Biochemical and Biophysical Research Communications 450 (2014) 532–537

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A soluble form of Siglec-9 provides an antitumor benefit against mammary tumor cells expressing MUC1 in transgenic mice

Yukiko Tomioka [a,b], Masami Morimatsu [a,c,*], Ken-ichi Nishijima [d], Tatsufumi Usui [b], Sayo Yamamoto [e], Haruka Suyama [e], Kinuyo Ozaki [e], Toshihiro Ito [b], Etsuro Ono [b,e,f,*]

[a] Division of Disease Model Innovation, Institute for Genetic Medicine, Hokkaido University, Sapporo 060-0815, Japan
[b] Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
[c] Laboratory of Laboratory Animal Science and Medicine, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[d] Department of Biotechnology, Graduate School of Engineering, Nagoya University, Nagoya 464-8603, Japan
[e] Center of Biomedical Research, Research Center for Human Disease Modeling, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
[f] Department of Biomedicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

ARTICLE INFO

Article history:
Received 26 May 2014
Available online 9 June 2014

Keywords:
Soluble Siglec-9
MUC1
Mammary tumor
Antitumor benefit
Transgenic mice

ABSTRACT

Tumor-associated MUC1 binds to Siglec-9, which is expected to mediate tumor cell growth and negative immunomodulation. We hypothesized that a soluble form of Siglec-9 (sSiglec-9) competitively inhibits a binding of MUC1 to its receptor molecules like human Siglec-9, leading to provide antitumor benefit against MUC1-expressing tumor, and generated transgenic mouse lines expressing sSiglec-9 (sSiglec-9 Tg). When mammary tumor cells expressing MUC1 were intraperitoneally transplanted into sSiglec-9 Tg, tumor proliferation was slower with the lower histological malignancy as compared with non-transgenic mice. The sSiglec-9 was detected in the ascites caused by the tumor in the sSiglec-9 Tg, and sSiglec-9 and MUC1 were often colocalized on surfaces of the tumor cells. PCNA immunohistochemistry also revealed the reduced proliferation of the tumor cells in sSiglec-9 Tg. In sSiglec-9 Tg with remarkable suppression of tumor proliferation, MUC1 expressions were tend to be reduced. In the ascites of sSiglec-9 Tg bearing the tumor, T cells were uniformly infiltrated, whereas aggregations of degenerative T cells were often observed in the non-transgenic mice. These results suggest that sSiglec-9 has an antitumor benefit against MUC1-expressing tumor in the transgenic mice, which may avoid the negative immunomodulation and/or suppress tumor-associated MUC1 downstream signal transduction, and subsequent tumor proliferation.

1. Introduction

The MUC1 is a major sialylated transmembrane glycoprotein that is expressed at the apical surface of normal epithelial cells, for protection from the external environment [1,2]. The MUC1 protein is cleaved into N- and C-terminal subunits (MUC1-N and MUC1-C) in the endoplasmic reticulum and the two subunits form a heterodimeric complex that is expressed at the cell membrane [3–5]. The MUC1 is overexpressed in various adenocarcinoma including breast carcinomas [1,6,7] and is involved in the tumor growth, tumorigenicity, and resistance to apoptosis, which is correlated with poor-prognosis of the tumors [8–13]. Recent studies have shown that transmembrane MUC1-C functions as an oncoprotein through interactions with EGFR, ErbB2, and other receptor tyrosine kinases [14]. Additionally, MUC1 directly bind to β-catenin, and blocks phosphorylation and degradation, contributing to the malignant phenotype of tumors [15]. Therefore, although many therapeutic approach targeting MUC1, using anti-MUC1 monoclonal antibody, anti-MUC1 vaccines, and agents that directly target MUC1, have been developed [14,16–22], all the details about molecular mechanisms of MUC1 in malignant tumor growth have not been yet revealed.

Recently, Ohta et al. [23] reported that tumor-produced mucins bound to the sialic acid-binding immunoglobulin-like lectin,

* Corresponding authors. Address: Laboratory of Laboratory Animal Science and Medicine, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan. Fax: +81 11 706 5107 (M. Morimatsu). Address: Department of Biomedicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan. Fax: +81 92 642 6165 (E. Ono).

E-mail addresses: ytomi@muses.tottori-u.ac.jp (Y. Tomioka), mmorimat@vetmed.hokudai.ac.jp (M. Morimatsu), nishijma@nubio.nagoya-u.ac.jp (K.-i. Nishijima), usutatsu@muses.tottori-u.ac.jp (T. Usui), ysayo@anim.med.kyushu-u.ac.jp (S. Yamamoto), sharuka@anim.med.kyushu-u.ac.jp (H. Suyama), k-ozaki@anim.med.kyushu-u.ac.jp (K. Ozaki), toshiito@muses.tottori-u.ac.jp (T. Ito), etsuro@anim.med.kyushu-u.ac.jp (E. Ono).

