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Immunization method for multi-pass membrane proteins using highly metastatic cell lines

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ABSTRACT

A novel method using metastatic breast cancer cell lines was established for producing monoclonal antibodies (mAbs) against multi-span membrane proteins. Grafting of metastatic cells (MCF7-14) into the mammary gland of BALB/cJ/nu/nu mice induced splenic hypertrophy ($1.6–3.0 \times 10^8$ cells/spleen [$n = 6$]). More than half of the mAbs against MCF7-14 cells reacted with the cell membrane. Inducing production of antibodies against the extracellular domain of multi-pass membrane proteins is difficult. Because the protein structure becomes more complex as the number of transmembrane domains increases, preparing antigens for immunization in which the original structure is maintained is challenging. Using highly metastatic MDA-MB231 cells as the host cell line, we produced mAbs against a 12 transmembrane protein, solute carrier family 6 member 6 (SLC6A6), as a model antigen. When SLC6A6-overexpressing MDA-MB231 cells were grafted into nude mice, the number of splenocytes increased to $2.7–11.4 \times 10^8$ cells/spleen ($n = 10$). Seven mAb-producing clones that not only recognized the extracellular domain of SLC6A6 but also were of the IgG subclass were obtained. Immunocytochemistry and flow cytometry analyses revealed that these mAbs recognized the native form of the extracellular domain of SLC6A6 on the cell surface. Our novel immunization method involving highly metastatic cells could be used to develop therapeutic mAbs against other multi-pass membrane proteins.

1. Introduction

Multi-pass membrane proteins, including G protein-coupled receptors and solute carriers (SLCs), are currently the most important class of therapeutic targets. Antibodies directed against these proteins are highly sought after for therapeutic, diagnostic, and research purposes. Despite substantial interest in these targets, the generation of high-quality antibodies against membrane proteins using conventional methods is challenging [1]. Conventional approaches include immunization with whole cells [2,3], reconstituted proteins, or peptides. However, these approaches are difficult to apply to some of the most important membrane protein targets because multi-pass membrane proteins are often expressed at low levels in cells, have high amino acid sequence homology with human antigens and their homologues in immunized animals, and are very unstable when purified.

In order to obtain antibodies reactive to the native extracellular structure of membrane proteins, immunization by injection of cultured cells expressing the antigen has generally been used [4]. However, it is usually necessary to inject a large number of cells (typically $10^6–10^8$ cells [4–400 mg protein] per animal) to induce antibody production, which increases the risk of anaphylactic shock and death upon boosting. Alternatively, various approaches to produce mAbs for multi-pass membrane proteins have been developed, such as those involving Ig-Fc-domain fused proteins, DNA immunization [5], antigen-comprising budding baculoviruses [6], and phage screening *in vivo* [7]. Although these methods have some advantages when used with membrane protein antigens that maintain their three-dimensional structure, their use involves complicated experiments, and their application is limited and does present some problems. In the case of Ig-Fc fused proteins, several complex steps are required, such as plasmid construction, effective overexpression and purification of the target proteins. Moreover, there is no guarantee that the protein structure will be maintained.

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DNA immunization requires effective electrotransfer of the target gene-encoding plasmid to animal tissues. Reconstruction of membrane proteins in budding baculoviruses involves highly complex experimental techniques and expensive equipment. Phage display systems require a reliable source of antibodies for each antigen, and the construction of libraries for screening is time consuming. Thus, for immunization with multi-pass membrane proteins, improved methods for obtaining IgG subclass antibodies are needed.

The SLC family of eukaryotic membrane transport proteins includes over 300 members organized into 52 subfamilies. SLCs control the uptake and efflux of various solutes, including amino acids, sugars, and drugs [8]. Recent research revealed that some SLCs, such as SLC35F2 [9], SLC5A5 [10], SLC22A5 [11], SLC45A3-BRAF fusion protein [12], and SLC44A4 (which is in the clinical study phase at Astellas Pharma Inc.) are highly expressed in prostate cancer patients and thus are potential targets of therapeutic antibodies.

In order to develop and evaluate a novel immunization method, we selected SLC family 6 member 6 (SLC6A6) as a model protein. Human SLC6A6 contains 12 transmembrane (TM) regions connected by intra- and extracellular loops, with the N- and C-termini located intracellularly. SLC6A6 is a Na^+- and Cl^--dependent taurine transporter and is induced by osmotic stress [13]. It has been reported that the concentration of taurine is increased in the serum of colorectal cancer patients [14] and is correlated with tumor cell density [15]. Thus, it is likely that the expression of SLC6A6 might be related to colorectal cancer. Polyclonal antibodies against the extracellular domain of SLC6A6 are available, but monoclonal antibodies (mAbs) against this protein are not available.

