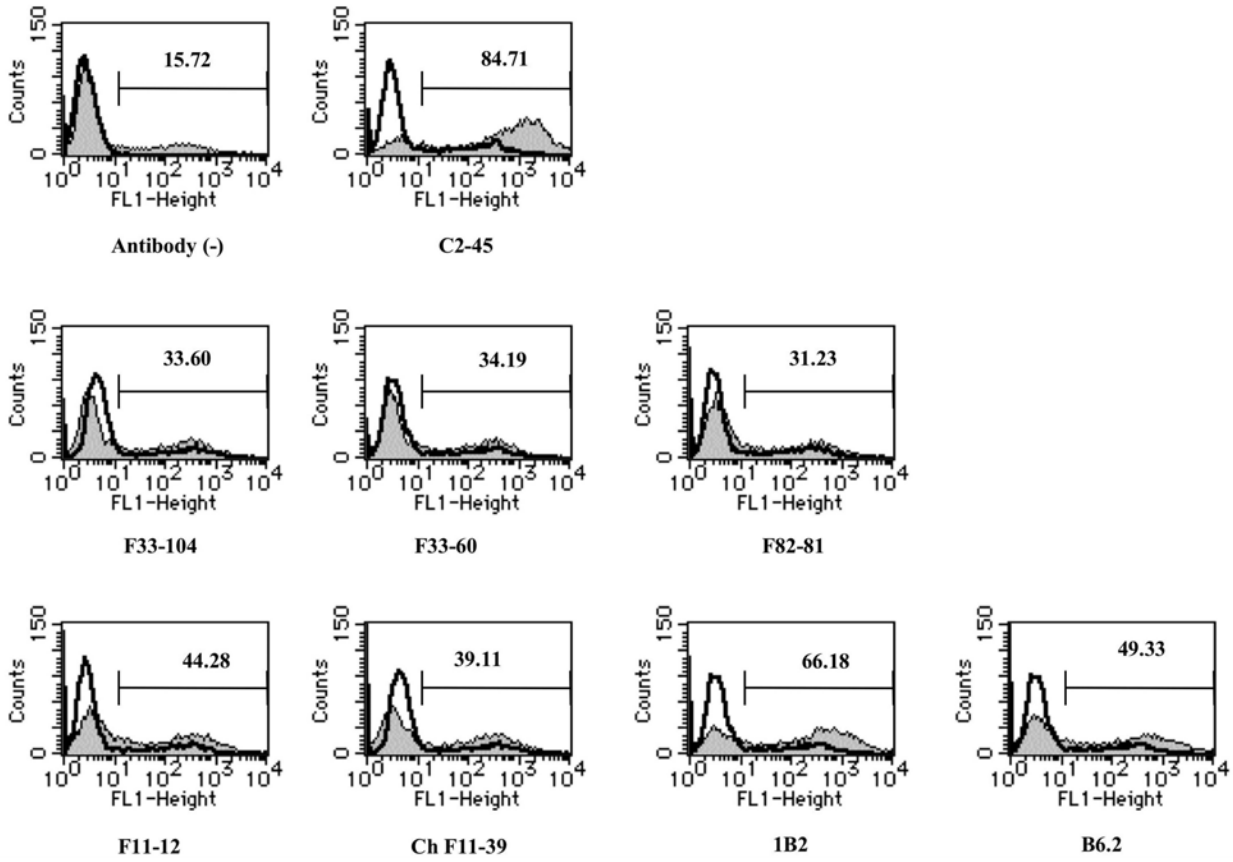

Figure 3. Adv-FZ33 EGFP gene transfer with various anti-CEA mAbs**.** Transduction efficiency in Ax3CAEGFP-Z33-infected CEA-CHO was evaluated by flow cytometry. Cells were infected with Ax3CAEGFP-FZ33 at 1,000 VP/cell after incubation with various anti-CEA or isotype control antibodies. Numbers, percentage of EGFP-positive cells. Open histograms, staining with an isotype control; Shaded histograms, staining with anti-CEA.

tried to develop a new effective gene delivery vehicle selective for cancer cells.

First, the relationship between gene expression efficiency by wild-type Ad5 fiber Adv-Fwt-lacZ virus and CAR expression was examined in a variety of cell lines. In SKOV3 and Has cells with weak CAR expression (Figure 4-a), gene expression was 10^4 VP/cell, which was 100 to 1,000-fold lower than that in U251 cells with strong CAR expression (Figure 4-b). These results were comparable to the gene expression efficiency when Adv-Z33-lacZ was used for infection without antibody. Next, ErbB2 expression was examined in SKOV3 and Has cells with low CAR gene expression, and it was found positive in SKOV3 cells but negative in Has cells (Figure 5).