http://dx.doi.org/10.1016/j.bbrc.2014.06.009
0006-291X/

Siglec-9, leading to negative immunomodulation. Siglec-9 is an inhibitory receptor expressed on immune cells and the binding of Siglec-9 to its ligands induces inhibition of NK cell and T cell antitumor immunity [24,25]. Therefore we hypothesized that a factor inhibiting the binding of Siglec-9 to MUC1 interfered the transmission of immunosuppressive signal, leading to provide antitumor benefits to the animals bearing MUC1-expressing tumors. However, the more recent study has revealed that binding of the soluble Siglec-9 to the MUC1 induces recruitment of β-catenin and subsequent cell growth [26]. Because both reports were performed *in vitro*, to clarify the antitumor potential of sSiglec-9 *in vivo*, we generated transgenic mouse lines expressing soluble Siglec-9 (sSiglec-9 Tg), which was expected to competitively inhibit binding of MUC1 to its receptor molecule similar to Siglec-9, such as CD33-related Siglec, but not mediate immunosuppressive signaling. Here we show that sSiglec-9 provides an antitumor benefit against proliferation of mammary tumor expressing MUC1 in the transgenic mice.

2. Methods and materials

2.1. Generation of transgenic animals

The transgene encoding the fusion protein consisting of the extracellular domain of human Siglec-9 gene (amino acids 1–342) and Fc region of human IgG2 was inserted into the pCXN2 vector [27]. The plasmid was designated pCXN2/sSiglec-9, which expressed sSiglec-9. The plasmid was digested with *Sal*I to obtain the transgene fragment containing the sSiglec-9 gene. Transgenic mice were generated by microinjection method. To put it briefly, approximately 500 copies of the *Sal*I transgene fragment containing the CAG promoter (cytomegalovirus IE enhancer and chicken β-actin promoter), the sSiglec-9 gene, and the rabbit β-globin polyA signal. The fragment was microinjected into the pronuclei of fertilized C57BL/6NJcl (CLEA Japan) mouse embryos. The FTA elute micro cards (Whatman) were used for isolation of genomic DNA for genotyping. Transgenes were detected by PCR analysis using specific primers; Sig9Ig-F2 1765–1787: 5′-GGAAATGGC TCATCTCTGTCACT-3′ and Sig9Ig-R2 2249–2227: 5′-GGCTTTGTCT TGGCATTATGCAC-3′. Blood biochemical examination about general screening items including serum proteins, nitrogen compounds, enzymes, lipids, glucose, were performed by a standard method at Oriental Kobo Life Science Laboratory. All mice were maintained in the animal facility at our institute and treated according to Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology in Japan.

2.2. Antibodies

For immunoblot, we used an anti-human IgG antibody (from rabbit, Sigma, 1:10,000) as a primary antibody for detection of sSiglec-9 and an HRP-labeled anti-rabbit IgG antibody (from goat, Bio-Rad, 1:50,000) as a secondary antibody. For immunohistochemistry, we used antibodies against human IgG (from goat, Sigma, 1:2000), proliferating cell nuclear antigen (PCNA) (from mouse, DAKO, 1:1000), human MUC1 (from rabbit, Lab Vision, 1:1000), and anti-human CD3 (from rabbit, DAKO, 1:500) as primary antibodies. Cy3-, (Jackson ImmunoResearch) and Alexa Fluor 488- (Invitrogen) labeled species-specific antibodies were used at a dilution of 1:200 as secondary antibodies for immunofluorescence.

2.3. Analysis of transgene expression

The sSiglec-9 protein for positive control of transgene expression was purified from a supernatant of the transformed MDCK cell line expressing sSiglec-9 by Protein G affinity chromatography. To confirm the presence of sSiglec-9 in sera and ascites of transgenic mice, Western blot analysis was performed by the method of Towbin et al. [28]. One microliter of each serum or supernatant of tumor ascites sample diluted with water by 100 times were fractionated by 10% SDS–polyacrylamide gel. Serially diluted sSiglec-9 was used for making a standard. Then the separated proteins were electrophoretically transferred to an Immobilon transfer membrane (Millipore). Blotted membrane was treated sequentially with blocking solution (5% skim milk in phosphate buffered saline with 0.05% Tween 20), primary antibody, and finally with HRP-labeled secondary antibody. Detection was performed using ECL Select Western Blotting Detection System (GE Healthcare). Densities of detected bands were semiquantified using NIH ImageJ software (version 1.41).

2.4. Cells

Mouse mammary tumor cell lines, MM46 and its mutant MM46-APR-MUC1cl.1. (MM46-MUC1), which overexpress extraneous human MUC1 (APR-MUC1), were obtained from Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, of Tohoku University and RIKEN BioResource Center, respectively. MM46 cells were cultured in RPMI-1640 (SIGMA) supplemented with 10% heat inactivated fetal bovine serum (GIBCO), 100 IU/mL penicillin, and 100 μg/mL streptomycin (GIBCO). MM46-MUC1 cells were cultured in same culture medium supplemented with 200 μg/mL geneticin (Wako).

