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# Adenovirus-mediated expression of pig $\alpha$(1, 3) galactosyltransferase reconstructs Gal a(1, 3) Gal epitope on the surface of human tumor cells

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## ABSTRACT

Gal $\alpha$(1, 3) Gal (gal epitope) is a carbohydrate epitope and synthesized in large amount by $\alpha$(1, 3) galactosyltransferase [$\alpha$(1, 3) GT] enzyme on the cells of lower mammalian animals such as pigs and mice. Human has no gal epitope due to the inactivation of $\alpha$(1, 3) GT gene but produces a large amount of antibodies (anti-Gal) which recognize Gal $\alpha$(1, 3) Gal structures specifically. In this study, a replication-deficient recombinant adenoviral vector Ad5sGT containing pig $\alpha$(1, 3) GT cDNA was constructed and characterized. Adenoviral vector-mediated transfer of pig $\alpha$(1, 3) GT gene into human tumor cells such as malignant melanoma A375, stomach cancer SGC-7901, and lung cancer SPC-A-1 was reported for the first time. Results showed that Gal epitope did not increase the sensitivity of human tumor cells to human complement-mediated lysis, although human complement activation and the binding of human IgG and IgM natural antibodies to human tumor cells were enhanced significantly after Ad5sGT transduction. Appearance of gal epitope on the human tumor cells changed the expression of cell surface carbohydrates reacting with Ulex europaeus I (UEA I) lectins, Vicia villosa agglutinin (VVA), Arachis hypogaea agglutinin (PNA), and Glycine max agglutinin (SBA) to different degrees. In addition, no effect of gal epitope on the growth in vitro of human tumor cells was observed in MTT assay.

**Key words:** *Adenoviral vector, galactosyltransferase, Gal a(1, 3) Gal, gene expression, human tumor cell.*

## INTRODUCTION

Gal $\alpha$(1, 3) Gal (gal epitope) is a carbohydrate epitope, which is produced in large amount on the cells of pigs, mice and New World monkey (monkey of South America) by the glycosylation enzyme Gal $\beta$1,4GlcNAc3-$\alpha$-D-galactosyltransferase [$\alpha$(1, 3)GT; EC2.4.1.51][1]. This enzyme is active in the Golgi apparatus of cells and transfers galactose from the sugar-donor uridine diphosphate galactose (UDP-galactose) to the acceptor N-acetyllactosamine residue (Gal $\beta$1-4GlcNAc-R ) on carbohydrate chains of glycolipids and glycoproteins, to form gal epitope (Gala1, 3Galb1-4GlcNAc-R) [1].

Gal epitope is completely absent in humans, apes, and Old World monkeys (monkey of Asia and Africa) because their genes encoding $\alpha$(1, 3)GT have become inactivated in the course of evolution [2],[3]. Since humans and Old World primates lack the gal epitope, they are not immunotolerant to it and produce anti-gal epitope antibodies (anti-Gal) throughout life in response to antigenic stimulation by gastrointestinal bacteria[4] and as many as 1% of circulating B cells are capable of producing this antibody[5].

Anti-Gal forms a major immunological obstacle for xenotransplantation of nonprimate mammalian vascularized organs, such as porcine organs, into human recipients[6]. The binding of anti-Gal to gal epitopes expressed on glycolipids and glycoproteins on the surface of endothelial cells in donor organs leads to activation of the complement cascade and hyperacute rejection, and also plays important role in occurrence of complement-independent delayed xenograft rejection[6]. Anti-Gal also prevents the use of retroviral vectors propagated in mouse cells for gene therapy, because natural

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anti-Gal binds to gal epitopes on the viral envelope glycoproteins and induces the destruction of these viruses[7].

In cancer research, studies on the immune response of tumor vaccines have indicated that a major prerequisite for the success of tumor vaccines is their effective uptake by antigen-presenting cells (APCs) and transportation of these APCs to the draining lymph nodes, where the processed and presented tumor-associated antigens (TAAs) activate tumor-specific naive T cells[8]. Galili et al[9] proposed that the immunogenicity of autologous tumor vaccines in human maybe augmented by engineering vaccinated tumor cells to express gal epitopes on membranes. Subsequent in situ binding of natural anti-Gal IgG molecules to these epitopes would result in the formation of immune complexes that target tumor vaccines for uptake by APCs via the interaction of the Fc portion of anti-Gal with Fcgreceptors on APCs. This hypothesis has been tested in vivo as well as in vitro[10],[11].

At present, a number of studies have suggested that human recombinant adenoviruses (rAdvs) are efficient vectors for the delivery of cloned genes to a variety of tumor cells and tissues[12]. Therefore, in the current study, a recombinant replication-defective adenovirus that expresses the pig $\alpha$(1, 3) GT gene was constructed. We evaluated the expression of gal epitope on the surfaces of several human tumor cell lines after adenoviral vector transduction.

## MATERIALS AND METHODS

### *Cell culture*

All cell lines were from Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The 293 cells (Immortalized human embryonic kidney cells) and COS cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco.BRL) supplemented with 10% heat-inactivated newborn bovine serum (NBS) in a humidified incubator with 5% $CO_2$ and 95% air at 37°C. Human lung cancer cells (SPC-A-1), melanoma cells (A375), and stomach cancer cells (SGC-7901) were maintained in RPMI-1640 (Gibco.BRL) supplemented with 10% NBS. 293 cells were used for recombinant adenovirus transfection, amplification, and titration.