In this study, we developed an immunization method using metastatic human breast cancer cell lines. Here, we demonstrate that this method can be used for the production of IgG subclass mAbs against multi-pass membrane proteins such as SLC6A6.

2. Material and methods

2.1. Cell lines

Human breast cancer cell line MCF7 was obtained from the Institute of Development, Aging and Cancer, Tohoku University. MCF7-derived MCF7-14 cells are described elsewhere [16]. MDA-MB231 cells were purchased (ATCC HTB-26, Manassas, VA). All cell lines were maintained in RPMI 1640 medium (Sigma–Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS) and an antibiotic–antimycotic.

2.2. Construction of the expression vector

RNA was extracted from MCF7-14 cells using an RNeasy Mini kit (Quiagen, Tokyo, Japan) and converted into cDNA using SuperScript III RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA). The resulting cDNA was used as a template. Amplicons of the human SLC6A6 gene (GenBank accession No.: NM_003043.3) were generated by polymerase chain reaction (PCR). PCR was performed with KOD Plus polymerase (TOYOBO, Osaka, Japan) using the following gene-specific primers: SLC6A6 (1863 bp) (forward) 5′-AAAGGATCCA TGGCCACCAAGGAGAAGCTGC-3′ and (reverse) 5′-AATCTAGACATCA TGGTCTCCACAATGATGT-3′. The amplified fragment was digested with *Bam*HI and *Xba*I and inserted into the pEF6-myc/HisA vector (Invitrogen) using the same restriction enzymes. The myc/His-tagged cDNA (pEF6-SLC6A6-myc/His) was introduced into MDA-MB231 cells using FUGENE 6 (Promega, Tokyo, Japan) according to the manufacturer's instructions. MDA-MB231 cells stably transfected with pEF6-SLC6A6-myc/His were selected for 3 weeks using Blasticidin S (10 μg/mL). Proteins were isolated from lysates of confluent cell cultures, subjected to SDS–PAGE, and transferred to PVDF membranes. The membranes were blocked (5% skim milk in TBS-T [25 mM Tris, 150 mM NaCl, 0.05% [v/v] Tween 20, pH 7.4]) and incubated with 1 μg/mL of anti-c-myc mAb (clone 9E10) (Santa Cruz, Dallas, TX) for 1 h, followed by incubation with the corresponding HRP-conjugated secondary antibody (1:200, BETHYL, Montgomery, TX) in TBS-T. Antibody complexes were detected using the Immobilon™ detection system (Millipore, Billerica, MA).

2.3. mAb production

We previously reported a method for grafting breast cancer cells into the fourth mammary gland of BALB/cJ/nu/nu mice [16]. All experiments were performed in compliance with both institutional guidelines and national laws and policies. MCF7-14 or MDA-MB231-SLC6A6-derived mouse spleen lymphocytes were fused in a standard manner with P3X63-Ag8 mouse myeloma cells (ATCC CRL-1580) using 50% (w/v) polyethylene glycol 4000 (Sigma). The fused cells were selected in HAT medium (Invitrogen). Culture supernatants were collected from the 96-well plates showing growth of the hybridoma cells and then analyzed for reactivity with intact colon cancer cells.

2.4. Titration of anti-sera (cell ELISA)

MCF7 and MCF7-14 cells were seeded at a concentration of 3000 cells/well in 96-well plates. After culturing, the cells were fixed with 10% (v/v) neutral buffered formalin solution (Wako, Osaka, Japan) for 10 min at room temperature. The plates were then washed with phosphate buffered saline (PBS[−]) and blocked (5% skim milk in TBS-T) for 30 min, after which 100 μL of mouse serum diluted 20,000-fold with TBS-T was added to each well. The plates were washed and reacted with the corresponding HRP-conjugated secondary antibody (1:2,000, BETHYL). Orthophenylenediamine (Sigma) was diluted with 50 mM carbonate–citrate buffer (pH 5.0) to a final concentration of 0.5 mg/mL and mixed with a 1/10,000 volume of 35% (w/w) aqueous hydrogen peroxide (Wako).