2.5. Transplantation of mammary tumor cells

For tumor transplantation, seven- to eight-week-old sSiglec-9 transgenic B6C3F1 mice (C57BL/6 background sSiglec-9 female mice × C3H/HeNJcl male mice, CLEA Japan) and the non-transgenic littermates (non-Tg) were used. MM46 or MM46-MUC1 cells (2×10^6 cells) were intraperitoneally transplanted into the mice under anesthesia by isoflurane inhalation. After transplantation, mice were observed daily for abnormal clinical signs such as weakness, dyspnea, or ascites accumulation throughout the experimental period until post-transplantation day (PTD) 28. The body weights were measured every day and the humane endpoints were a failure to eat or drink and the 30% weight gain by the tumor proliferation.

2.6. Histopathological analyses

Under deep anesthesia by isoflurane inhalation, the whole blood collecting from the hearts were performed for serum samples. The mice were sacrificed by cervical dislocation, and the tumor ascites were collected for examination of gene expression and histopathological analyses. The precipitations of tumor ascites were immersed overnight in 4% paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (PB). The fixed tumor cells were dehydrated and then embedded in paraffin-wax in the usual manner. Paraffin sections (2–4 μm in thick) were cut and stained with haematoxylin and eosin (H. E.). Immunoperoxidase staining by the streptavidin–biotin method was performed using a Histofine SAB-PO kit (Nichirei) and 3,3′-Diaminobenzidine tetrahydrochloride (DAB) detection. The PCNA index (%) was calculated as the average of PCNA-positive tumor cells/all tumor cells × 100 in 4 fields at a high magnification. In immunofluorescence, sections were immunoreacted overnight with a mixture of primary antibodies, and then incubated with a mixture of fluorescent species-specific secondary antibodies for 2 h.

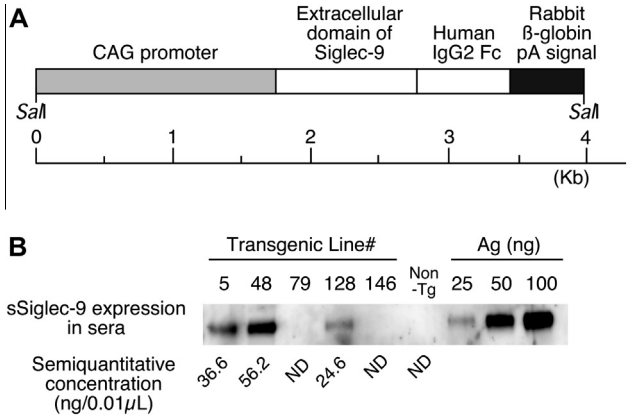

Fig. 1. Generation of sSiglec-9 transgenic mice. (A) The transgene fragment contains the CAG promoter (cytomegalovirus IE enhancer and chicken β-actin promoter), the sSiglec-9 (extracellular domain of human siglec-9 gene and Fc region of human IgG2) and the rabbit β-globin polyA signal. (B) Detection of sSiglec-9 secreted in the sera of transgenic mouse lines by Western blot analysis. Semiquantitative concentration (ng/0.01 μL serum) was indicated at the bottom. ND, not detected.

2.7. MTT assay

Antitumor properties of sSiglec-9 expressed in sera of transgenic mice were estimated using the Cell Proliferation Kit I (MTT) (Roche). Briefly, 5×10^3 of MM46-MUC1 or MM46 cells were seeded in 96-well plates, and the sera of sSiglec-9 Tg or non-Tg littermates were diluted directly into the medium to a final concentration of 5.0%. Cells were incubated for 72 h at 37 °C, and then 10 μL of MTT labeling solution to a final concentration of 0.5 mg/mL was added. Plates were incubated for an additional 4 h, and 100 μL of solubilization solution was added for overnight incubation. The amount of dissolved formazan dye was measured at A550 nm, using an ELISA plate reader.

2.8. Statistics

The values were expressed as the averages of at least three times ± standard deviations. Statistical analysis was performed by Student's *t*-test.

3. Results

3.1. Generation of sSiglec-9 mice

We generated five transgenic mouse lines with sSiglec-9 transgene under the control of CAG promoter (Fig. 1A), which allows an expression in all cell types [27]. Western blot analysis using an anti-human IgG rabbit antibody revealed that the transgene expression was confirmed in the sera of three of five transgenic lines and their concentrations of sSiglec-9 protein were relatively high and variable (2.46–5.62 mg/mL) (Fig. 1B). By immunohistochemistry with an anti-human IgG goat antibody, sSiglec-9 were expressed in many tissues including various secretory and mucosal epithelium in the mice of sSiglec-9 transgenic lines. However, no significant abnormalities in the sSiglec-9 mice were observed in histological examinations with H.E. staining and general blood biochemical examinations (data not shown). For the following transplant experiment, we used the mice of sSiglec-9 line 48, which expressed the highest level of sSiglec-9 protein.