### *Construction of recombinant adenoviral vector expressing pig a(1, 3)GT gene*

Plasmid pcDNA3-sGT1100[13] was digested by Xho I and blunted with Klenow enzyme, and then digested by BamH I. the resulting 1.19 kb fragment of pig α (1, 3)GT cDNA was subcloned into BamH I-EcoR V site in pBluescript II KS+/- to produce pKS-sGT, and then subcloned into the Hind III-Not I site in pAdCMV(s)-BGHpA containing 0 to 17 map units (mu) human type 5 adenovirus genome with a human CMV promoter, multicloning site and bovine growth hormone polyA signal inserted in the E1 region of viral genome to obtain the recombinant plasmid pAd-sGT (Fig 1). This plasmid was cotransfected into 293 cells along with a plasmid pJM17[14] containing most of the rightward sequences (3.7 to 100 mu) of human type 5 adenovirus genome with a partial deletion in the E3 region. The recombinant replication-defective adenovirus Ad5sGT was res-

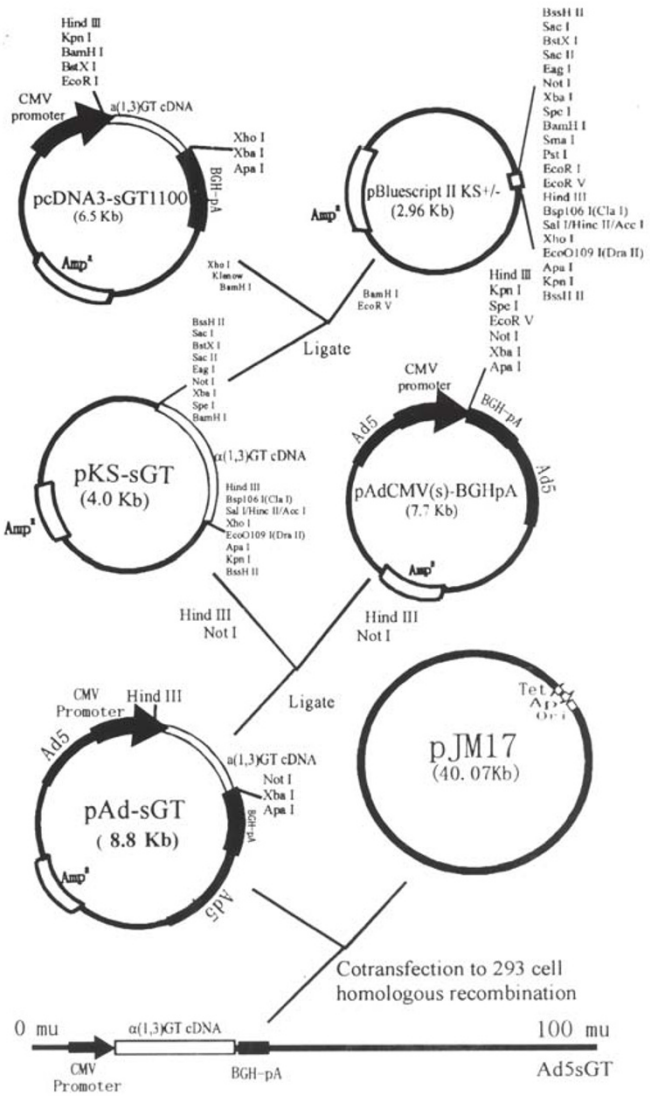


**Fig 1. Construction of recombinant adenovirus Ad5sGT expressing pig α (1, 3)GT gene** The recombinant plasmid pAd-sGT was constructed by inserting pig a(1, 3)GT cDNA into the multicloning site of a shuttle vector pAdCMV(s)-BGHpA which harbored a cytomegalovirus promoter (CMV) and a bovine growth hormone polyA signal (poly A). The recombinant adenovirus Ad5sGT was generated by homologous recombination after cotransfecting 293 cells with pAd-sGT and a virus-rescuing vector pJM17.

cued by homologous recombination (Fig 1). The replication-deficient recombinant adenoviruses, Ad5 $\beta$ gal and Ad5null, used as the control throughout the study, have been previously described[15]. High titers of recombinant adenoviruses were amplified, purified, titered, and stored as previously described [16].

### *Characterization of adenoviral vector expressing pig a(1, 3)GT gene*

The genomic DNA extracted from Ad5sGT-infected 293 cells was digested with Hind III and the fragments were analyzed by agarose gel electrophoresis and Southern hybridization. COS cells were infected with Ad5sGT at multiplicity of infection (MOI) of 40. Flow cytometric analysis and the direct fluorescence of cell surface carbohydrate epitope on Ad5sGT-infected COS cells were performed with fluorescein isothiocyanate (FITC)-conjugated Bandeiraea simplicifolia isolectin B4 (BS-IB4) lectins (Sigma) specific for gal epitope.

### *Southern hybridization*

Southern blot analysis was performed according to the standard procedures using pig $\alpha$(1, 3)GT cDNA released from pcDNA3-sGT1100 as probe. The probeswere labeled with $\alpha$-$^{32}$P dATP using Random primed DNA labeling kit (Boehringer Mannheim, GmbH, Germany). DNA fragments separated by agarose gel electrophoresis were transferred onto Hybond-N membranes (Amersham) by capillary transfer method and were subsequently hybridized with the radiolabeled probes.

### *Flow cytometric analysis*

A375, SGC-7901 and SPC-A-1 cells were detached from the tissue culture flasks 48 h after Ad5sGT infection by brief treatment with 5 mM EDTA in phosphate buffered saline (PBS, pH 7.4), centrifuged for 10 min at 250 g in the presence of 10%NBS, and resuspended at $10^6$ cells/ml in cold (4°C) PBS, 1% bovine serum albumin (BSA), 0.1% $NaN_3$ (Sigma). 100 ml of cells in triplicate were incubated with the primary antibody for 1 h at 4°C, and then washed three times with cold PBS/BSA/NaN3 before being resuspended in the same buffer containing second antibody (FITC-conjugated rabbit anti-human IgG and IgM antibodies, DAKO) at the dilutions recommended by the manufacturer. Following incubation at 4°C for 1 h in the dark, the samples were again washed three times with cold PBS/BSA/NaN3 and resuspended in 100 $\mu$l of ice-cold PBS, 1% formaldehyde. FITC-conjugated Ulex europaeus I (UEA I), Vicia villosa agglutinin (VVA), Arachis hypogaea agglutinin (PNA), Glycine max agglutinin (SBA), and BS-IB4 lectins were used at 5 $\mu$g/ml in PBS/NBS/NaN3. A375, SGC-7901 and SPC-A-1 cells were labeled for 1 h at 4°C in the dark and then washed and fixed as above. Each individually labeled sample was analyzed separately by flow cytometry using a Becton Dickinson FACScan cytometer.