Alternatively, the TMB One-Step Substrate System (DAKO, Carpinteria, CA) was used. The plates were reacted at room temperature for 10 min. After stopping the reaction by the addition of 25 μL of 3 N sulfuric acid (Wako), the absorbance of each well was measured with a plate reader (SpectraMaxPure384, Molecular Devices, Tokyo, Japan).

2.5. SLC6A6-Loop2 recombinant protein

The construct SLC6A6-Loop2, corresponding to amino acids 143–217 of the full-length gene, was amplified by PCR using following gene-specific primers: 5′-ATAGGATCCGGCCTGGGCCACAT ATCACCTG-3′ (forward) and 5′-TATGAATTCGCTTTCAGAGAGCCTGG GTGGTC-3′ (reverse). The PCR fragment was subcloned into the pET-32b expression vector (Novagen, San Diego, CA) downstream of the T7 promoter using the *Eco*RI and *Bam*HI restriction sites. The construct, which is fused to a Trx (Thioredoxin), S, and His (polyhistidine) tags at the N- and C-termini, was transformed into *Escherichia coli* BL21 (DE3) (Novagen). After induction with 1 mM isopropyl-1-thio-β-D-1-galactopyranoside (Melford Laboratories, Ipswich, UK) for 3 h, bacterial cells were harvested and lysed using 10 volumes of lysis buffer (10 mM Tris–HCl, 10 mM EDTA, 150 mM NaCl, 10 mM dithiothreitol [DTT], pH 8.0) and then mechanically disrupted by sonication. Cell debris was removed by centrifugation, and protein was then extracted from the inclusion bodies in wash buffer containing 1 M guanidine hydrochloride for 1 h at

37 °C. For refolding, the protein was gently diluted to 1 L in refolding buffer (50 mM Tris–HCl, 150 mM NaCl, 5% glycerol [Wako], 0.4 mM glutathione oxidized [Sigma], pH 8.5) and stirred for 18 h. Next, the diluted sample was loaded onto a column containing 2 mL of FastFlow His6-binding resin (GE Healthcare UK Ltd, Bucks, England), washed with washing buffer (50 mM potassium phosphate buffer, 150 mM NaCl, 10 mM imidazole, pH 8.0), and eluted with elution buffer (50 mM potassium phosphate buffer, 150 mM NaCl, 200 mM imidazole, pH 8.0). Following dialysis against elution buffer without imidazole, purified SLC6A6-Loop2 protein was obtained.

For determining the titer of mAbs against the SLC6A6-Loop2 recombinant protein using an ELISA, 96-well plates were coated with 500 ng/well of the protein at 4 °C overnight.

2.6. Immunofluorescence staining

Microscope slides (Superfrosty Plus, Fisher Scientific, Waltham, MA) were incubated overnight with 10 μg/mL of collagen in HCl (pH 3.0). Human HT-29, LoVo, and HCT15 colorectal cancer cells were seeded in wells and fixed with 10% (v/v) neutral buffered formalin solution. After blocking (5% skim milk in TBS-T), cells were incubated with hybridoma culture supernatant or 10 μg/mL of purified mAb for 1 h at room temperature. After washing, the cells were incubated with Alexa Fluor-488-conjugated goat anti-mouse IgG (1:200, Molecular Probes, Invitrogen) for 30 min at room temperature. The cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole. The stained cells were then observed by fluorescent microscopy (Axiovert 200 M, Carl Zeiss, Jena, Germany).