3.2. sSiglec-9 mice were significantly resistant to proliferation of MM46-MUC1 tumor cells not MM46 cells

To find out whether the sSiglec-9 mice were resistant to proliferation of tumor cells expressing MUC1, MM46-MUC1 and MM46

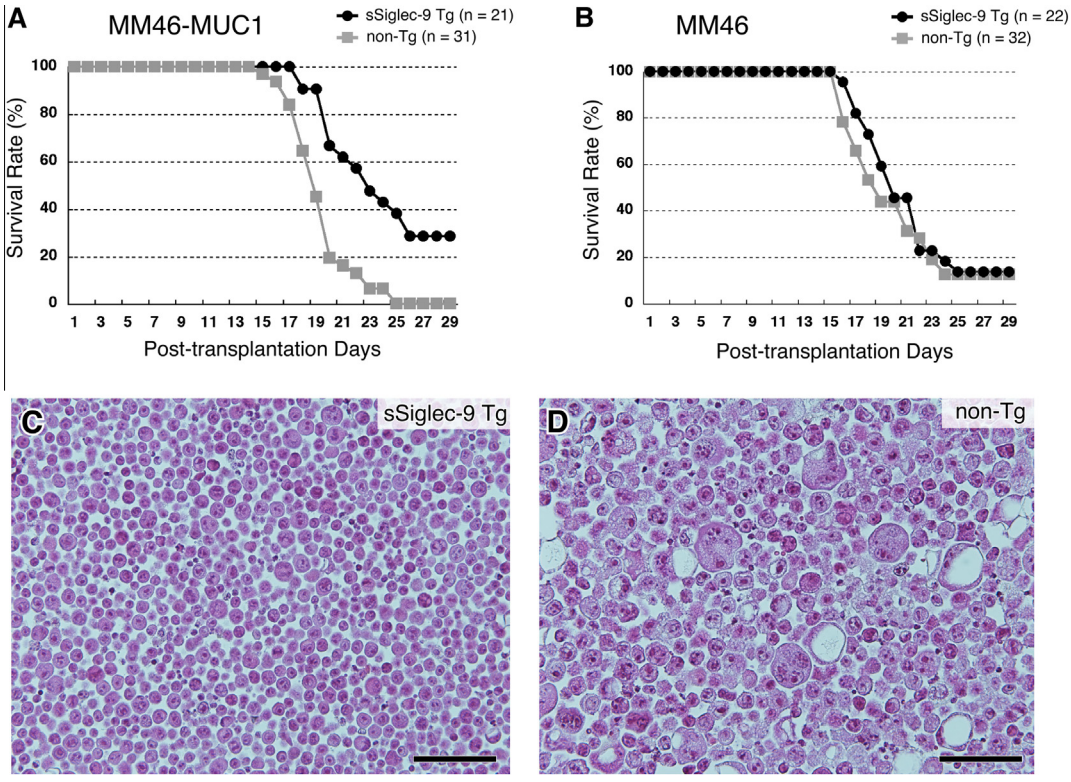

Fig. 2. sSiglec-9 mice were resistant to proliferation of MM46-MUC1 tumor cells. (A and B) Survival curves of the sSiglec-9 and non-Tg mice after the transplantation of MM46-MUC1 (A) and MM46 (B). (C and D) Histopathology of transplanted MM46-MUC1 in sSiglec-9 mice (C) and non-Tg mice (D). Bars, 50 μm.

cells were i.p. inoculated into the sSiglec-9 mice and their non-Tg littermates. The survival curves demonstrated that the tumor proliferation causing death in sSiglec-9 Tg was significantly slower than that in non-Tg littermates (Fig. 2A). The sSiglec-9 mice began to die three days (PTD 17) later after non-Tg did (PTD 14). Furthermore, 28.6% of sSiglec-9 mice survive throughout the experimental period until PTD 28 and the survived mice had only a small amount of ascites. All of non-Tg mice died or were sacrificed at the humane endpoints as above mentioned after transplantation of MM46-MUC1 within experimental period until PTD 28. In contrast, there was not much difference between sSiglec-9 and non-Tg mice after transplantation of MM46 cells without forcibly expressing MUC1 (Fig. 2B). The histological examination revealed that MM46-MUC1 cells in ascites of sSiglec-9 mice were relatively small and uniform in size and shape (Fig. 2C), whereas the tumor cells in non-Tg mice were pleomorphic with enlargement of many cells (Fig. 2D).

3.3. Soluble Siglec-9 expression and histopathology in ascites tumor cells of the sSiglec-9 mice

The expressions of sSiglec-9 were detected in all of the ascites of MM46-MUC1 transplanted mice by Western blot and there was no significant difference in the concentration of sSiglec-9 between the sera and ascites (Fig. 3A). The sSiglec-9-positive signals were observed to cover the surface of the tumor cells in ascites of the sSiglec-9 Tg (Fig. 3B). Non-specific signals were not detected in the non-Tg littermates. When immunofluorescence for sSiglec-9 (green), MUC1 (red), and DAPI (blue) was applied to the MM46-MUC1 cells in the sSig-9 Tg ascites, the colocalizations of sSiglec-9 and MUC1 on the cell surfaces were often detected (Fig. 3C).