### *Efficiency of recombinant adenovirus infection*

Bacterial $\beta$-galactosidase ($\beta$-gal) gene (lacZ gene) expression was used as a marker of viral infection efficiency. COS, A375, SGC-7901 and SPC-A-1 cells were tested using Ad5 $\beta$ gal containing E. coli lacZ gene. Exponentially growing cells were seeded in duplicate in 24-well tissue culture plates at a density of $4\times10^4$ cells/well and infected with Ad5 $\beta$ gal virus at different MOIs at the same time. After 48 h cells were fixed with 0.5% glutaraldehyde and then stained with X-gal solution (Sigma). Positive cells infected with Ad5$\beta$gal expressed bgal activity and developed blue in color. The results were analyzed qualitatively by visualization and captured by photomicrography.

### *C3c binding assay*

A375, SGC-7901 and SPC-A-1 cells were washed and then incubated in 200 ml of normal human serum (NHS) at concentration of 10% for 10 min at 37°C, washed again, and then incubated with FITC-conjugated rabbit anti-human C3c antibody (Dakopatts, Denmark) at 1:50 dilution at 4°C for 30 min. Stained cells were analyzed using a FACScan cytometer.

### *Pig α(1, 3)GT gene expression at different postinfection time points*

A375, SGC-7901 and SPC-A-1 cells were seeded in tissue culture flasks and infected with Ad5sGT at a MOI of 20 for A375, a MOI of 40 for SGC-7901, and a MOI of 45 for SPC-A-1. Cells were collected at 1, 2, 3, 4, 5, 6 and 7 d from the time the culture was initiated and stained with FITC-conjugated BS-IB4 lectins. Flow cytometric analysis was performed to detect the expression of gal epitope on the cell surface.

### *Determination of the effect of normal human serum (NHS) on human tumor cells*

Human tumor cells A375, SGC-7901 and SPC-A-1 were seeded in tissue culture flasks and infected with Ad5sGT at MOIs as described above. After 48 h, cells were incubated in the presence of 10%, 20% or 40% NHS (blood group B) at 37°C for 30 min, and then stained with 0.4% trypan blue. The numbers of viable and nonviable cells were counted and recorded as the percentage of viable cells (viable cells/total cells counted).

### *Effect of gal epitope expression on cell growth in vitro*

A375, SGC-7901 and SPC-A-1 cells were seeded at a density of $2\times10^3$ cells/well in 96-well plates in octuple after infection with Ad5sGT at MOIs as described above. The MTT assay was employed to account the viable number of cells at 1, 2, 3, 4, 5, 6 and 7 d after Ad5sGT infection. A growth curve was plotted for each cell line depicting the viable number of cells versus the duration of postinfection. Cell growth assay was repeated for three times under the same experimental condition.

### *Statistical analysis*

Data in this study were expressed as means and standard deviations and analyzed by Student' s t test.

## RESULTS

### *Characterization of adenoviral vector express-*

*ing pig a(1, 3)GT gene*

Upon hybridization to pig $\alpha$(1, 3)GT cDNA probe, a specific hybrid band was detected in Hind III-digested genomic DNAs from 293 cells infected by the recombinant adenovirus expressing pig $\alpha$(1, 3)GT gene (Ad5sGT) (Fig 2A), corresponding to about 5.3 kb fragment of viral DNA as predicted. These results demonstrated the incorporation of pig a(1, 3)GT cDNA into the adenoviral genome.

Since COS cells were demonstrated previously that no gal epitope was expressed on the cell membrane[17], these cells were therefore used in this study to determine the expression of pig $\alpha$(1, 3)GT gene incorporated in the adenoviral vector Ad5sGT. To verify transgene expression, COS cells were infected with Ad5sGT at 40 MOI. At 48 h after infection, the expression of $\alpha$(1, 3)GT gene in Ad5sGT-infected COS cells was detected in both the direct immunofluorescence of cell surface carbohydrates (Fig 2B) and flow cytometric analysis (Fig 2C) using the FITC-conjugated BS-IB4 lectin specific for gal epitope. These data display the efficacy of pig $\alpha$(1, 3)GT gene expression in various cell types, driven by the CMV promoter contained in this adenoviral vector.

*Recombinant adenovirus infection*

Following infection with Ad5$\beta$gal, COS, SPC-A-1, SGC-7901 and A375 cells exhibited different efficacies of recombinant adenovirus infection. However, more than 90% of infectivity was achievable for all these cell lines at different optimum MOIs (Fig 3). The optimum MOI was 40 for COS and SGC-7901 cells, 20 for A375 cells, and 45 for SPC-A-1 cells.

*Expression of gal epitope on human tumor cells*

To determine whether $\alpha$(1, 3)GT expressed from Ad5sGT could effectively synthesize gal epitope on the membrane of human tumor cells, three tumor cell lines were analyzed. The results revealed that all the tumor cells infected with Ad5sGT expressed high level of gal epitope recognizable by BS-IB4 lectin on the cell membrane. In contrast, original tumor cells and Ad5null-infected tumor cells did not express gal epitope, as indicated by a lack of interaction with BS-IB4 lectin (Fig 4).

