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Research Article

Producing defucosylated antibodies with enhanced in vitro antibody-dependent cellular cytotoxicity via *FUT8* knockout CHO-S cells

To engineer a host cell line that produces defucosylated mAbs with superior antibody-dependent cellular cytotoxicity, we disrupted α -1, 6 fucosyltransferase (*FUT8*) gene in CHO-S (CHO is Chinese hamster ovary) cells by clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9. The gene knockout cell line was evaluated for growth, stability, and product quality. The growth profile of *FUT8* gene knockout CHO-S (*FUT8*^{−/−}) cells was comparable with wild type CHO-S cells. *FUT8* catalyzes the transfer of a fucose residue from GDP-fucose to *N*-glycans residue. Defucosylated IgG1 antibodies produced by *FUT8*^{−/−} cells showed increased binding affinities to human FcγRIIIa and higher activities in mediating antibody-dependent cellular cytotoxicity, comparing with conventional fucosylated IgG1. Our results demonstrated the potential of using the clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9 technology in cell line engineering for biopharmaceutical industrial applications.

Keywords: α -1, 6 fucosyltransferase (*FUT8*) / Antibody-dependent cellular cytotoxicity (ADCC) / CRISPR/Cas9 / Defucosylated antibodies / Gene knockout

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1 Introduction

Therapeutic mAbs have dominated a large proportion in pharmaceutical market over the past decades. mAb therapy is increasingly used for treating cancer and autoimmune disorders. In addition, market data forecast that mAbs would grow at a higher rate than any other therapeutic biological medicines [1]. The mechanism of antibody-activated immune system mainly includes complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) [2]. ADCC is mediated by antibody Fc portion binding to FcγRIIIa receptor on NK cell. The efficacy of natural killer (NK) cell recruitment and activation is determined by the affinity of IgG Fc for FcγRIIIa. It was reported that the affinity was related to the presence or absence of fucose on the oligosaccharide of the IgG Fc [3, 4]. Shields

demonstrated that defucosylated *N*-oligosaccharide attached to Fc at asparagine 297 enhanced ADCC activities, through improving the binding affinity to FcγRIIIa [5].

Mogamulizumab, a defucosylated, humanized, anti-chemokine receptor 4 mAb, was approved in Japan in March 2012 for treating relapsed or refractory C-C chemokine receptor type 4 (CCR4)-positive T-cell leukemia-lymphoma. Removal of fucose has increased mogamulizumab's ADCC activity. Six other defucosylated mAbs are currently in clinical studies [6, 7]. Chinese hamster ovary (CHO) cells have become the most widely used industrial mammalian cell line to produce antibodies. Among the therapeutic antibodies launched all over the world, 35.5% are produced by CHO cells [8–10]. However, more than 80% IgG1 produced by wild-type CHO cells are fucosylated [5]. The transfer of a fucose residue from guanosine diphosphate (GDP)-fucose to *N*-glycans residue is catalyzed by α -1, 6 fucosyltransferase (*FUT8*) [11, 12]. The disruption of *FUT8* gene in CHO cells would eliminate the fucose from the IgG1 oligosaccharide [5, 13].

CHO-S cell line was chosen as model cell line in this study for its wide application in industry. Cell line with two *FUT8* gene modification created by homologous recombination (HR) and Zinc-finger nucleases (ZFNs) were reported, respectively. However, the use of HR and ZFNs is limited by their shortcomings. HR is an inefficient and time-consuming process. ZFNs are difficult to design and expensive to make. Those all