3.4. Soluble Siglec-9 was not cytotoxic for MM46-MUC1 cells in vitro, but reduced cell proliferation activities and MUC1 expression of MM46-MUC1 cells in vivo

In order to examine whether sSiglec-9 was cytotoxic and can suppress the proliferation of MM46-MUC1 cells *in vitro*, MTT assays were performed using mice sera and cultured MM46-MUC1 cells. In the MTT assays, no significant difference was observed in the proliferation activities between MM46-MUC1 and MM46 cells with sSiglec-9 Tg sera vs. non-Tg sera (Fig. 4A). In contrast, the PCNA immunohistochemistry revealed that the reduced proliferation of ascites cells in sSiglec-9 Tg mice (Fig. 4B and C). The MUC1 expressions were also reduced in sSiglec-9 Tg mice (Fig. 4D). Additionally, viable, CD3-positive T lymphocytes were comparative uniformly infiltrated into the tumor ascites of

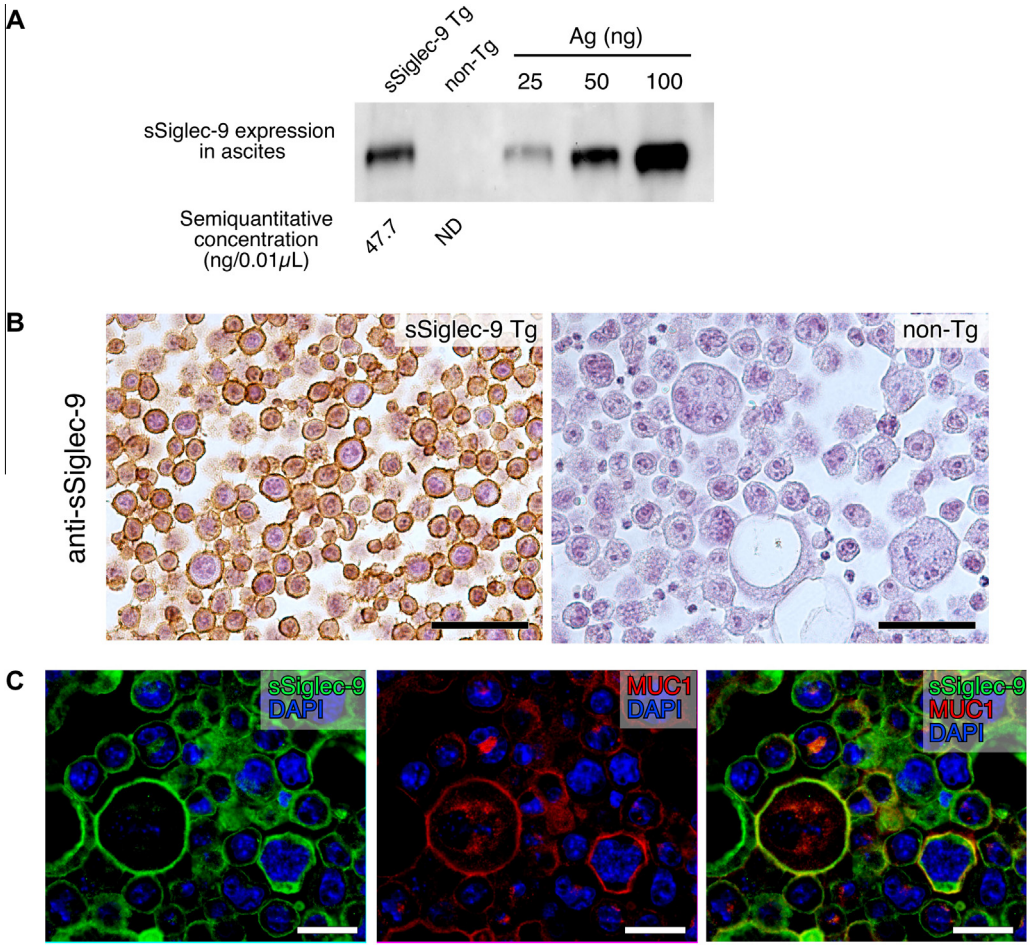

Fig. 3. sSiglec-9 expression in ascites of the MM46-MUC1 tumor-bearing sSiglec-9 mice. (A) A representative result of the expressions of sSiglec-9 in the ascites supernatant of MM46-MUC1 transplanted mice by Western blot. Semiquantitative concentration (ng/0.01 μL ascite) was indicated at the bottom. ND, not detected. (B) Immunohistochemistry for the sSiglec-9 of the MM46-MUC1 tumor cells transplanted in the sSiglec-9 Tg mice (left) and non-Tg (right). Bars, 100 μm. (C) Immunofluorescence for sSiglec-9 (green), MUC1 (red), and DAPI (blue) of the transplanted MM46-MUC1 cells. Bars, 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