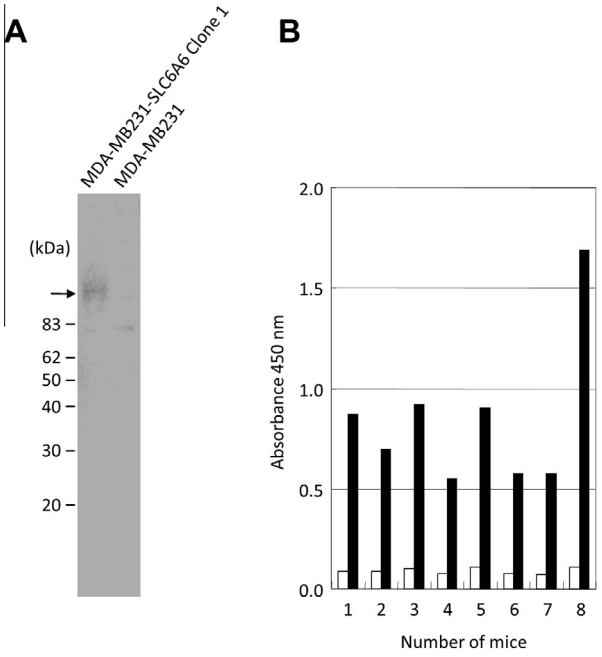

Fig. 2. Immunization with MDA-MB231-SLC6A6 cells. (A) MDA-MB231 cells were transfected with a plasmid encoding myc-tagged SLC6A6. A stable clone (clone 1) was established after drug selection. The cells were lysed and the isolated proteins were separated by SDS–PAGE and analyzed by Western blotting. Molecular weight markers are indicated on the left. Arrow indicates full-length myc-tagged SLC6A6 protein. (B) MDA-MB231-SLC6A6 clone 1 cells were injected into the mammary fat pads of eight mice. After 7 months, serum samples were collected and titrated using the SLC6A6-Loop2 recombinant protein (■). An uncoated plate was used as a negative control (□).