In SKOV3 cells with weak CAR expression but positive ErbB2 expression, it was examined whether or not selective and high efficiency gene transfer was possible by a combination of Adv-FZ33 and antibody for ErbB2. As a result, in SKOV3 cells with ErbB2 expression, gene transfer efficiency was enhanced 17-fold compared with the control (without antibody or with isotype control IgG + Adv-Z33). On the other hand, gene transfer efficiency was not improved in Has cells without ErbB2 expression (Figure 6).

After antibody to ErbB2 was attached *in vitro* to Z33 adenovirus Adv-Z33-UP that expressed UPRTase, which would efficiently convert 5-FU to the active form, FUMP, SKOV3 cells were infected with the virus, followed by treatment with 5-FU (0.01 μM – 100 μM). As a result, the cytotoxicity of 5-FU was enhanced 100-fold in terms of IC_{50}, which suggested a potential novel therapy that would overcome the resistance of cancer cells to 5-FU (Figure 7). These results indicated that use of a combination of antibody for ErbB2 and FZ33-modified adenovirus was a potent and selective therapy for cancer cells expressing ErbB2.

7. SYSTEMATIC SEARCH FOR NOVEL TARGET MOLECULES

Our results have shown that an appropriate cell surface molecule for targeted cancer therapy was present and that a combination of a high-performance antibody for the molecule and Z33-Adv was able to provide highly efficient gene transfer with high specificity for tumor cells. Currently, we are doing a comprehensive