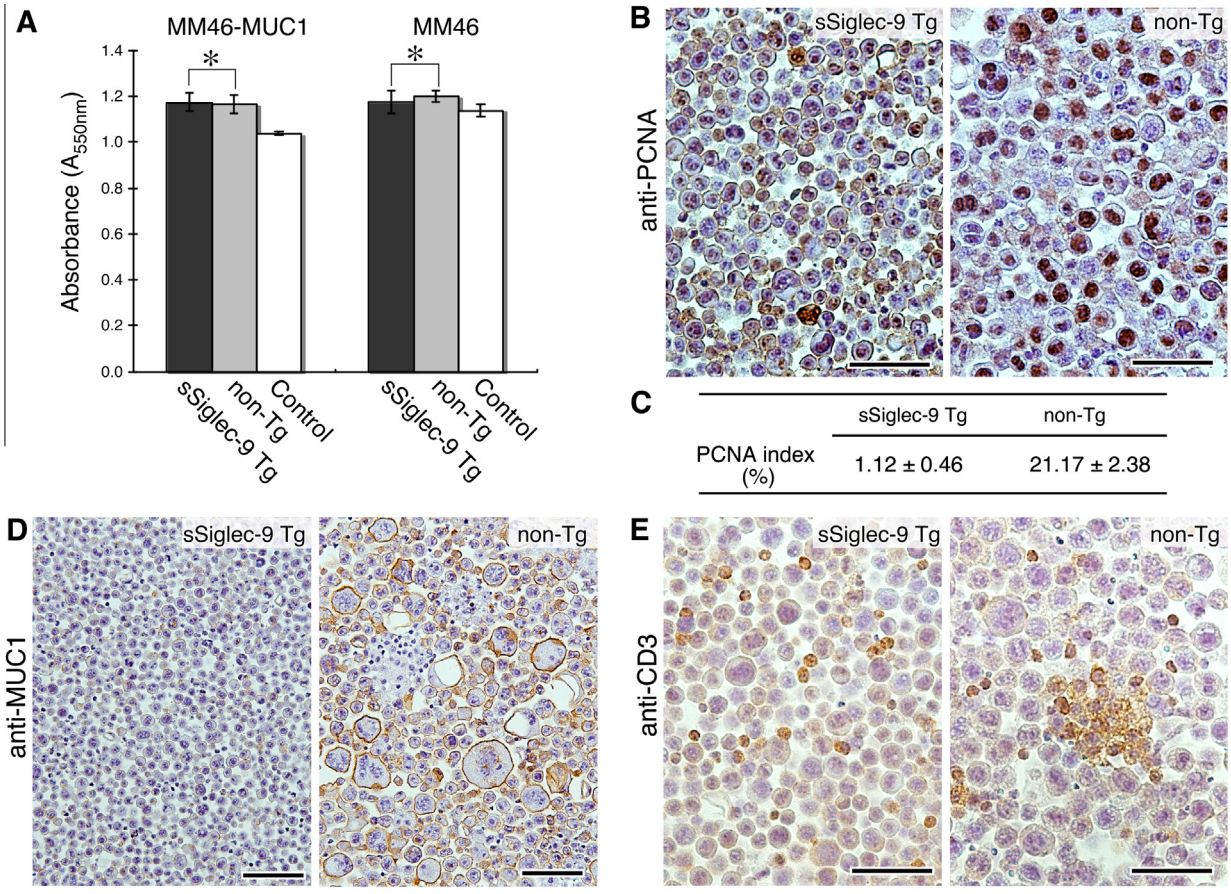

Fig. 4. The influences of sSiglec-9 on the tumor cell proliferation and histopathology. (A) MTT assay. No significant difference was observed in the proliferation activities between cells with sSiglec-9 Tg sera vs. non-Tg sera (*$P > 0.2$). (B) Immunohistochemistry for PCNA of the ascites cells in the sSiglec-9 Tg mice (left) and non-Tg (right), MM46-MUC1 transplanted. Bars, 100 μm. (C) PCNA labeling index (%). (D) Immunohistochemistry for MUC1 of the ascites cells in the sSiglec-9 Tg mice (left) and non-Tg (right), MM46-MUC1 transplanted. Bars, 50 μm. (E) Immunohistochemistry for CD3 of the ascites cells in the sSiglec-9 Tg mice (left) and non-Tg (right), MM46-MUC1 transplanted. Bars, 100 μm.

sSiglec-9 Tg, although the aggregations of degenerative CD3-positive cells were often observed in the non-Tg (Fig. 4E).