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Abbreviations: 2-AB, 2-aminobenzamide; ADCC, antibody-dependent cellular cytotoxicity; CHO, Chinese hamster ovary; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9; *FUT8*, α -1, 6 fucosyltransferase; HR, homologous recombination; LCA, Lens culinaris agglutinin; PEI, polyethylenimine; sgRNA, single guide RNA; ZFN, zinc-finger nucleases

limited their widespread use [14]. In contrary, clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9 (CRISPR/Cas9) system only requires guide sequence RNA, which is easy to design, highly efficient, and inexpensive to make. CRISPR/Cas9 system was used to modify *FUT8* gene in CHO-S cells. Paired CRISPR/Cas9 cleavages in different target region have some obvious advantages. Clones with appropriate deletion could be conveniently identified via PCR. Even though adequate biallelic deletion clones could not be obtained easily, a monoallelic deletion missing functionality would enrich for deletion diversity. Therefore, two exonic single guide RNAs (sgRNAs) were designed to create intervening chromosomal segment deletions, so as to improve the efficiency of gene knockout [9, 15]. Recent reports by Bachu R, Ronda C and Sun T (from our lab) respectively presented successful applications of CRISPR/Cas9 technology to disrupt *FUT8* gene [16–18]. Here we studied the growth characteristics of CRISPR/Cas9 generated *FUT8*^{−/−} CHO-S cells and the ADCC activity of the antibodies produced in this cell line. We modified CHO-S cells with *FUT8* gene completely disrupted via CRISPR/Cas9 technology. Loss of function clones was enriched with the assist of Lens culinaris agglutinin (LCA), and stable *FUT8*^{−/−} clones were selected. The genetic modifications had no detrimental effects on cell growth and viability. Defucosylated antibodies produced in *FUT8*^{−/−} CHO-S cells were comparable to those from wild-type cells in physiochemical properties, and with immensely improved ADCC activities.

2 Materials and methods

2.1 Plasmid construction

There are 11 exons in CHO-S *FUT8* gene. The exon 9 of *FUT8* (GeneBank ID: 100751648) was selected as the target site for mutagenesis since exon 9 encoded for the catalytic site of *FUT8* (Fig. 1A) [4]. Primers *FUT8*-F (5'-CTGTTGATTCCAGGTTCCCATATA-3') and *FUT8*-R (5'-TTGAATGATGACTGCTAGTGATGCT-3') amplified a 685-bp fragment from wild-type CHO-S genome. sgRNAs were located in the upstream of a protospacer adjacent motif (PAM). Paired sgRNAs approach would increase efficiency and minimize detection work, so two sgRNAs were designed to have an interval of 125-bp targeting sequence. The plasmid pX330 was digested with *Bbs*I (New England Biolabs, Shanghai, China). Forward and reverse sgRNAs were annealed and then cloned into linearized pX330 plasmid. All cloning steps were confirmed by DNA sequencing, and the generated plasmids were named pX330-sgRNA1 and pX330-sgRNA2.

2.2 Cell culture and transfection

CHO-S cells were purchased from Invitrogen and grown in CD-CHO Medium (Life Technologies, Shanghai, China) supplemented with 8 mM glutamine (Life Technologies), as recommended by the vendor. Cells were amplified in shake flasks in a humidified incubator (Thermo Fisher Scientific, Shanghai, China) at 120 rpm, 37°C, and 5% CO₂. Cell growth was

monitored using a cell counter and cells were subcultured into fresh medium every 2 to 3 days with seeding densities at 3–4 × 10⁵ cells/mL.

CHO-S cells were co-transfected with plasmids pX330-sgRNA1 and pX330-sgRNA2 directly in CD-CHO medium using polycation compound polyethylenimine (PEI, Polysciences, Shanghai, China) under the following conditions: cell density was 3 × 10⁶ cells/mL; the ratio of DNA and PEI was 1:2; DNA concentration was 4 µg/mL.

2.3 Clone screening

After 48-h transfection, the cells transfected with plasmids pX330-sgRNA1 and pX330-sgRNA2 were selected with 100 µg/mL LCA (Vector Laboratories, Shanghai, China). Wild-type cells were also applied with LCA at the same concentration as control. After 10 days of pressured selection, a small proportion of cells in the experiment group proliferated normally. Two rounds of limiting dilution were performed to screen double-alleles knockout clones in 96-well plates. When reached 50% confluence, cells were tested by PCR using the primers *FUT8*-F and *FUT8*-R to verify double-alleles' knockout. PCR amplifications were TA-cloned using TA cloning kit (Takara, Shanghai, China). Ten monoclonal colonies were picked from transformation for DNA sequencing.