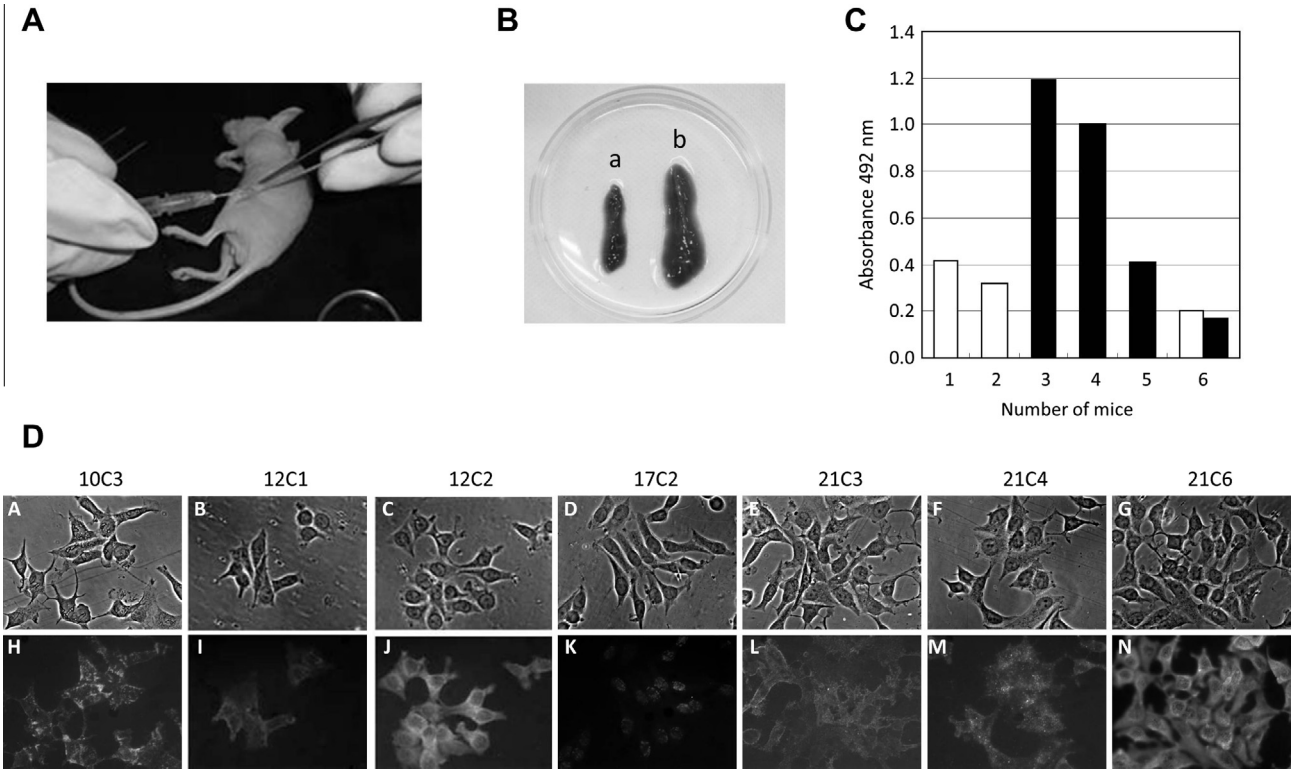

Fig. 1. Immunization with metastatic breast cancer cells. (A) Cells were injected into the fourth mammary fat pad of a BALB/cJ/nu/nu mouse. (B) Comparison of the spleen size of (a) an unimmunized mouse and (b) an MCF7-14-grafted mouse. (C) Serum samples were collected from MCF7-grafted mice (mice 1 and 2), MCF7-14 mice (numbers 3–5), and an unimmunized mouse (number 6). The sera were diluted (1:20,000) and titrated against formalin-fixed MCF7 (□) and MCF7-14 cells (■). (D) Monoclonal antibodies were analyzed by immunocytochemistry. Representative light microscopic (upper panels, A–G) and fluorescence microscopic (lower panels, H–N) images of MCF7-14 cells. The clone used to stain the MCF7-14 cells is indicated above each set of panels. Plasma membrane-staining was observed with clones 10C3 (H), 12C1 (I), 21C3 (L), and 21C4 (M). Alternatively, nuclear or cytoplasmic staining was observed with clones 17C2 (K), 12C2 (J), and 21C6 (N).

2.7. Flow cytometry

Four human colon cancer cell lines (HCT15, HT-29, HCT116, and SW480) were harvested using Cell Dissociation Buffer (Life Technologies) and then resuspended (2×10^5 cells/100 μL) with PBS(−) containing 1% FBS (BD Biosciences, San Jose, CA). Alexa Fluor-488-conjugated goat anti-mouse IgG (1:200) was added to the cell suspension at the concentration recommended by the manufacturer, and the suspension was incubated at 4 °C in the dark for 60 min. The labeled cells were analyzed using a FACS Calibur system (BD Biosciences).

3. Results

3.1. Immunization using metastatic breast cancer cells

MCF7 and MCF7-14 human breast cancer cells were suspended with matrigel and grafted into the fourth mammary gland of BALB/cJ/nu/nu mice (Fig. 1A). After 3 months, MCF7-grafted mice ($n = 5$), MCF7-14-grafted mice ($n = 6$), and unimmunized mice ($n = 8$) were sacrificed and their spleens were surgically removed. The spleens of MCF7-14-grafted mice were clearly hypertrophic compared with those of unimmunized mice (Fig. 1B). The number of splenocytes harvested from MCF7-14-grafted mice was >2-fold higher (median value; 2.0×10^8 cells/spleen) than the number harvested from MCF7-grafted or unimmunized mice (median values: 0.7 and 0.8×10^8 cells/spleen, respectively).

We determined the titer of antisera against the grafted cell lines using a cell ELISA. The sera of three MCF7-14-grafted mice showed significant reactivity against MCF7-14, however, the sera from MCF7-grafted or unimmunized mice reacted weakly or not at all with the grafted cell lines (Fig. 1C). Following fusion of splenocytes and myeloma cells, 12 mAbs against MCF7-14 were established. Seven clones were randomly selected and analyzed by immunocytochemistry (Fig. 1D), which identified antibodies that reacted with the plasma membrane (clones 10C3, 12C1, 21C3, and 21C4), cytoplasm (clones 12C2 and 21C6), and nucleus (clone 17C2). More than half of the antibodies reacted with the plasma membrane of MCF7-14 cells. These results indicate that metastatic breast cancer cell lines could be used for effective induction of antibodies against multi-pass membrane proteins.

3.2. Establishment of mAbs against a multi-pass membrane protein

Next, we used highly metastatic MDA-MB231 cells [17] as hosts and challenged them to produce mAbs against the multi-pass membrane protein SLC6A6 as a model. SLC6A6 is a taurine transporter that contains 12 TM regions connected by intra- and extracellular loops, with the N- and C-termini located intracellularly [13]. After transfection of MDA-MB231 cells with the pEF6-SLC6A6-myc/His expression vector, we obtained MDA-MB231