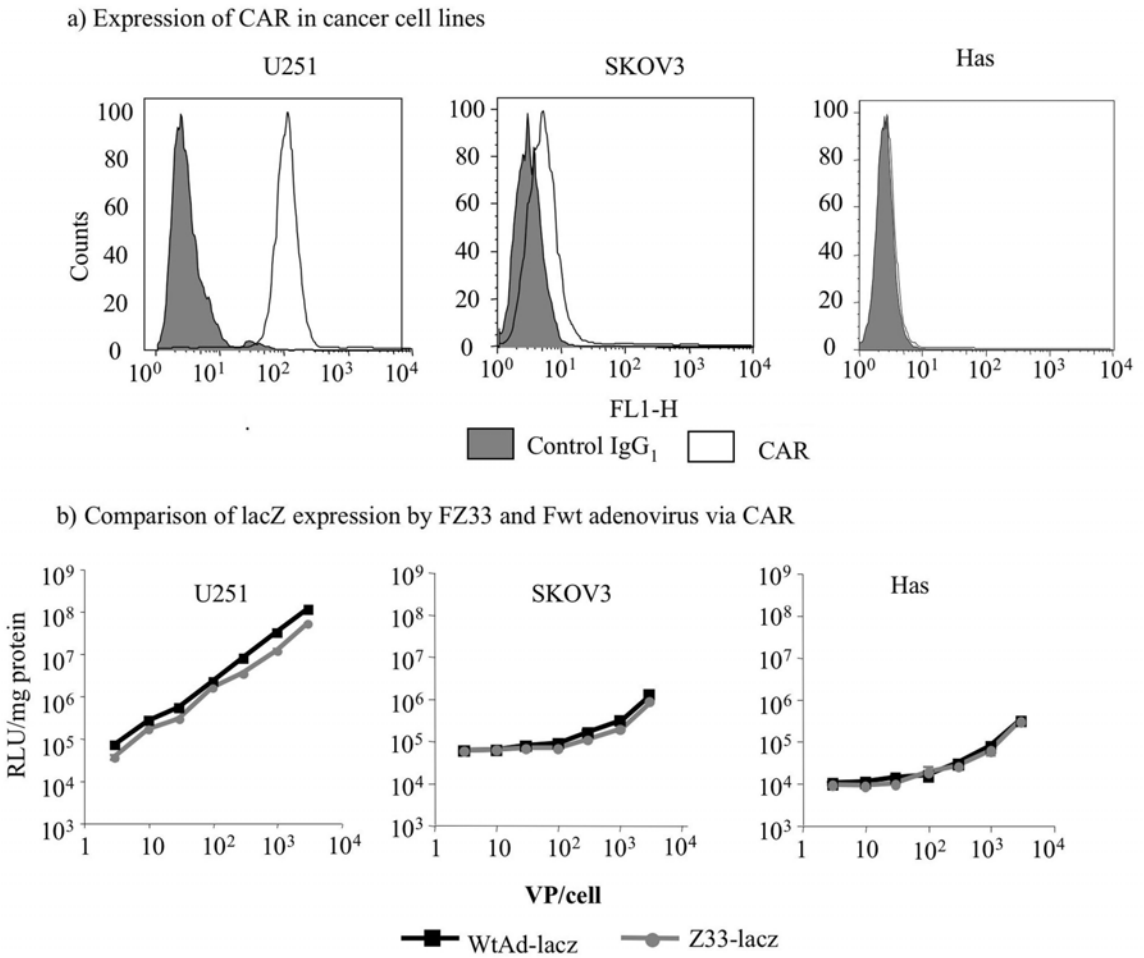

Figure 4. (a) Expression of CAR in cancer cell lines. Analysis of the expression of CAR on cancer cell lines by flow cytometry. Shaded histograms, staining with an isotype control, IgG1k; open histograms, staining with anti-CAR. (b) Comparison of lacZ expression by FZ33 and Fwt adenovirus via CAR. Cells were infected with AxCAZ3-FZ33 or AdvFwt-lacZ at 0 to 10,000VP/cell. Beta-gal activity is measured by chemiluminescence assay. Points, mean; bars, SD; RLU, relative light units

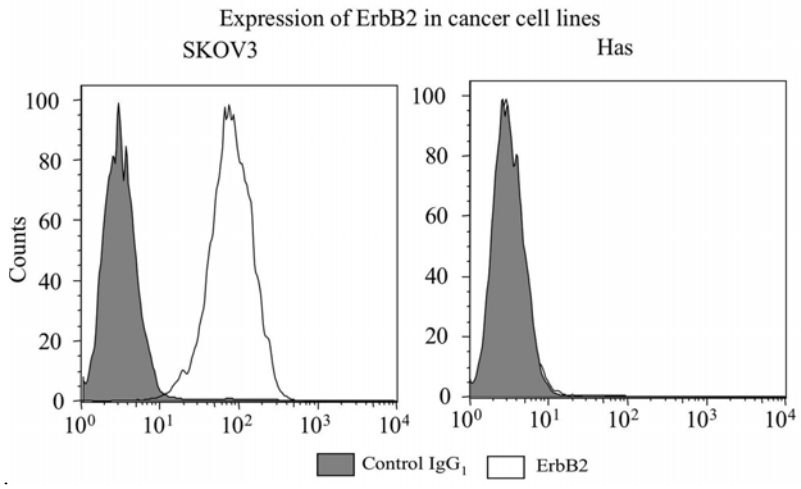

Figure 5. Expression of ErbB2 in cancer cell lines. Analysis of the expression of ErbB2 on cancer cell lines by flow cytometry. Shaded histograms, staining with an isotype control, IgG1k; open histograms, staining with SER4.