2.4 LCA binding assay

Cells were seeded at a density of 3 × 10⁵ cells/well in a 24-well plate and incubated for 45 min at room temperature in media containing 5 µg/mL FITC-LCA (Vector Laboratories). Cells were washed three times with 1 × PBS. Then 1 × 10⁴ stained cells were analyzed by flow cytometer.

2.5 Culture viability and growth curve

Wild-type and *FUT8*^{−/−} CHO-S cell growth were tested in shake-flask. Cells were seeded at a density of 3 × 10⁵ cells/mL in CD-CHO medium supplemented with 8 mM glutamine and grown in 125 mL shake-flasks with a working volume of 20 mL. Culture viability was measured daily by Trypan blue dye exclusion method.

2.6 Monoclonal antibody production in *FUT8* knockout CHO-S clones

Humanized anti-HER2 antibody (Trastuzumab, Drug Bank: DB00072) was chosen as a model antibody. The heavy chain and light chain were cloned into pcDNA3.1 vector (Invitrogen, Shanghai, China), respectively. All cloning steps were confirmed by DNA sequencing. Constructed plasmids were named as pcDNA3.1-HC and pcDNA3.1-LC. Wild-type and *FUT8*^{−/−} CHO-S cells were cotransfected with expression vectors pcDNA3.1-HC and pcDNA3.1-LC by PEI. When cell viabilities were below 50%, culture supernatants were collected. Antibody IgG1s were purified using Protein A column (GE Healthcare

Figure 1. Sequence-level view of sgRNAs binding/cutting site in CHO-S *FUT8* gene and the modification site in CHO-S *FUT8* gene. (A) CHO-S *FUT8* gene has 11 exons, sgRNAs binding/cutting site located in exon 9, primers *FUT8*-F (5'-CTGTTGATTCCAGGTTCCCATATA-3') and *FUT8*-R (5'-TTGAATGATGACTGCTAGTGATGCT-3') amplify a 685-bp fragment from wild-type CHO-S genome. Guide RNAs located in the upstream of a protospacer adjacent motif (PAM). The sequence in the red box refers to PAM. (B) Interpretation of amplified PCR products observed via agarose gel electrophoresis. Lane 1, 2-kb DNA marker; lane 2, PCR result of *FUT8* gene fragment in CHO-S; lane 3, PCR result of *FUT8* gene fragment in *FUT8*^{-/-}. (C) Sequence alignment of gene sequences of *FUT8* from CHO-S and *FUT8*^{-/-} clone. (D) Sequence alignment of amino acid sequences of *FUT8* from CHO-S and *FUT8*^{-/-} clone.

Life Sciences, Beijing, China) with AKTA purifier system (GE Healthcare Life Sciences) [19]. The product concentration was measured by BCA assay (Beyotime, Shanghai, China).

2.7 N-glycans analysis

Glycans were released from 200 μ g IgG samples by incubating with 1000 U of the PNGaseF (New England Biolabs) in the reaction buffer at 37°C for 12 h following manufacturer's protocol. Samples were purified to remove proteins using cold ethanol precipitation and dried under vacuum. The purified N-glycans were labeled with 5 μ L 2-aminobenzamide (2-AB; Prozyme, Shanghai, China) at 65°C for 3 h and excess 2-AB was removed by passing the labeling mixture through a column, which used hydrophilic interaction LXC method combined with SPE (HILIC SPE; Waters, Shanghai, China). The purified, 2-AB-labeled glycans were dried under vacuum. The samples were reconstituted in 50 μ L of 60% v/v acetonitrile in water, of which 10 μ L was injected on Agilent 1260 (Agilent Technologies, Shanghai, China) for glycans analysis through fluorescence detection by the HPLC.