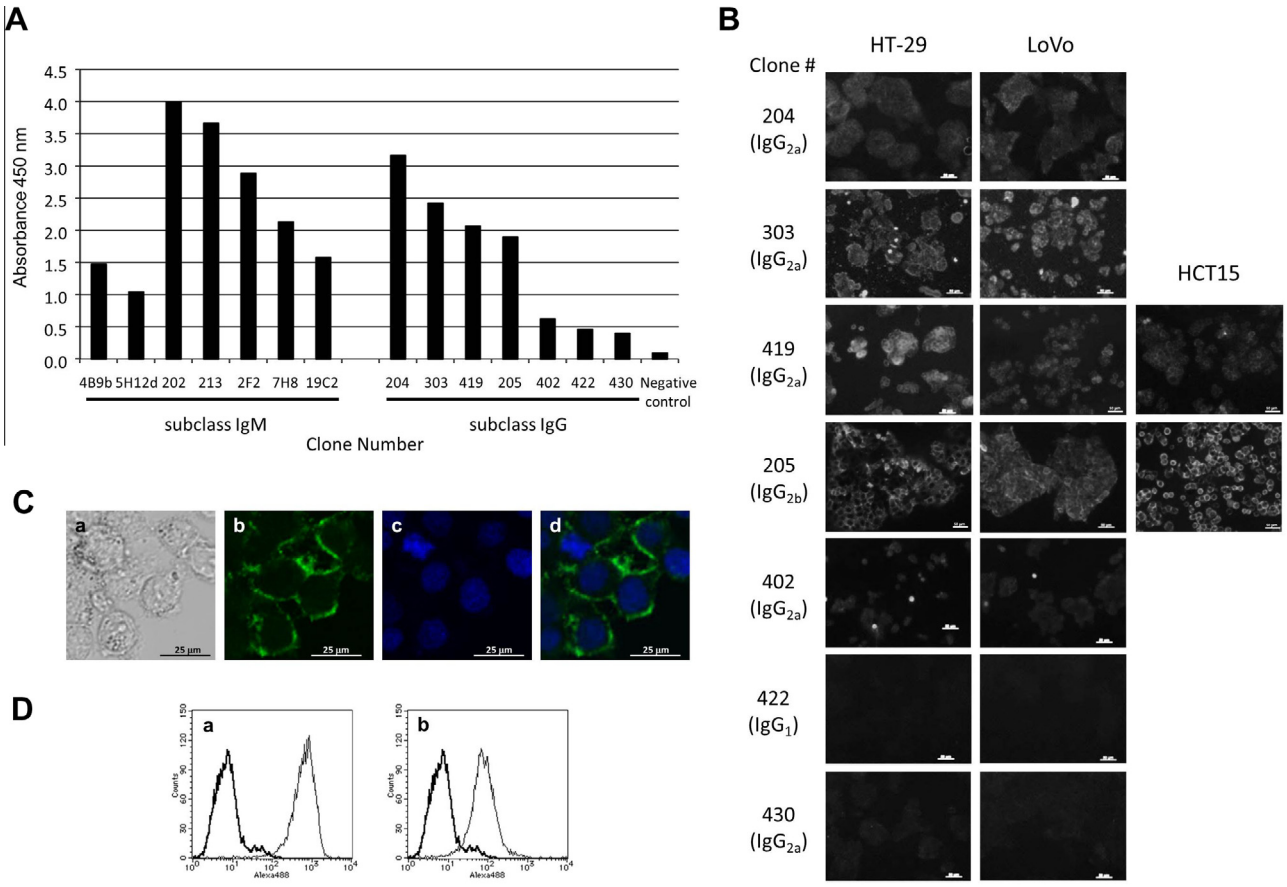

Fig. 3. Analysis of anti-SLC6A6 monoclonal antibodies. (A) Supernatants of hybridoma cultures were analyzed by ELISA using SLC6A6-Loop2 recombinant protein. HT medium was used as a negative control. Seven IgG subclass mAbs (clones 204, 303, 419, 205, 402, 422, and 430) were obtained. (B) The supernatants were analyzed by immunocytochemistry against HT-29 (left panels), LoVo (middle panels), and HCT15 (right panels) colorectal cancer cells; bar = 50 μm. Clone number and IgG subclass are indicated to the left side of each set of panels. (C) Purified antibody (clone 419) was analyzed by confocal laser scanning microscopy: (a) phase contrast image, (b) immunofluorescence showing the presence of SLC6A6 on the membrane surface, (c) 4′,6-diamidino-2-phenylindole-staining fluorescence image, and (d) immunofluorescence image merged with (b); bar = 25 μm. (D) Flow cytometry analysis of the reaction of intact cells of clones 419 (a) and 205 (b) with HCT15 cells. Shown are isotype control mouse IgG_{2b} (thick lines) and anti-SLC6A6 antibodies (thin lines).