*Binding of human IgG and IgM natural antibodies to human tumor cells and complement activation after Ad5sGT infection*

To determine whether expression of $\alpha$(1, 3)GT gene in human tumor cells could result in binding of human natural antibodies to cell surface, SGC-7901, SPC-A-1 and A375 cells were infected with Ad5sGT and Ad5null, respectively, and then incubated with 10% normal human serum (NHS). Rabbit anti-human IgG and IgM antibodies were used as second antibodies to detect the binding of human Igs to the cell surface. Flow cytometry was performed as described in Materials and Methods. As shown in Fig 5A, the tumor cells expressing gal epitope exhibited a significant increase in the level of human Igs (IgG and IgM) binding when compared with Ad5null-infected cells which did not express gal epitope.

Next we determined the effect of increased binding of human Igs on human complement activation. The tumor cells from each cell line at 48 h after Ad5sGT infection were incubated in the presence of NHS at concentrations of 10%, and then the deposition of C3, a component of activated complement, was determined as a measure of cell surface complement activation by Flow cytometric analysis. As shown in Fig 5B, C3c deposition was higher on cells expressing gal epitope when compared with control.

*Effect of $\alpha$(1, 3)GT gene expression on expression of cell surface carbohydrates*

To investigate whether the gal epitope expression could alter the expression of cell surface carbohydrates, the expression of cell surface carbohydrates, particularly those reacting with UEA-I, VVA, PNA and SBA lectins, on the surface of human tumor cells A375, SGC-7901 and SPC-A-1 was first determined by flow cytometry using FITC-conjugated lectins. The results revealed that all these tumor cell lines expressed the UEA-I, SBA, PNA and VVA lectin binding sites on the membrane at different levels (Tab 1). In the presence of gal epitope on tumor cell surface, the expression of UEA I lectin binding site, known as the blood group H antigen[18] which was expressed at the highest level on SGC-7901 cells, became decreased significantly. However, no effect of gal epitope expression on blood group H antigen expression was

detected on A375 and SPC-A-1 cells which have rather lower level of blood group H antigen. The appearance of gal epitope also resulted in the decrease of VVA and PNA lectin binding sites on A375 and SGC-7901 cells, but not on SPC-A-1 cells. PNA lectin binding sites on SPC-A-1 cells and SBA lectin binding sites on A375 and SPC-A-1 cells were up-regulated by the expression of gal epitope (Tab 2).

*Effectiveness of gal epitope in inducing de-*

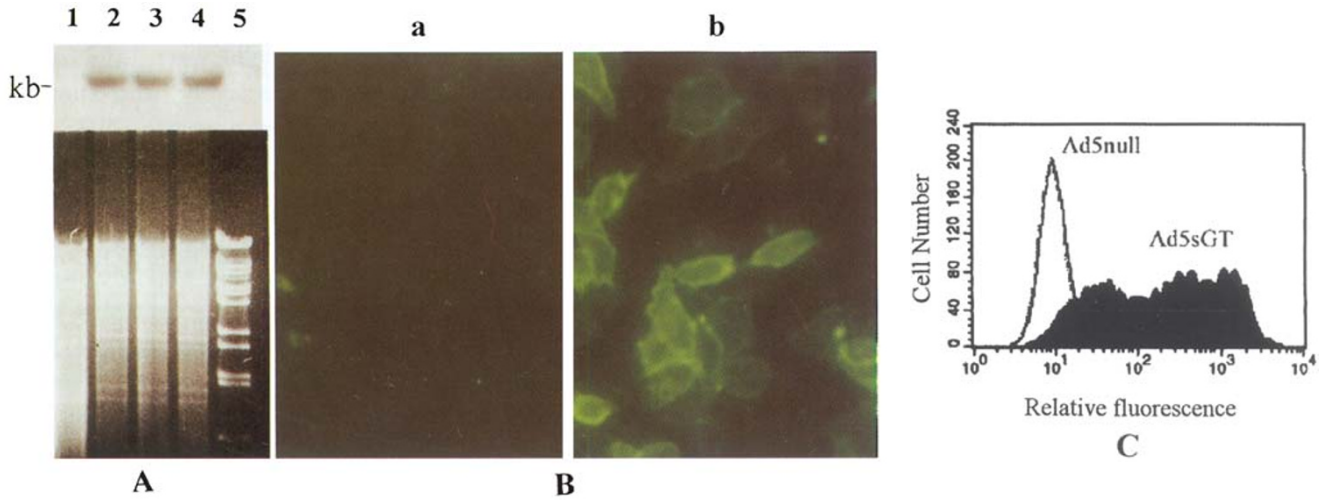


**Fig 2. Characterization of recombinant adenovirus Ad5sGT** **(A)** The genomic DNA extracted from Ad5sGT-infected 293 cells was digested with Hind III. The resultant fragments were gel-separated and hybridized to a pig α(1, 3)GT cDNA probe in Southern hybridization. From left, Lane 1, genomic DNA from control adenoviral vector Ad5null-infected 293 cells; lane 2, 3, and 4, genomic DNA from Ad5sGT-infected 293 cells; lane 5, a DNA ladder as a control and size marker (lDNA/Eco91 I). **(B)** Cell surface staining of recombinant adenovirus infected COS cells. FITC-conjugated BS-IB4 lectin staining of the surface of COS cells after Ad5null infection (a), and Ad5sGT infection (b). Results are representatives of at least 4 experiments. ×280 **(C)** Flow cytometric analysis of gal epitope expression on the surface of COS cells after Ad5sGT infection using FITC-conjugated BS-IB4 lectin specific for gal epitope. Ad5null infection was shown as bold line (control), and Ad5sGT infection was shown as filled histogram.