The HPLC mobile phase A consisted of 10 mM NH₄AC in ACN–water (80:20, v/v) and mobile phase B consisted of 10 mM NH₄AC in ACN–water (50:50, v/v). A SeQuant ZIC-HILIC column (5 μ m, 250 $\times$ 4.6 mm; Waters) was equilibrated with 80% phase A overnight. Gradient elution was applied at a flow rate of 1 mL/min through the column at 35°C. The elution gradients included 0 min, 20% B; 10 min, 35% B; 35 min, 60% B; 36 min, 100% B; 42 min, 100% B; and 43 min, 20% B. Gradient 20% B was kept for 17 min for column equilibration. The HPLC column was equipped with a fluorescence detector set at an excitation wavelength of 330 nm and an emission wavelength of 420 nm [20, 21].

2.8 Fc γ RIIIa binding assay

The HER2 binding affinities of antibodies produced in *FUT8*^{-/-} CHO-S (IgG1-*FUT8*^{-/-}) and wild-type CHO-S (IgG1-WT) to antigen Fc γ RIIIa (176 Phe CD16a, Sino Biological, Beijing, China) were determined using BIAcore T200 (GE Healthcare Life Sciences). A BIAcore sensor chip CM5 (GE Healthcare Life Sciences) was treated with 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (GE Healthcare Life Sciences) and N-hydroxysuccinimide (GE Healthcare Life Sciences). The concentration of ligand Fc γ RIIIa used for coupling was 3.33 μ g/mL. The pH of the coupling buffer was 5.5. The assays were performed with a constant flow rate (60 μ L/min) at 20°C. Various concentration of antibodies IgG1-*FUT8*^{-/-} (29.3–333.3 nM) and IgG1-WT (105–1200 nM) were injected, respectively, as the analytes. The chemical binding groups were regenerated by sequentially washing out the analytes with 10 mM glycine–HCl. The affinity between Fc γ RIIIa and antibodies was calculated using the kinetic analysis function in the BIAcore software [22].

2.9 ADCC assay

In vitro ADCC activities were tested on high HER2-expressing breast cancer cells (SK-BR-3) and hepatocarcinoma cells (HepG2). SK-BR-3 cells were grown in McCoy's 5a medium supplemented with 10% fetal bovine serum (Invitrogen). Cells were passaged in 1:2 every 3 days. HepG2 cells were grown in DMEM medium supplemented with 10% fetal bovine serum (Invitrogen). Cells were passaged in 1:5 every 3 days. All cultures were incubated at 37°C and 5% CO₂.

During the assay, SK-BR-3 and HepG2 cells were seeded at 1×10^4 cells/well respectively in 96-well plates as target cells.

Cells were pre-incubated with the antibody IgG1-*FUT8*^{-/-} or IgG1-WT at various concentrations at 37°C for 20 min. Human peripheral blood mononuclear cells (PBMCs) from donors were added to the cell suspensions in a 10:1 E/T ratio and the cell suspensions were incubated at 37°C for 20 h. Cytotoxicity was measured in terms of LDH activity released from dead cells using CytoTox 96 Non-Radioactive Cytotoxicity Assay Kit (Promega, Shanghai, China) [23–25]. Cytotoxicity measurements were done in triplicate. The percentage of cytotoxicity was calculated as Eq. (1).

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental} - \text{Effector spontaneous} - \text{Target spontaneous})}{(\text{Target maximum} - \text{Target spontaneous})} \times 100 \quad (1)$$

ADCC activity mediated by IgG1 on SK-BR-3 was tested at different E:T ratios. SK-BR-3 (1×10^4 cells/well) as target cells were incubated with the antibody IgG1-*FUT8*^{-/-} and IgG1-WT at 1 ng/mL at 37°C for 20 min. Human PBMCs from donors were added to the cell suspensions at the E/T ratio of 1:1, 2.5:1, 5:1, 10:1, 25:1, and 50:1, respectively [23, 24, 26].