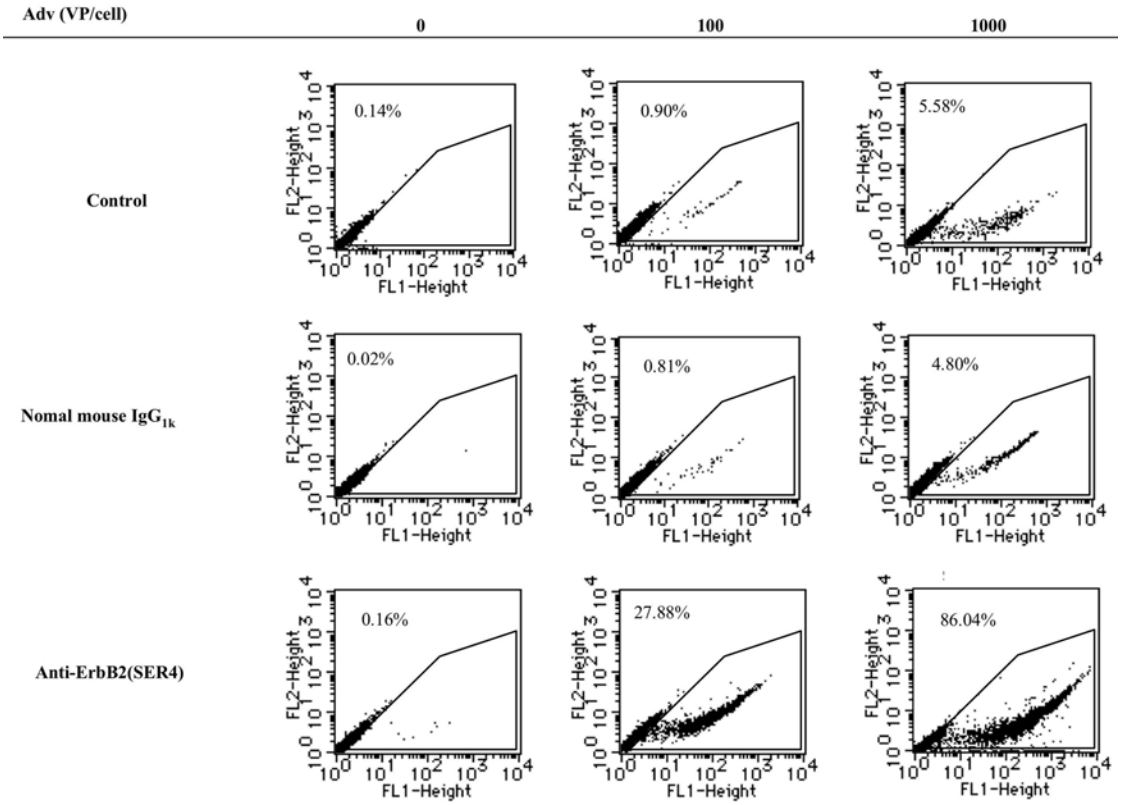

Figure 6. Anti-ErbB2(SER4)-mediated EGFP gene transfer into SKOV3. Transduction efficiency in Ax3CAEGFP-Z33-infected SKOV3 was evaluated by flow cytometry. Cells were infected with Ax3CAEGFP-FZ33 at 0 to 1,000 VP/cell after incubation with SER4 or isotype control IgG1k. Numbers, percentage of EGFP-positive cells.

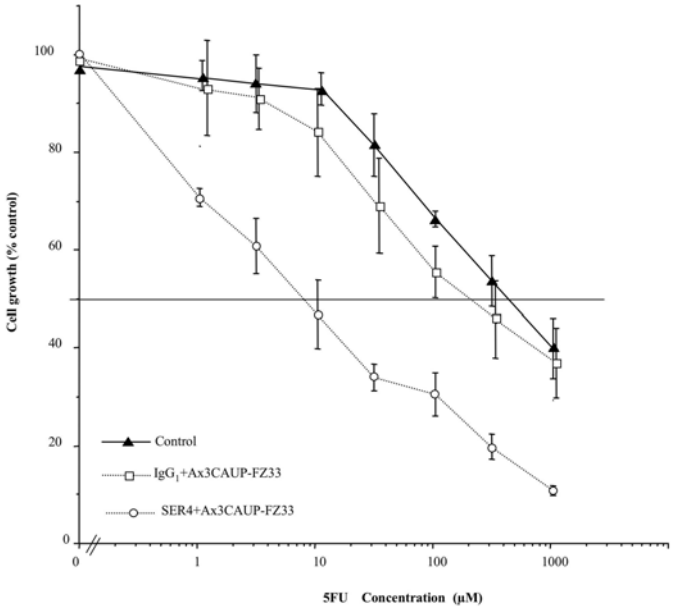

Figure 7. *In vitro* sensitivity to 5-FU following gene transfer with Ax3CAUP-FZ33. SKOV3 (ErbB2-positive) cells were infected with Adv-FZ33 encoding the UPRT gene from E.coli and tested in vitro for their sensitivity to 5-FU. Cells were infected with Ax3CAUP-FZ33 at 300VP/cell after incubation with SER4 (○) or isotype control antibody (□) or without antibody (▲). After infection, the cells were cultured in medium containing 5-FU (0-1,000 μM) for 5 days. Cell viability was determined by a WST-1 assay. The percentage of surviving cells was measured. Points, mean; ber, SD.