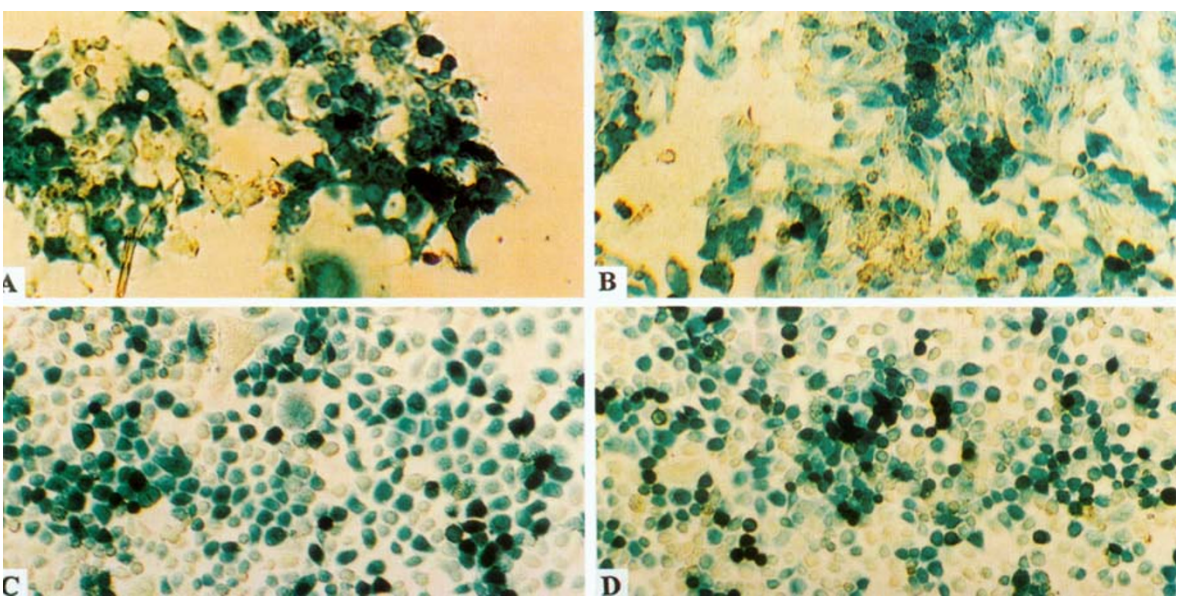


**Fig 3.** β-gal gene expression 48 h after infection with Ad5 bgal in COS cells at MOI of 40 (A), in A375 cells at MOI of 20 (B), in SGC-7901 cells at MOI of 40 (C), and in SPC-A-1 cells at MOI of 45 (D). ×140

*struction of human tumor cells by normal human serum (NHS)*

To test the effect of NHS on human tumor cells expressing gal epitope, A375, SGC-7901 and SPC-A-1 cells were infected with Ad5sGT and then incubated in the presence of NHS at different concentrations. Cells without Ad5sGT infection were used as negative controls. As shown in Tab 3,

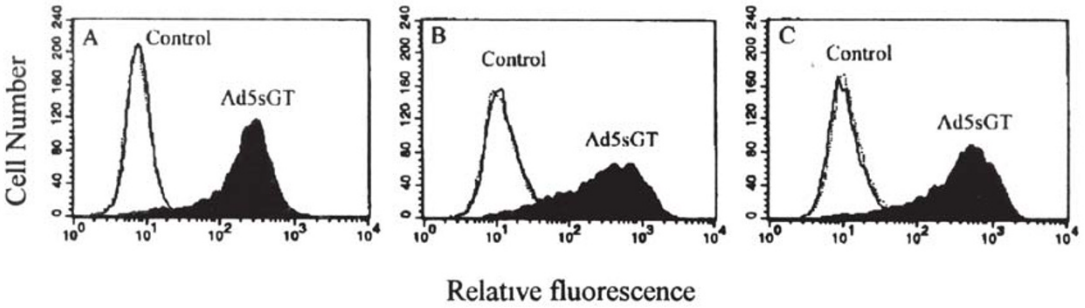


**Fig 4.** Expression of gal epitope on human tumor cells. Human tumor cells A375 (A), SGC-7901 (B) and SPC-A-1 (C) were infected with Ad5sGT at corresponding optimum MOIs and collected at 48 h, stained with FITC-conjugated BS-IB4 lectin. Flow cytometric analysis was carried out using a fluorescence-activated cell sorter (FACScan, Beckton Dickinson). Mock infection was shown in broken line (control), Ad5null infection in bold line (control), and Ad5sGT infection in filled histogram.

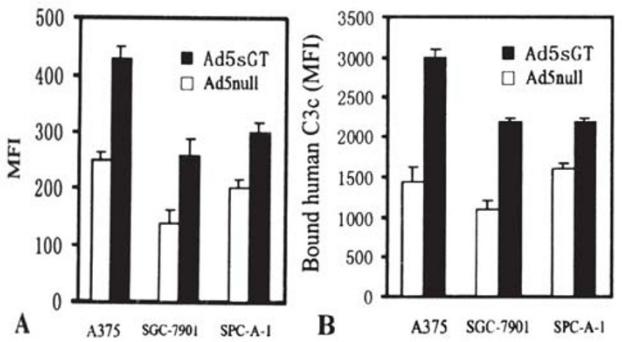


**Fig 5.** Binding of human IgG and IgM natural antibodies to human tumor cells and their complement activation after recombinant adenovirus infection. Human tumor cells A375, SGC-7901 and SPC-A-1 were infected with Ad5null and Ad5sGT respectively and collected at 48 h postinfection. **(A)** The binding of human natural antibodies. Cells were incubated with 10% normal human serum (NHS) as the first antibody and then with FITC-conjugated rabbit anti-human IgG and IgM antibodies as second antibodies. Stained cells were analyzed by flow cytometry (Ad5null, Ad5sGT). **(B)** C3c-binding assay. Cells were incubated in 10% NHS for 10 min at 37°C, and then washed, examined for deposition of the C3 component of complement by staining with FITC-labeled rabbit anti-human C3c antibody, and analyzed by flow cytometry. Data shown in Fig 5 (A) and (B) represent means and standard deviations of triplicate mean fluorescence intensity (MFI) values for flow cytometry histograms.