3 Results

3.1 Gene editing and generation of *FUT8*^{-/-} clones

After transfection 48 h, 100 µg/mL LCA was applied to select genetically modified cells. LCA is a plant lectin with high specificity to bind to *FUT8* on oligosaccharides. After 10 days' incubation, all of untransfected cells in the control group were dead. A small proportion of cells in the experimental group that could not express fucose propagated normally. Cells were cultivated in 96-well plates with two-rounds of limiting dilution. At the first round of screening, after about 4 weeks' growth in 96-well plates, the cell clones reached a 50% confluence and clones' genetic modifications were screened by PCR using the primers *FUT8*-F and *FUT8*-R. Four modified clones were identified from 25 monoclonal colonies. We selected the best clone based on cell growth and FITC-LCA binding for the next round of screening. Monoclonal colonies were picked from 96-well plates, and tested by PCR. To test double-alleles' knockout in the clones, PCR amplifications were tested by DNA gel electrophoresis. The results showed that the band in the *FUT8*^{-/-} sample (lane 3) was lower than that of wild-type (lane 2), indicating the occurrence of sequence deletion (Fig. 1B). Sequencing results showed that *FUT8* alleles were deleted 122 and 117 bps, respectively (Fig. 1C). The amino acid sequences were translated from DNA sequences, and the alignment indicated that a 39-aa fragment was deleted in one allele, and a frameshift was introduced in the other (Fig. 1D).

The binding of LCA to cells could indicate the presence of α-1,6-fucosylated in N-linked oligosaccharides. We tested the *FUT8*^{-/-} clone by FITC-LCA resistance phenotype method. It was confirmed by flow cytometry that *FUT8*^{-/-} clone completely lost the ability to bind to FITC-LCA completely (Fig. 2A and B).

To evaluate the effect of *FUT8* gene knockout on cell growth, the *FUT8*^{-/-} clone was cultivated along with wild-type CHO-S

cell line. As shown in Fig. 3, both cell densities reached 7×10^6 cells/mL, and viabilities maintained about 90% after 1 week. *FUT8*^{-/-} clones were cultured for 15 passages and were still stable as shown by DNA sequencing.

3.2 N-glycans analysis of IgG1-*FUT8*^{-/-}

Humanized anti-HER2 IgG1 Trastuzumab, an approved therapeutic antibody, is commercially produced in CHO-S cell lines.

Figure 2. LCA-binding of CHO-S and *FUT8*^{-/-}. (A) CHO-S; (B) *FUT8*^{-/-}. Cells were stained with FITC-labeled LCA (dotted line) or no stained as a negative control (solid line).

Figure 3. Growth and viability of *FUT8*^{-/-} and wild-type CHO-S cells. (A) Cell growth; (B) Viability. Measurements were done in triplicate and the results are expressed as the mean $\pm$ SD.

To investigate the N-linked oligosaccharide structure of *FUT8*^{-/-} produced glycoproteins, Trastuzumab was used as a model to be expressed in *FUT8*^{-/-} cells. The vectors encoding IgG1 heavy and light chains were transiently co-transfected into *FUT8*^{-/-} clone and wild-type CHO-S cell line concurrently. Culture supernatants were collected when the cell viability dropped below 50%. Harvested antibodies were purified through protein A chromatography and the concentrations of IgG1-WT and IgG1-*FUT8*^{-/-} were determined by BCA assay as 13.775 and 10 mg/mL, respectively.

Two hundred micrograms of IgG1-*FUT8*^{-/-} and IgG1-WT were used for testing N-linked oligosaccharides' structure using the HPLC method. Figure 4A showed HPLC analysis of the N-glycans released from IgG1 produced in those two cells. The percentage of fucose in IgG1-WT in all N-glycans was over 98%, but completely absent in IgG1-*FUT8*^{-/-}. It further demonstrated that the *FUT8* alleles had been disrupted and completely defucosylated antibodies were produced.