4. Discussion

In the present study, we generated transgenic mouse lines with sSiglec-9 transgene under the control of CAG promoter (sSiglec-9 Tg). It has been reported that tumor-produced MUC1 bound to Siglec-9, leading to negative immunomodulation [23]. Siglec-9 is an inhibitory receptor expressed on immune cells and the binding of Siglec-9 to its ligands induces inhibition of NK cell and T cell antitumor immunity [24,25]. We hypothesized that a factor inhibiting the binding of Siglec-9 to MUC1 interferes the transmission of the immunosuppression signal, leading to prevent antitumor immunity, and transplanted MM46-MUC1 mammary tumor cells to sSiglec-9 Tg mice intraperitoneally. The sSiglec-9 Tg mice were resistant to proliferation of MM46-MUC1 tumor cells compare with non-Tg littermates. These antitumor benefits were not observed in the sSiglec-9 Tg mice bearing MM46 cells without forcibly expressing MUC1. In the transgenic mouse lines, histopathological and blood biochemical abnormalities were not observed. Furthermore, the expression of sSiglec-9 had no significant effect on a number of lymphocyte homing into lymph nodes at least (data not shown). Taken together, these data suggest that the sSiglec-9 may be a medical agent against the tumor expressing MUC1, which did not produce side effects expected on the immune system.

The sSiglec-9 in mice sera or purified from supernatant from stably expressing cell line was not cytotoxic for MM46-MUC1 cells *in vitro*. However, proliferative activities of MM46-MUC1 cell transplanted in the sSiglec-9 Tg were lower than those in non-Tg. These results may suggest that sSiglec-9 *in vivo* suppressed the proliferation of MM46-MUC1 cell by inhibition the MUC1 downstream signal transduction, due to direct suppression of MUC1 expression and/or competitive inhibition of MUC1 binding to CD33-related Siglec similar to human Siglec-9. Recently, Tanida et al. [26] reported that binding of soluble Siglec-9 to the MUC1 induces recruitment of β-catenin and subsequent cell growth *in vitro* rather than suppression of the tumor growth, which results disagree with our ones *in vivo*. The effect of sSiglec-9 in cell proliferation may depend on various factors such as its localization, concentration, and microenvironment including the presence of endogenous CD33-related Siglec or similar molecules on immune cells.

Histologically, the viable CD3-positive T lymphocytes were infiltrated through the tumor ascites of sSiglec-9 Tg, whereas aggregations of degenerative CD3-positive cells were often observed in the non-Tg. In addition, the proliferative activities of splenic T lymphocytes separated from the sSiglec-9 Tg were slightly higher than non-Tg (data not shown). Yurugi et al. reported that bindings of Siglec-9 to prohibitins negatively regulate TCR signaling [29]. Taken together, sSiglec-9 in the Tg mice may competitively inhibit the CD33-related Siglec similar to human Siglec-9 bindings to prohibitins, resulting in the histopathological

difference in the T cell infiltration into the ascites. Also it has been reported that cancer-associated MUC1 inhibit proliferation of T lymphocytes and induce apoptosis of T lymphocytes [30,31]. Possibly, suppressed expression of MUC1 in sSiglec-9 Tg mice indirectly affected activity and proliferation T cells in the tumor.

In summary, we conclude that sSiglec-9 provides an antitumor benefit against mammary tumor cells expressing MUC1 in the transgenic mice. The present findings suggest that a sSiglec-9 suppress tumor-associated MUC1 downstream signal transduction, following the tumor proliferation, and might be a medical agent against the tumor expressing MUC1.