**Tab 1.** Expression of cell surface carbohydrates reacting with UEA I, VVA, PNA and SBA lectins.

| | A375 | SPC-A-1 | SGC-7901 |
|---|---|---|---|
| control | 3+_1 | 2+_1 | 3+_2 |
| UEA I | 22+_5[a] | 63+_8[a] | 422+_35[a] |
| VVA | 55+_10[a] | 1799+_150[a] | 30+_40[a] |
| SBA | 58+_8[a] | 380+_40[a] | 90+_15[a] |
| PNA | 129+_15[a] | 110+_20[a] | 42+_7[a] |

Data shown represent means and standard deviations of triplicate mean fluorescence intensity values for flow cytometric histograms. [a]$P < 0.01$ vs control (no lectins).

**Tab 2.** Effect of expression ofa(1, 3)GT gene on the expression of cell surface carbohydrates

| | A375 | | SPC-A-1 | | SGC-7901 | |
|---|---|---|---|---|---|---|
| | Ad5null | Ad5sGT | Ad5null | Ad5sGT | Ad5null | Ad5sGT |
| UEA I | 20+3 | 20+6[b] | 62+10 | 60+8[b] | 400+40 | 100+10[a] |
| VVA | 59+7 | 40+5[a] | 1800+200 | 1838+70[b] | 327+30 | 170+19[a] |
| SBA | 60+10 | 130+17[a] | 400+32 | 520+29[a] | 85+15 | 84+14[b] |
| PNA | 120+12 | 80+7[a] | 100+14 | 150+10[a] | 42+5 | 30+4[a] |

Data shown represent means and standard deviations of three experiments. [a]$P < 0.01$, [b]$P > 0.05$ vs corresponding control group (Ad5null group).

**Tab 3.** Sensitivity of human tumor cells to normal human serum treatment

| Cell | Treatment | 10%NHS | 20%NHS | 40%NHS |
|---|---|---|---|---|
| line | group | viable cell(%) | viable cell(%) | viable cell(%) |
| SGC-7901 | control | 99.25+0.95 | 99.00+1.41 | 98.75+1.50 |
| | Ad5sGT | 98.75+0.95[b] | 98.50+1.73[b] | 99.25+0.95[b] |
| SPC-A-1 | control | 98.00+0.81 | 98.75+1.25 | 98.75+1.89 |
| | Ad5sGT | 98.75+0.95[b] | 99.00+1.41[b] | 98.7+1.50[b] |
| A375 | control | 97.50+1.29 | 98.00+2.16 | 96.75+1.71 |
| | Ad5sGT | 98.00+1.8[b] | 98.25+0.95[b] | 95.50+1.29[b] |

Data represent means and standard deviations of four experiments. [b]$P > 0.05$ vs corresponding control group.

trypan blue exclusion did not detect the significant difference in sensitivity to NHS-mediated lysis between human tumor cells with and without gal epitope.

*Expression of gal epitope at different postinfection time points*

In all three tumor cell lines, gal epitope levels were analyzed by flow cytometry using FITC-conjugated BS-IB4 lectin and were substantially high at 24 h after Ad5sGT infection. The gal epitope levels progressively increased with time and reached a maximum at 48 h in A375 and SPC-A-1 cells, and at 72 h in SGC-7901 cells. After these time points, gal epitope level decreased with time and became reduced to background level at 7 d after Ad5sGT infection (Fig 6).

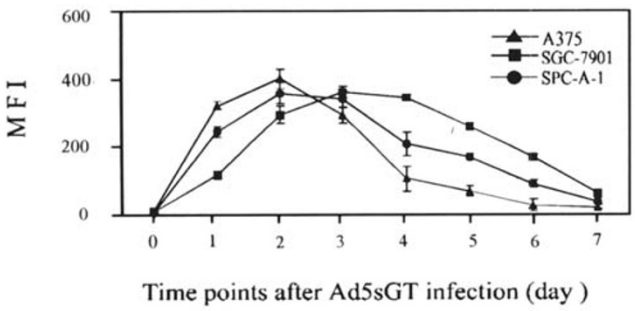


**Fig 6.** Gal epitope expression after adenovirus-mediated pig α(1, 3)GT gene transfer in vitro. A375, SGC-7901 and SPC-A-1 cells were infected with Ad5sGT containing pig α(1, 3)GT cDNA at 20, 40 and 45 MOIs respectively. Cells were collected at different time-points and stained with FITC-conjugated BS-IB4 lectin, and then analyzed by flow cytometry (MFI, mean fluorescence intensity).

*Tumor cell growth in vitro following gal epitope expression*

The effect of gal epitope expression on the proliferative capacity of human tumor cells, including SGC-7901, SPC-A-1 and A375, was judged using MTT assay. The results showed that cells expressing gal epitope had the same proliferative capacity as their parent and the Ad5null-infected cells (Fig 7). In addition, the morphology of these human tumor cells remained unchanged following gal epitope expression under light microscope.