The binding affinity of antibody IgG1-*FUT8*^{-/-} and IgG1-WT to antigen Fc γ RIIIa was measured using BIAcore (Fig. 4B and C). As shown in Table 1, the dissociation constant of antibody expressed by IgG1-*FUT8*^{-/-} ($K_D = 2.049 \times 10^{-7}$ M) was seven times lower than that produced by wild-type IgG1-WT (K_D

$= 1.443 \times 10^{-6}$ M), which indicated stronger binding affinity between Fc γ RIIIa and the Fc region of defucosylated antibody.

3.3 In vitro ADCC activity of defucosylated antibody

ADCC activity of antibodies without fucose would be higher than that of fucosylated IgG1-WT as reported previously. In this study, the ADCC activities of humanized anti-HER2 IgG1 expressed by *FUT8*^{-/-} clone and wild-type CHO-S cells were compared.

ADCC activities on target cells were tested at different IgG1 concentrations with E: T ratios being 10:1. As shown in Fig. 5A and B, defucosylated IgG1 had a pronounced superiority in ADCC compared with wild-type fucosylated IgG1. Defucosylated IgG1 had a higher cytotoxicity even at much lower antibody concentrations. The ADCC activities increased by about 25-fold on SK-BR-3 and over eightfold on HepG2 with IgG1-*FUT8*^{-/-} (Fig. 5D). ADCC activity was also tested on SK-BR-3 at different E:T ratios. Based on previous results, we used 1 ng/mL as the experimental concentration. Statistically significant increase in cytotoxicity was observed at all E:T ratios with IgG1-*FUT8*^{-/-} (Fig. 5C). The increase was ratio-dependent in the LDH release assay, suggesting NK-cell number was a limiting factor in ADCC activity.

4 Discussion

Glycosylation of IgGs at Asn297 helps to maintain the tertiary structure of their CH2 domains. The fucosylation of glycoconjugates is related to a wide variety of biological processes and some severe diseases [27]. The slightly change of antibody glycoforms may have impact on the ADCC [28]. T. Shinkawa demonstrated that the improvement of ADCC was caused by the absence of fucose but not the presence of galactose or bisecting N-acetyl glucosamine in human IgGs [13].

In this study, we presented completely disrupting of *FUT8* gene in CHO-S cell line by CRISPR/cas9 technology and established a new host cell line for producing defucosylated antibodies. Glycan composition was analyzed by HPLC methodology to confirm the complete disruption of the *FUT8*. Furthermore, we analyzed IgG1-*FUT8*^{-/-} and confirmed antibody's improvement in ADCC activity. The new host line was characterized from growth and viability, genetic stability, and product quality.

Previously, we used CRISPR/cas9 technology with one sgRNA to delete *FUT8* gene in CHO-K1 cells, which express antibody with lower fucosylated antibodies below LOD with FITC-LCA lectin blot [17]. In order to obtain an industrially applicable host cell line that can produce completely defucosylated antibodies, the genetic disruption method was improved by using two sgRNAs. In this study, two sgRNAs were designed to delete a 125 bps in *FUT8* exon 9 that encodes for the catalytic site. The modification results were identified by PCR more effectively.

Fc region binding affinity of antibodies to antigen Fc γ RIIIa was tested using BIAcore. Comparing with ELISA, the interaction of antigen with the corresponding partner is monitored in real time [24, 29]. None of the interacting molecules needs to be labeled and it is not necessary to determine the concentration

Figure 4. Glycans structures and Fc γ RIIIa binding assay of IgG1 produced in *FUT8*^{-/-} and wild-type CHO-S cells. (A) HPLC analysis of the N-glycans released from antibodies IgG1-*FUT8*^{-/-} and IgG1-WT. (B) BIAcore analysis of antibody IgG1-*FUT8*^{-/-} binding to antigen Fc γ RIIIa. Various concentration of antibodies IgG1-*FUT8*^{-/-} is from 29.3 to 333.3 nM. (C) BIAcore analysis of antibody IgG1-WT binding to antigen Fc γ RIIIa. Various concentration of antibodies IgG1-WT is from 105 to 1200 nM. The analysis at steady state response units equilibrium (RUeq).