Acknowledgments

We thank RIKEN BioResource Center and Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, of Tohoku University for providing us with MM46-APR-MUC1cl.1. and MM46, respectively. We are deeply grateful to Toshihiko Iwanaga for his technical help. This work was supported by Grant-in-Aid for Scientific Research (C) (Grant Number 26450398) from The Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] D. Kufe, G. Inghirami, M. Abe, et al., Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors, Hybridoma 3 (1984) 223–232.
[2] J. Wesseling, S.W. van der Valk, H.L. Vos, et al., Episialin (MUC1) overexpression inhibits integrin-mediated cell adhesion to extracellular matrix components, J. Cell Biol. 129 (1995) 255–265.
[3] M.J. Ligtenberg, L. Kruijshaar, F. Buijs, et al., Cell-associated episialin is a complex containing two proteins derived from a common precursor, J. Biol. Chem. 267 (1992) 6171–6177.
[4] F. Levitin, A. Baruch, M. Weiss, et al., A novel protein derived from the MUC1 gene by alternative splicing and frameshifting, J. Biol. Chem. 280 (2005) 10655–10663.
[5] B. Macao, D.G.A. Johansson, G.C. Hansson, et al., Autoproteolysis coupled to protein folding in the SEA domain of the membrane-bound MUC1 mucin, Nat. Struct. Mol. Biol. 13 (2006) 71–76.
[6] E.A. Rakha, R.W.G. Boyce, D. Abd El-Rehim, et al., Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer, Mod. Pathol. 18 (2005) 1295–1304.
[7] J.J. Rahn, L. Dabbagh, M. Pasdar, et al., The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the literature, Cancer 91 (2001) 1973–1982.
[8] M.A. McGuckin, M.D. Walsh, B.G. Hohn, et al., Prognostic significance of MUC1 epithelial mucin expression in breast cancer, Hum. Pathol. 26 (1995) 432–439.
[9] D.W. Kufe, Mucins in cancer: function, prognosis and therapy, Nat. Rev. Cancer 9 (2009) 874–885.
[10] S. Yonezawa, M. Goto, N. Yamada, et al., Expression profiles of MUC1, MUC2, and MUC4 mucins in human neoplasms and their relationship with biological behavior, Proteomics 8 (2008) 3329–3341.
[11] D. Raina, S. Kharbanda, D. Kufe, The MUC1 oncoprotein activates the anti-apoptotic phosphoinositide 3-kinase/Akt and Bcl-xL pathways in rat 3Y1 fibroblasts, J. Biol. Chem. 279 (2004) 20607–20612.
[12] L. Yin, L. Huang, D. Kufe, MUC1 oncoprotein activates the FOXO3a transcription factor in a survival response to oxidative stress, J. Biol. Chem. 279 (2004) 45721–45727.
[13] X. Wei, H. Xu, D. Kufe, Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response, Cancer Cell 7 (2005) 167–178.
[14] D.W. Kufe, MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches, Oncogene 32 (2013) 1073–1081.
[15] L. Huang, D. Chen, D. Liu, et al., MUC1 oncoprotein blocks glycogen synthase kinase 3beta-mediated phosphorylation and degradation of beta-catenin, Cancer Res. 65 (2005) 10413–10422.
[16] M.D. Pegram, V.F. Borges, N. Ibrahim, et al., Phase I dose escalation pharmacokinetic assessment of intravenous humanized anti-MUC1 antibody AS1402 in patients with advanced breast cancer, Breast Cancer Res. 11 (2009) R73.
[17] N.K. Ibrahim, K.O. Yariz, I. Bondarenko, et al., Randomized phase II trial of letrozole plus anti-MUC1 antibody AS1402 in hormone receptor-positive locally advanced or metastatic breast cancer, Clin. Cancer Res. 17 (2011) 6822–6830.
[18] J.L. Gulley, P.M. Arlen, K.-Y. Tsang, et al., Pilot study of vaccination with recombinant CEA–MUC-1–TRICOM poxviral-based vaccines in patients with metastatic carcinoma, Clin. Cancer Res. 14 (2008) 3060–3069.
[19] M. Mohebtash, K.-Y. Tsang, R.A. Madan, et al., A pilot study of MUC-1/CEA/TRICOM poxviral-based vaccine in patients with metastatic breast and ovarian cancer, Clin. Cancer Res. 17 (2011) 7164–7173.
[20] D. Raina, R. Ahmad, M.D. Joshi, et al., Direct targeting of the mucin 1 oncoprotein blocks survival and tumorigenicity of human breast carcinoma cells, Cancer Res. 69 (2009) 5133–5141.
[21] Y. Zhou, H. Rajabi, D. Kufe, Mucin 1 C-terminal subunit oncoprotein is a target for small-molecule inhibitors, Mol. Pharmacol. 79 (2011) 886–893.
[22] M.D. Joshi, R. Ahmad, L. Yin, D, MUC1 oncoprotein is a druggable target in human prostate cancer cells, Mol. Cancer Ther. 8 (2009) 3056–3065.
[23] M. Ohta, A. Ishida, M. Toda, et al., Immunomodulation of monocyte-derived dendritic cells through ligation of tumor-produced mucins to Siglec-9, Biochem. Biophys. Res. Commun. 402 (2010) 663–669.
[24] Y. Ikehara, S.K. Ikehara, J.C. Paulson, Negative regulation of T cell receptor signaling by Siglec-7 (p70/AIRM) and Siglec-9, J. Biol. Chem. 279 (2004) 43117–43125.
[25] T. Avril, H. Floyd, F. Lopez, et al., The membrane-proximal immunoreceptor tyrosine-based inhibitory motif is critical for the inhibitory signaling mediated by Siglecs-7 and -9, CD33-related Siglecs expressed on human monocytes and NK cells, J. Immunol. 173 (2004) 6841–6849.
[26] S. Tanida, K. Akita, A. Ishida, et al., Binding of the sialic acid-binding lectin, Siglec-9, to the membrane mucin, MUC1, induces recruitment of β-catenin and subsequent cell growth, J. Biol. Chem. 288 (2013) 31842–31852.
[27] H. Niwa, K. Yamamura, J. Miyazaki, Efficient selection for high-expression transfectants with a novel eukaryotic vector, Gene 108 (1991) 193–199.
[28] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 4350–4354.
[29] H. Yurugi, S. Tanida, K. Akita, et al., Prohibitins function as endogenous ligands for Siglec-9 and negatively regulate TCR signaling upon ligation, Biochem. Biophys. Res. Commun. 434 (2013) 376–381.
[30] B. Agrawal, M.J. Krantz, M.A. Reddish, et al., Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2, Nat. Med. 4 (1998) 43–49.
[31] C.D. Gimmi, B.W. Morrison, B.A. Mainprice, et al., Breast cancer-associated antigen, DF3/MUC1, induces apoptosis of activated human T cells, Nat. Med. 2 (1996) 1367–1370.