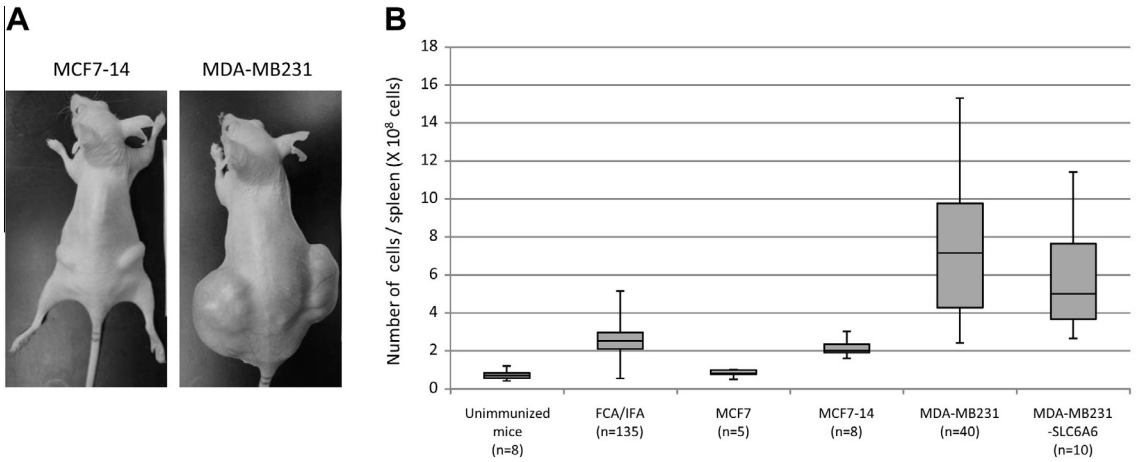

Fig. 4. Statistical analysis of splenocyte numbers. (A) Photographs of MCF7-14- and MDA-MB231-grafted mice at 3 month after grafting. (B) Box plot showing the number of splenocytes/spleen in each group of mice. The bottom and top of each box marks the 25th and 75th percentiles, respectively, and minimum and maximum values are shown as vertical lines below and above the boxes, respectively. The number of mice in each group is shown below the plot.

clones that stably expressed SLC6A6 under drug selection. The SLC6A6 cDNA was directly tagged with a myc/His epitope at the C-terminus so that expression of the transfected gene could be evaluated using an anti-myc tag antibody. Western blot analysis of the cell extracts of transfected clones using anti-myc tag antibody (9E10) revealed binding in MDA-MB231-SLC6A6 clone 1 but not in MDA-MB231 cells (Fig. 2A). The SLC6A6-postive cells (clone 1) were grafted into the fourth mammary gland of BALB/cJ/nu/nu mice. These tumor-bearing mice survived for over 7 months.

A recombinant protein containing the SLC6A6 extracellular domain (SLC6A6-Loop2) was generated to analyze the titer of each serum sample against SLC6A6. Loop 2, which is located between TM domains 3 and 4, is the largest extracellular domain of SLC6A6, consisting of 75 amino acids. ELISA clearly revealed that anti-sera harvested from MDA-MB231-SLC6A6-grafted mice reacted with the SLC6A6-Loop2 recombinant protein (Fig. 2B).

After fusion of splenocytes and myeloma cells, hybridoma cell lines producing mAbs that reacted with the SLC6A6-Loop2 recombinant protein were selected by ELISA. A total of 14 hybridoma clones were established, and we selected seven IgG subclass clones (numbers 204, 303, 419, 205, 402, 422, and 430) for further analyses (Fig. 3A). Immunocytochemical analysis indicated that clones 419 and 205 reacted strongly with HT-29, LoVo, and HCT15 human colorectal cancer cells, whereas clones 204, 303, and 402 reacted moderately with each cell line (Fig. 3B). Clones 422 and 430 reacted weakly or not at all with the formalin-fixed cells. Staining of the membrane region was clearly observed on confocal laser scanning microscopic analysis of HCT15 cells upon reaction with clone 419 (Fig. 3C). FACS analysis of intact HCT15 cells using the antibodies of clones 419 or 205 showed that these mAbs reacted with the native form of the protein on the cell surface (Fig. 3D). These results confirmed that we successfully established mAbs against the extracellular domain of SLC6A6.

3.3. Production of mAbs using SLC6A6-Loop2 recombinant protein

A series of mAbs against the partial recombinant SLC6A6 protein (SLC6A6-Loop2, 75 amino acids) were also established. BALB/cJ mice were immunized with the SLC6A6-Loop2 protein in an emulsion containing Freund's adjuvant. A total of 19 hybridoma clones that reacted with SLC6A6-Loop2 recombinant protein in ELISA were obtained (Supplemental Fig. S1A). However, immunocytochemical and flow cytometry analyses using HCT15 human colorectal cancer cells revealed only one weakly reactive clone (Supplemental Fig. S1B and S1C). These results indicate that the SLC6A6-Loop2 recombinant protein induces little or no production of antibodies reactive to the native extracellular structure of SLC6A6, even if the SLC6A6-Loop2 recombinant protein is refolded and solubilized.