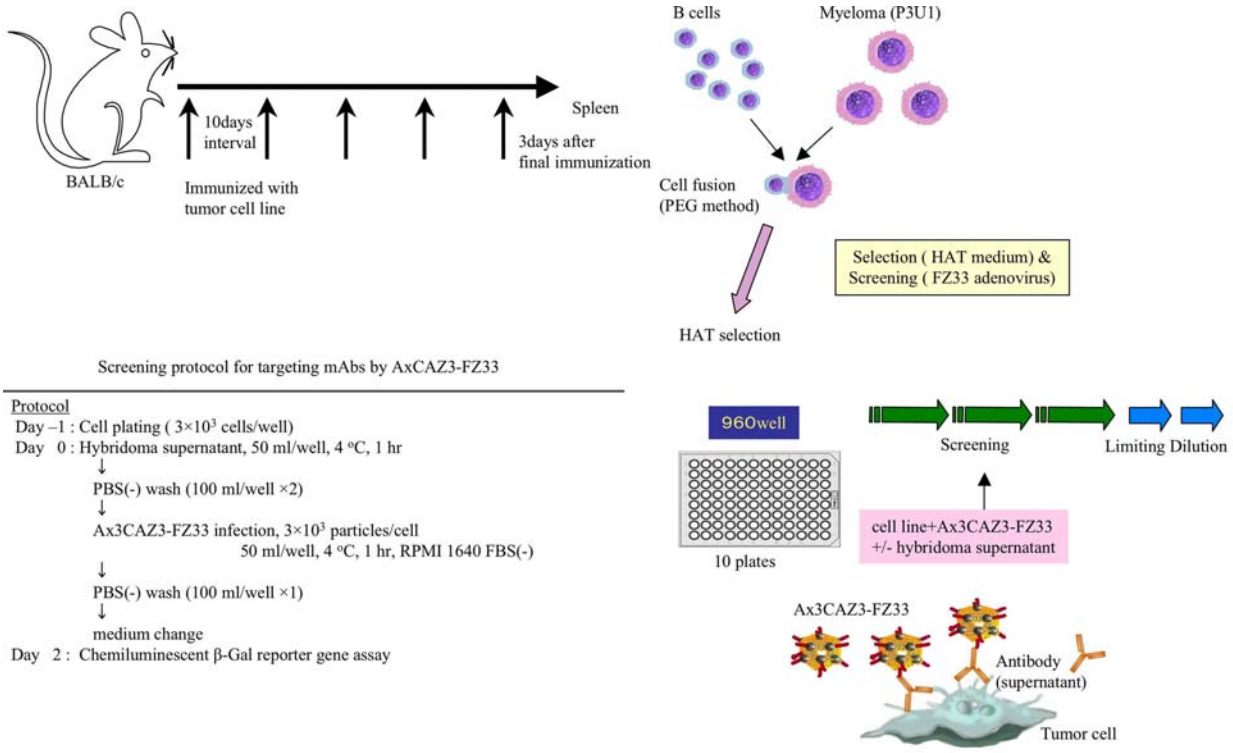

Figure 8. Methods of screening for novel cancer-targeting antibodies using AxCAZ3-FZ33.

search for novel candidate molecules for targeted therapy that would enable selective gene transfer to cancer cells (Figure 8).

To search for molecules suitable for targeted tumor therapy, experiments are being performed as follows: (a) Mice are immunized with certain tumor cells, and a library of monoclonal antibodies for the tumor is constructed; (b) Antibodies that enhance gene transfer efficiency by bridging fiber-modified FZ33 adenovirus and tumor cells are screened; (c) A band obtained by immunoprecipitation is excised and the target molecule recognized by the antibody is identified by mass-spectrometry. (d) Expression of candidate surface molecules for selective targeting of tumor cells is examined by tissue array and in culture cells and surgically resected specimens. (e) Potential candidate antibodies for targeted therapy are examined. A hybridoma library for production of antibodies for melanoma, ovarian cancer, multiple myeloma, malignant mesothelioma, and lung cancer in addition to human prostatic and pancreatic cancer antigens is screened and analyzed to identify those that would enhance gene transfer efficiency. In the ongoing project directed at constructing antibodies targeted at human prostatic cancer antigens, about 200 antibody-producing hybridoma cell lines have been established.

8. CONCLUSIONS

It was possible to enhance gene transfer efficiency to cancer cells by using a combination of fiber-modified adenovirus and antibody to a target molecule on the tumor surface. In addition, candidate target molecules were screened and several antibodies have been established to date. On the basis of these results, we would like to develop selective and effective cancer gene therapy by combining identification of individually expressed target antigens and selective gene transfer with antibody and adenovirus,.

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Abbreviation: CAR: coxsackie-adenovirus receptor

Key Words: Adenovirus vector, Gene therapy, Targeting vector, Review

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