Table 1. The binding affinity of IgG1-*FUT8*^{-/-} and IgG1-WT to human Fc γ RIIIa antigen

Antibody	K_a (1/Ms)	K_d (1/s)	K_D (M) ^{a)}	Ratio
IgG1- <i>FUT8</i> ^{-/-}	2.033×10^5	0.04166	2.049×10^{-7}	×7
IgG1-WT	8.847×10^4	0.1276	1.443×10^{-6}	

^{a)} K_D , affinity equilibrium dissociation constant; $K_D = K_d/K_a$.

of the bound molecules in advance [30]. The binding affinity to Fc γ RIIIa of the defucosylated antibody was seven times higher than the control wild-type. The increased affinity may be a result of the reduction or absence of steric inhibition at the receptor–ligand interface.

Niwa R. Sakurada reported that low-fucose IgG1s could mediate ADCC at low antigen densities while corresponding high-fucose counterparts did not induce ADCC at the same concentration [31]. We observed that defucosylated antibodies were superior to fucosylated in ADCC activity to destroy breast

cancer. Furthermore, significant E:T ratio-dependent increase in cell cytotoxicity was observed. These characters of defucosylated antibodies could be beneficial to cancers' therapy because clinical tumors often display the heterogeneity in antigen expression levels and the number of effector cells is unstable.

Besides of breast cancer, it has been recognized that HER2 overexpression occurs in other types of cancers such as stomach, ovary, uterine serous endometrial carcinoma, colon, bladder, lung, uterine cervix, head and neck, liver, and esophagus [32–35]. We also tested the ADCC activities in a liver cancer cell

Figure 5. In vitro ADCC activities of IgG1-FUT8^{-/-} and IgG1-WT. (A) ADCC activities on SK-BR-3 cells at different concentrations, cytotoxicity were detected using LDH release assay. Effectors PBMC, E:T ratio 10:1, time point of 20 h. (B) ADCC activities on HepG2 cells at different concentrations, cytotoxicity were detected using LDH release assay. Effectors PBMC, E:T ratio 10:1, time point of 20 h. (C) ADCC activity on SK-BR-3 cells at different E:T ratios, Effectors PBMC, target SK-BR-3, time point of 20 h, LDH release assay. (D) Data of LDH release assay refer to A and B. Cytotoxicity measurements were done in triplicate and the results are expressed as the mean ± SD. **p* < 0.05.

line (HepG2), and observed that anti-HER2 IgG1 expressed by FUT8^{-/-} cell lines had a superior effect in ADCC compared with wild-type fucosylated IgG1 in liver tumor. Potentially, the defucosylated antibodies could be used to treat tumor types other than the primary indication.

A stable FUT8^{-/-} cell line was engineered using CRISPR/Cas9 saving time and cost compared with HR and ZFNs gene targeting technologies. The IgG1 antibodies produced by FUT8^{-/-} cell line were completely defucosylated and had better activity in vitro. The genetically engineered cell line could potentially be further developed for industrial applications.

Practical application

Fucosylation could hinder the Fc region binding to Fc receptors and lead to lower antibody-dependent cellular cytotoxicity effect. In Chinese hamster ovary (CHO) cells, α -1, 6 fucosyltransferase (FUT8) is responsible for adding fucose to N-glycans on antibody molecules. We engineered a stable FUT8^{-/-} CHO cell line by deleting the FUT8 gene using the clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9 system. The FUT8^{-/-} cells have similar growth profiles compared to wild-type CHO-S cells. Glycoform assay with HILIC-HPLC confirmed no fucose present in mAbs expressed in the FUT8^{-/-} cells. The antibodies expressed by FUT8^{-/-} cells binding to Fc γ RIIIa were sevenfold stronger in BIAcore assay than those from wild-type CHO-S cells. A 25-fold higher antibody-dependent cellular cytotoxicity activity was observed with defucosylated antibodies.

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The authors have declared no conflict of interest.

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