## DISCUSSION

Data in this study showed that human tumor cells SGC-7901, SPC-A-1 and A375 expressed high level of gal epitope on the membrane after Ad5sGT infection. In parallel, the ability of the binding of human IgG and IgM natural antibodies to gal epitope expressing cells was also enhanced. This suggested that the immune complexes of human tumor cells could be formed easily using human anti-Gal natural antibodies after Ad5sGT transduction. Link et al[19] has utilized gal epitope and anti-Gal antibodies reaction as a gene therapy for treatment of human cancer, in which human

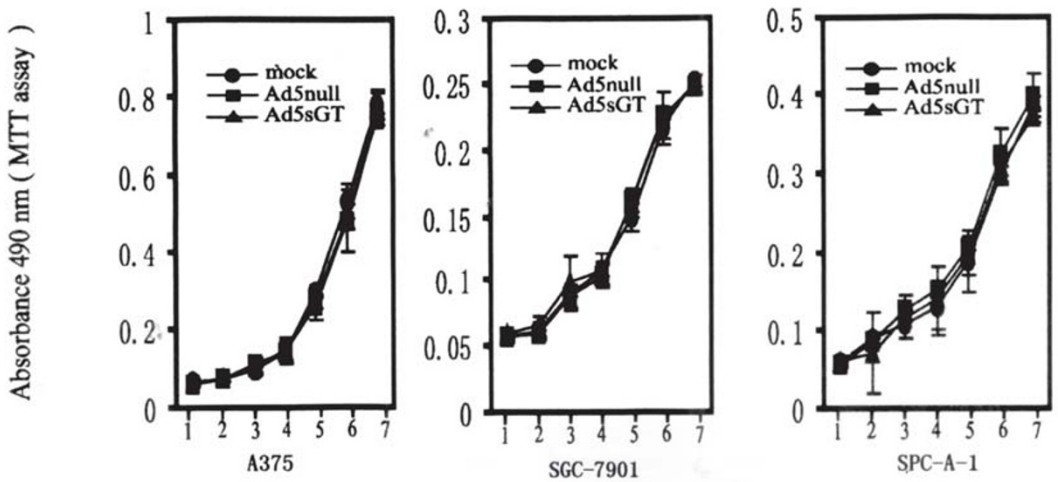


**Fig 7.** Human tumor cell growth in vitro following gal epitope expression over 1-7 d in MTT assay. A375, SGC-7901 and SPC-A-1 cells were infected with Ad5null or Ad5sGT at optimum MOIs as determined in previous experiments, and then cultured in 96-well plates. MTT assay was performed on each day, and growth curves were plotted to determine the growth potential of cells expressing gal epitopes. Data represent means of octuple values and standard deviations.

complement-mediated lysis of human melanoma A375 cells was induced by engineering melanoma cells to express gal epitope followed by incubation with NHS. In this study, we also examined the susceptibility of human tumor cells SGC-7901, SPC-A-1 and melanoma A375 to lysis by normal human serum following gal epitope expression. Cells were analyzed for gal epitope expression by flow cytometry and at the same time were incubated at 37°C for 30 min in the presence of NHS at 10%, 20% and 40% concentrations respectively, and then the number of viable cells was counted by the method of trypan blue exclusion. However, no significant difference in susceptibility to lysis by NHS was observed in each of three tested human tumor cell lines between gal epitope expressing cells and its corresponding parental cells, although activation of complement was enhanced. Because complement-mediated cell lysis is inhibited by the complement regulatory proteins expressed on the cell surface[20], no sensitization of these human tumor cells to NHS may be the results of high level expression of complement inhibitory molecules on these human tumor cells.

The biosynthesis of carbohydrate epitopes in cells is regulated by glycosyltransferases, which are responsible for the addition of carbohydrates to the oligosaccharide chain on glycolipids and glycoproteins in a sequential manner[21]. The newly introduced carbohydrate epitope had been observed to alter the expression of other carbohydrates. Gorelik et al[22] transfected murine melanoma cells, which do not express the gal epitope as well as carbohydrates reacting with SBA, PNA, and VVA lectins, with $\alpha$(1, 3)GT cDNA. This resulted in the appearance of gal epitopes as well as carbohydrates reacting with SBA, PNA or VVA lectins. Appearance of SBA, PNA and VVA lectin binding carbohydrates in the $\alpha$(1, 3)GT gene-transfected melanoma cells was suggested to be the results of reduction of cell membrane sialylation and subsequent unmasking of these carbohydrates due to the competition between $\alpha$(1, 3)GT with $\alpha$(2, 3) sialyltransferase or $\alpha$(2, 6) sialyltransferase for the common acceptor N-acetyllactosamine in the Golgi apparatus[22]. In this study, unlike murine melanoma cells used in Gorelik's report[22], human tumor cells SGC-7901, SPC-A-1 and A375 express carbohydrates reacting with SBA, PNA and VVA lectins on their surface but do not express gal epitope due to the inactivation of $\alpha$(1, 3)GT gene in humans. After transduction with Ad5sGT, expression of carbohydrates reacting with PNA and VVA lectins on SGC-7901 and A375 cells was reduced to different degrees, but SBA and PNA lectin binding carbohydrates on SPC-A-1 cells were increased. The results reflected that expression of gal epitope has different effects on other carbohydrates expression depending on the features of carbohydrates on different cell types.

The $\alpha$(1, 2) fucosyltransferase gene expression has been reported to resultin a drastic suppression of the gal epitope on mouse cells due to the enzymatic competition between the $\alpha$(1, 3)GT and $\alpha$(1, 2) fucosyltransferase for the common acceptor substrate[23], [24]. In contrast to the mouse cells, SGC-7901, SPC-A-1 and A375 all express the blood group H antigen on the surface due to the $\alpha$(1, 2) fucosyltransferase activity[18, 25]. Following gal epitope expression, the blood group H antigen was also reduced significantly on SGC-7901 cells. No changes in blood group H antigen expression on A375 and SPC-A-1 cells following gal epitope expression probably represented the limitation of H antigen which could be replaced by newly introduced gal epitopes. In this study, we also investigated whether expression of gal epitope could affect the growth of human tumor cells in vitro. The results showed that the expression of gal epitope on human tumor cells did not result in changes in cell growth in vitro as determined by MTT assays.

