Efficient Gene Transfer into Human Trophoblast Cells with Adenovirus Vector Containing Chimeric Type 5 and 35 Fiber Protein

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Recombinant adenovirus (Ad) vectors based on Ad type 5 have been widely used for gene transfer experiments. Conventional Ad type 5 vectors have a narrow range of tropism and are limited by the size of the transgene that can be packaged. To overcome these limitations, we previously developed an Ad vector (Ad5/35 vector) containing a chimeric Ad type 5 and 35 fiber protein. In the current study, we evaluated the ability of the Ad5/35 vector to transfer genes into human trophoblast cell lines (JAR, JEG-3 and BeWo cells), which are used as in vitro models of human placenta. We compared the gene transfer efficiency of Ad5/35 to that of conventional Ad vector. We found that expression of CD46, which are receptors for Ad5/35 vector, is higher than that of coxsackievirus and adenovirus receptor in all 3 trophoblast cell lines, as determined by flow cytometry. Next, we compared the transducing activity of Ad5 vector and Ad5/35 vector that each expressed luciferase as a reporter gene. Ad5/35 vector had greater gene transfer activity than the conventional Ad vector in all 3 trophoblast cell lines (1.82-fold in JAR cells, 5.37-fold in BeWo cells, 6.11-fold in JEG-3 cells). Thus, Ad vector that contains chimeric type 5 and 35 fiber protein can be a powerful tool for gene transfer experiments in human trophoblast cell lines.

Key words adenovirus vector; chimeric fiber; gene therapy; trophoblast

The placenta, which is responsible for the development of the fetus, has broad-ranging functions that include transporting nutrients from maternal fluid into the fetus, secreting hormones, and preventing the transfer of toxic substances into the fetus. 1) The placenta contains a variety of cell types, including trophoblast cells, endothelial cells and epithelial cells. Trophoblast cells are believed to be important for fetal development because they transport nutrients from the mother to the fetus. 1) Human trophoblast cell lines functionally expressed transporters of monocarboxylic acids, folic acid and anti-cancer drugs. 2–5) Gene transfer into trophoblast cells can be a useful tool for clarifying the biology of placenta, but methods to transfer gene into trophoblast cells have never been fully investigated.

Recombinant adenovirus (Ad) vectors can introduce genes of interest into cells and tissues. There are more than 51 serotypes of Ad. Ad type 5 (Ad5) vector has been frequently used in basic research and clinical work. 6) Ad5, which belongs to subgroup C, has been used to prepare recombinant Ad vectors because its genetic and biological characteristics are extensively studied. There are at least two steps to the infection of cells with Ad5. The first step is the attachment of the virus to coxsackievirus and adenovirus receptor (CAR) on the cell membrane via the knob domain of the fiber. 7,8) Then, Ad5 is internalized into the cell through the interaction of RGD (Arg-Gly-Asp) motifs on the penton base of the Ad5 surface with αvβ3- and αvβ5-integrins on the cell membrane. 9,10) Transgenes delivered by a conventional Ad5 vector are limited to a size of 8.1–8.2 kb, 11) and Ad5 has poor transduction efficiency in CAR-negative cells. 12) Thus, conventional first-generation Ad vectors have a limited transgene size as well as limited tropism.

To overcome these limitations, we previously developed Ad vectors containing chimeric type 5 and 35 fiber protein. 13,14) Ad type 35 (Ad35), which belongs to subgroup B, was initially isolated from the kidneys and lungs of a renal transplant patient. 15) CD46, which is a receptor for Ad35, 16) is ubiquitously expressed in human cells. 17) The Ad5/35 vector can package 8.8 kb of foreign DNA and can transduce CAR-negative cell lines and various human cell lines more effectively than Ad5 vector. 13,14) Thus, Ad5/35 vector is a promising candidate for mediating efficient gene transfer into human trophoblast cell lines.

In the present study, we examined the expression of CD46 in 3 human trophoblast cell lines (JAR, BeWo and JEG-3), which are used in human trophoblast research. We also evaluated the ability of Ad5/35 vector to transfer genes into the human trophoblast cell lines.

MATERIALS AND METHODS

Cell Culture The BeWo cells (clone b30) were obtained from Dr. Alan Schwartz (Washington University, MO, U.S.A.). BeWo cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% MEM non-essential amino acid solution (Gibco, MD, USA), 1.6 g/l sodium bicarbonate, 0.584 g/l l-glutamine and 3.5 g/l d-glucose. JAR and JEG-3 were obtained from American Type Culture Collection (Manassas, VA, U.S.A.) and were cultured with Minimum essential Eagle’s medium (MEM) supplemented with 10% FBS and RPMI-1640 supplemented with 10% FBS, respectively.