Table 1
Number of splenocytes.

Immunization method	Number of mice	Average number of splenocytes ($\times 10^8$ cells)	Maximum number of splenocytes ($\times 10^8$ cells)
Unimmunized mice	8	0.7	1.2
FCA/IFA	135	2.6	5.2
MCF7	5	0.8	1.0
MCF7-14	8	1.8	2.2
MDA-MB231	40	7.4	15.3
MDA-MB231-SLC6A6	10	5.9	11.4

3.4. Number of splenocytes

We expected that the level of immune response would be related to the tumorigenicity and metastatic potential of the grafted cell lines. Indeed, tumor formation in MDA-MB231-grafted mice was faster than in MCF7-14-grafted mice at 3 months after grafting (Fig. 4A). Data regarding the number of splenocytes are shown in the box plot of Fig. 4B and in Table 1. The median number of splenocytes was 0.7×10^8 cells/spleen in unimmunized mice ($n = 8$), 2.5×10^8 cells/spleen in mice immunized using a conventional method involving an emulsion containing complete and incomplete Freund's adjuvant ($n = 135$), 0.8×10^8 cells/spleen in MCF7 (parental cell line of MCF7-14)-grafted mice ($n = 5$), 2.0×10^8 cells/spleen in MCF7-14-grafted mice ($n = 8$), and 7.2×10^8 cells/spleen in MDA-MB231-grafted mice ($n = 40$). These results indicate that grafted highly metastatic cells constantly induce hypertrophy of the spleen. It was noted that formation of tumors in the spleen was not observed in all cases.

4. Discussion

In this study, we developed an effective immunization method that uses metastatic cancer cell lines such as MCF7-14 and MDA-MB231 to induce production of antibodies against the extra-

cellular domain of multi-pass membrane proteins. Grafting of highly metastatic cells resulted in significant splenic hypertrophy and the generation of a large number of splenocytes ($\sim 15.3 \times 10^8$ cells/spleen) as well as the production of a high titer of antibodies that reacted with the intact extracellular domain of the multi-pass membrane protein SLC6A6.

We believe that a strong immune response was induced because the grafted cells continuously metastasized through the lymph nodes. The metastasized cells are presumably continuously attacked, destroyed, and taken up by antigen-presenting cells. It was previously reported that metastasis of breast cancer cells to sentinel lymph nodes is associated with maturation arrest of dendritic cells in clinical samples [18]. Dendritic cells are professional antigen-presenting cells that play a critical role in inducing adaptive immune responses [19]. In addition, the intravenous injection of dead cells can induce cell-associated antigen-specific immunosuppression or tolerance, but dead tumor cells transported via lymphatic flow are phagocytosed by $CD169^+$ macrophages and subsequently cross-presented as tumor antigens to $CD8^+$ T cells [20]. Although the mechanism through which antibody production is induced in our method is unclear, metastasis through the lymph nodes might be an essential component.

BALB/cJ/nu/nu mice are immunodeficient and have an abnormal thymus. Although T cell-dependent antigens such as proteins and hapten–protein conjugates reportedly fail to induce a response, T cell-independent antigens stimulate an excellent antibody response in BALB/cJ/nu/nu mice [21]. T cell-independent antigens stimulate antibody production in the absence of MHC class II-restricted T cell help but do need T cell-derived factors. In addition, thymus-independent antigens induce production of IgG_2 as the predominant IgG subclass. Indeed, the anti-SLC6A6 mAbs produced using our method were IgG_{2a} (204, 303, 419, 402, and 430) or IgG_{2b} (205), excepting clone 422 (IgG_1). The type of antigen associated with the metastasizing cells has not been determined, but we hypothesize that our novel method involves a T cell-independent antibody response.

The metastatic cell-mediated immunization protocol developed in this study is simple to perform and induces efficient production antibodies against the extracellular domain of multi-pass membrane proteins in their native form. Furthermore, the mAbs produced in the study reacted not only with the HT-29, LoVo, and HCT15 colorectal cancer cell lines (Fig. 3B) but also with the HCT116, SW480, and SW620 cell lines (data not shown). These results indicate that the anti-SLC6A6 mAbs we generated could be used for colorectal cancer therapy.

Author contributions

H.S. designed the metastatic cell line immunization and screening method; Y.O. and Y.T. isolated the antibodies; T.U., M.O., and Y.M. helped draft the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.05.065.

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