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## REFERENCES

[1] Blanken WM, Van den Eijnden DH. Biosynthesis of terminal Gal $\alpha$(1-3) Gal $\beta$(1-4) GlcNAc-R oligosaccharide sequences on glycoconjugates: Purification and acceptor specificity of a UDP-Gal:N-acetyllactosaminide

a1-3-galactosyltransferase from calf thymus. J Biol Chem 1985; **260**:12927-34.
[2] Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. Man, apes, and Old World monkeys differ from other mammals in the expression of a-galactosyl epitopes on nucleated cells. J Biol Chem 1988; **263**:17755-62.
[3] Larsen RD, Rivera-Marrero CA, Ernst LK, Cummings RD, Lowe JB. Frameshift and nonsense mutations in a human genomic sequence homologous to a murine UDP-Gal:β-D-Gal(1, 4)-D-GlcNAc α(1, 3)-galactosyltransferase cDNA. J Biol Chem 1990; **265**: 7055-61.
[4] Galili U, Mandrell RE, Hamadeh RM, Shohet SB, Griffiss JM. Interaction between human natural anti-a-galactosyl immunoglobulin G and bacteria of the human flora. Infect Immun 1988; **56**:1730-7.
[5] Galili U, Anaraki F, Thall A, Hill-Black C, Radic M. One percent of human circulating B lymphocytes are capable of producing the natural anti-Gal antibody. Blood 1993; **82**:2485-93.
[6] Cooper DKC. Xenoantigens and xenoantibodies. Xenotransplantation. 1998; **5**:6-17.
[7] Takeuchi Y, Porter CD, Strahan KM, Preece AF, Gustafsson K, Cosset FL, et al. Sensitization of cells and retroviruses to human serum by α(1-3) galactosyltransferase. Nature 1996; **379**:85-8.
[8] Schweighoffer T, Schmidt W, Buschle M, Birnstiel ML. Depletion of naive T cells of the peripheral lymph nodes abrogates systemic antitumor protection conferred by IL-2 secreting cancer vaccines. Gene Ther 1996; **3**:819-24.
[9] Galili U, LaTemple DC. Natural anti-Gal antibody as a universal augmenter of autologous tumor vaccine immunogenicity. Immunol Today 1997; **18**:281-5.
[10] LaTemple DC, Abrams JT, Zhang SY, Galili U. Increased immunogenicity of tumor vaccines complexed with anti-Gal: studies in knockout mice for α(1,3) galactosyltransferase. Cancer Res 1999; **59**:3417-23.
[11] LaTemple DC, Henion TR, Anaraki F, Galili U. Synthesis of a-galactosyl epitopes by recombinant α(1, 3) galactosyl transferase for opsonization of human tumor cell vaccines by anti-galactose. Cancer Res 1996; **56**:3069-74.
[12] Zhang WW. Development and application of adenoviral vectors for gene therapy of cancer. Cancer Gene Ther. 1999; **6**:113-38.
[13] Xing L, Xia GH, Bai XF, Fei J, Guo LH. Adenovirus-mediated expression of RNA transcripts complementary to the pig α(1, 3) galactosyltransferase mRNAs inhibits the expression of Gal α(1, 3) Galepitope. Acta Pharmacol Sin 2000; **21**:1005-10.
[14] McGrory WJ, Bautista DS, Graham FL. A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology. 1988; **163**:614-7. [15] Xing L, Xia GH, Fei J, Bai XF, Guo LH. Adenovirus-mediated expression of human secretor type a(1, 2) fucosyltransferase leads to reduction in the level of Gal α(1, 3) Gal epitope. Acta Pharmacol Sin 2000; **21**:807-13.
[16] Hitt M, Bett AJ, Addison CL, Prevec L, Graham FL. Techniques for human adenovirus vector construction and characterization. in: Methods in molecular genetics. Academic Press; 1995; **7**:13-45.
[17] Osman N, McKenzie IF, Ostenried K, Ioannou YA, Desnick RJ, Sandrin MS. Combined transgenic expression of α-galactosidase and α(1, 2)-fucosyltransferase leads to optimal reduction in the major xenoepitope Gal α(1, 3) Gal. Proc Natl Acad Sci USA 1997; **94**:14677-82.
[18] Larsen RD, Ernst LK, Nair RP, Lowe JB. Molecular cloning, sequence, and expression of a human GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase cDNA that can form the H bood group antigen. Proc Natl Acad USA 1990; **87**:6674-8.
[19] Link CJ Jr, Seregina T, Atchison R, Hall A, Muldoon R, Levy JP. Eliciting hyperacute xenograft response to treat human cancer: α(1, 3) galactosyltransferase gene therapy. Anticancer Res 1998; **18**:2301-8.
[20] Mollnes TE, Fiane AE. Xenotransplantation: how to overcome the complement obstacle? Mol Immunol 1999; **36**:269-76.
[21] Roth J. Subcellular organization of glycosylation in mammalian cells. Biochim Biophys Acta 1987; **906**: 405-36.
[22] Gorelik E, Duty L, Anaraki F, Galili U. Alterations of cell surface carbohydrates and inhibition of metastatic property of murine melanomas by α(1, 3) galactosyltransferase gene transfection. Cancer Res 1995; **55**:4168-73.
[23] Cohney S, McKenzie IF, Patton K, Prenzoska J, Ostenreid K, Fodor WL, et al. Down-regulation of Gal α(1, 3) Gal expression by α(1, 2) fucosyltransferase: Further characterization of α(1, 2) fucosyltransferase transgenic mice. Transplantation 1997; **64**:495-500.
[24] Sharma A, Okabe J, Birch P, McClellan SB, Martin MJ, Platt JL, et al. Reduction in the level of Gal α(1, 3)Gal in transgenic mice and pigs by the expression of an a(1, 2) fucosyltransferase. Proc Natl Acad Sci USA 1996; **93**: 7190-5.
[25] Xing L and Guo LH. FUT2 gene involved in expression of H blood group antigen on surface of human tumor cell lines BEL-7404, SGC-7901, and SPC-A-1. Acta Pharmacol Sin 2000; **21**:997-1001.