Preparation of Ad Vectors Ad-L2, which is the conventional Ad vector derived from Ad type 5, and Ad-F35-L2, which contains chimeric type 5 and 35 fiber protein, were purified as previously described. 13,18,19) Both vectors expressed luciferase. The virus particle titer and infectious (plaque forming unit: PFU) titer were spectrophotometrically deter-
mined by the methods of Maizel et al.\textsuperscript{20} and by the method of Kanegae et al.;\textsuperscript{21} respectively. The PFU to particle ratio was 1 : 14 for Ad-L2 and 1 : 15 for Ad-F35-L2.

**Adenovirus-Mediated Gene Transduction into Human Trophoblast Cells** Cells (1×10^6 cells) were seeded into a 96-well dish. On the following day, they were transduced with Ad-L2 or Ad-F35-L2 (3000 vector particles per cell) for 1.5 h. After culture for 48 h, luciferase production in the cells was measured using a luciferase assay system (PicaGene LT2.0, Toyo Inki Co., Ltd., Tokyo, Japan).

**Flow Cytometry** To detect the expression of human CAR on the membrane, cells were labeled with mouse monoclonal antibody RmcB (kindly provided by Dr. J. M. Bergelson, The Children’s Hospital of Philadelphia, PA, U.S.A.). The cells were then incubated with fluorescein-labeled secondary antibody (Pharmingen, San Diego, CA, U.S.A.). To detect the expression of human CD46, the cells were labeled with fluorescein-conjugated anti-human CD46 (E4.3; Pharmingen). Labeled cells were analyzed by flow cytometry (FACSCalibur, Becton Dickinson, Tokyo, Japan).

**Statistical Analysis** The significant difference was calculated using one-way ANOVA followed by Dunnnett’s test.

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![Fig. 1. Flow Cytometric Analysis of CAR and CD46 Expression in Human Trophoblast Cell Lines](image)

Cells were incubated with: (1) anti-CAR antibodies followed by fluorescein-labeled secondary antibody, (2) fluorescein-labeled anti-CD46 antibody or (3) fluorescein-labeled goat IgG. Then, labeled cells were detected by flow cytometry. The dashed regions indicate cells labeled by anti-CD46, the shaded regions indicate cells labeled by goat IgG and the solid regions indicate cells labeled by anti-CAR antibody.

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**RESULTS AND DISCUSSION**

To overcome the limited tropism of conventional Ad5 vectors, we previously developed Ad5/35 vectors that contain chimeric type 5 and type 35 fiber.\textsuperscript{13,14} These chimeric Ad vectors can infect cells via CD46, a receptor of the type 35 fiber.\textsuperscript{15} First, we investigated the expression of CD46 and CAR on the membranes of JAR, JEG-3 and BeWo cells. Flow cytometry analysis showed that CD46 and CAR were expressed on the membranes of all 3 trophoblast cell lines (Fig. 1), suggesting that Ad5/35 vector can infect with the trophoblast cell lines in a CD46-dependent fashion.

To compare the transgene activities of the chimeric Ad5/35 vector (Ad-F35-L2) and the conventional Ad5 vector (Ad-L2), we used luciferase as a reporter gene. Ad5/35 mediated greater transgene activity than Ad5 in JAR (1.82-fold), JEG-3 (6.11-fold) and BeWo cells (5.37-fold) (Fig. 2). We previously reported that Ad5/35 vector mediated 100-fold greater expression of reporter gene than Ad5 vector in CAR-negative LN444 cells.\textsuperscript{13} Taken together, Ad5/35 may infect with the trophoblast cells via different receptors from CAR. Although CD46 is known to be a receptor for Ad type 35,\textsuperscript{16} the involvement of unidentified receptors for Ad type 35 in the infection of Ad5/35 vectors is not negligible. Indeed, Erikson et al. indicated Ad 35 infected with the cells via a trypsin-insensitive receptor.\textsuperscript{22} The different transgene activity among JEG-3, JAR and BeWo cells may be caused by different expression profiles of receptors for Ad type 35 among them.

In summary, this is the first report of efficient gene delivery into JAR, JEG-3 and BeWo cells by Ad vector containing chimeric type 5 and type 35 fiber protein. The chimeric

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![Fig. 2. Comparison of Luciferase Production in Human Trophoblast Cells Transduced by Ad-L2 or Ad-F35-L2](image)

Cells were transduced with 3000 vector particles per cell of Ad-L2 or Ad-F35-L2 for 1.5h. After culture for 48h, luciferase production was measured by a luminescent assay. The data are expressed as mean±S.D. (n=4). * Significant difference from the Ad-L2-transduced group (p<0.01).
Ad5/35 vector can package a gene of up to 8.8 kb and has broad tropism. Thus, Ad5/35 vectors may be powerful tools for gene transfer experiments in human trophoblast cells.

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