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Prophylactic effect of a peptide vaccine targeting TM4SF5 against colon cancer in a mouse model

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ABSTRACT

Expression of transmembrane 4 superfamily member 5 protein (TM4SF5) was implicated in hepatocellular carcinoma (HCC) and colon cancer. Previously, we have shown that immunization with TM4SF5 peptide-CpG-DNA-liposome complex induces production of TM4SF5-specific antibodies and protects mice from HCC progression in an allograft model. Here, we confirmed expression of TM4SF5 in the mouse colon cancer cell line CT-26 and found that anti-TM4SF5 antibody inhibits growth of CT-26 cells. We then immunized mice with TM4SF5 peptide-CpG-DNA-liposome complex and transplanted CT-26 cells to investigate the vaccination effects. Robust production of TM4SF5-specific antibodies was induced by challenge with CT-26 cells and the tumor growth was significantly suppressed in the immunized mice. The peptide vaccine targeting TM4SF5 consequently showed a prophylactic effect against colon cancer development in a mouse model. These results suggest that the peptide vaccine can be potentially applied in humans to treat colon cancer.

1. Introduction

Colorectal cancer, commonly known as colon cancer, is the third most prevailing cancer in the world with high mortality, and it is more common in developed countries than undeveloped countries [1]. Colon cancer most frequently results from the abnormally activated Wnt-APC-β-catenin signaling pathway involving mutation of genes such as APC, β-catenin, AXIN1, AXIN2, TCF7L2, or NKD1 [2–5]. Mutations in apoptotic pathway regulators such as p53, TGF-β, and SMAD were also implicated [6–8]. Some mutated genes are oncogenes such as KRAS, RAF, and PI3K, which induce over-activation of cell proliferation [9,10].

Tetraspanins have four hydrophobic transmembrane domains generating two extracellular loops, and thereby they are also referred to as transmembrane 4 superfamily (TM4SF) members. Tetraspanins are widely involved in cell differentiation, activation, growth, migration, and regulation of cell signaling [11,12]. This multiple function may be related with the characteristics that tetraspanins associate with various molecules including integrins, extracellular matrix proteins, and other tetraspanins as an essential component of tetraspanin-mediated microdomains [13]. Recently, tetraspanins are also implicated in tumor progression and metastasis [14]. Therefore, tetraspanins have been suggested as diagnostic and prognostic markers and therapeutic targets for tumor treatment [15].

The mRNA expression of transmembrane 4 superfamily member 5 protein (TM4SF5) was previously reported in human cancers such as pancreatic cancer, hepatocellular carcinoma (HCC), and colon cancer [16,17]. It was also reported that TM4SF5 plays an important role in HCC formation by epithelial-mesenchymal transition, uncontrolled cell proliferation, and angiogenesis [17–19]. Therefore, TM4SF5 may be considered as a novel molecular target for the clinical development of HCC therapeutics [20]. In previous studies, we confirmed that immunization with a TM4SF5 B-cell epitope-CpG-DNA-liposome complex had preventive and thera-

Abbreviations: TM4SF5, transmembrane 4 superfamily member 5 protein; Lipoplex(O), phosphodiester-backbone CpG-DNA coencapsulated in DOPE:CHEMS liposomes; DOPE:CHEMS, phosphatidyl-β-oleoyl-γ-palmitoyl ethanolamine : cholesterol hemisuccinate.

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peutic effects on tumor growth in a mouse HCC model [21,22]. Considering that TM4SF5 is also expressed in colon cancer tissues, we can expect that the vaccine has similar anti-tumor effects in colon cancer.

In this study, we investigated expression of TM4SF5 in colon cancer cells and confirmed the efficacy of a vaccine composed of TM4SF5 B-cell epitope and Lipoplex(O) in an mouse model transplanted with mouse colon cancer cells.

2. Materials and methods

2.1. Cell culture

The mouse colon cancer cell line CT-26 was obtained from the Korean Cell Line Bank. CT-26 cells were maintained in DMEM medium containing 10% fetal bovine serum (FBS; Hyclone), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37 °C in an atmosphere containing 5% CO_2.

2.2. Detection of TM4SF5 expression

To analyze the TM4SF5 expression, we performed RT-PCR and FACS analyses. Normal mouse colon tissues were cut out from BALB/c mice and chopped up with scissors. After washing with DMEM medium, 200 μg/ml collagenase IV (Bioshop) was added and incubated for 10 min at 37 °C. After washing with DMEM medium, colon tissue debris were filtered with a cell strainer and dissociated cells were harvested by centrifugation. Total RNAs were extracted with an RNeasy Mini Kit (Qiagen), and the cDNA was generated as described previously [23]. The standard PCR reaction was performed for 25 cycles with the following primer sets: mouse GAPDH, 5′-ATGGTGAAGGTCGGTGTGAACG-3′ and 5′-GTTGTCATGGATGATCTTGGCC-3′(501 bp); mouse TM4SF5, 5′-cgcttacttgcgaaatgaca-3′, and 5′-TTTCCTGCAATCGCCACACA-3′ (174 bp). The expression of TM4SF5 protein in CT-26 cells was confirmed by a FACS analysis using Cy5.5 (Promega)-conjugated anti-TM4SF5 antibody, which was prepared according to the manufacturer's instructions.

2.3. Preparation of B cell epitope and CpG-DNA co-encapsulated in DOPE:CHEMS complexes

The B cell epitope peptide of human TM4SF5 (hTM4SF5R2-3, ^{138}NRTLWDRCEAPPRV^{151}) was selected and produced as previously described [21,24]. A natural phosphodiester bond CpG-DNA, specifically MB-ODN 4531(O), consists of 20 bases containing three CpG motifs (underlined): AGCAGCGTTCGTGTCGGCCT [21]. Complexes consisting of B cell epitope and CpG-DNA (MB-ODN 4531(O)) co-encapsulated with phosphatidyl-β-oleoyl-γ-palmitoyl ethanolamine: cholesterol hemisuccinate (DOPE:CHEMS) liposomes (Lipoplex(O)) were prepared as reported previously [21].

2.4. Animals

Four-week-old male BALB/c mice were obtained from Central Lab. Animal Inc., and the mice were maintained under a specific-pathogen-free condition. All procedures involving animal studies are in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Veterinary Research & Quarantine Service of Korea with the approval of the Institutional Review Board in Hallym University (Hallym2011-89). The mice were sacrificed under Zoletil 50+ Rompun anesthesia, and all efforts were made to minimize suffering.

2.5. ELISA

Mouse sera were obtained by orbital bleeding before each injection as well as by sacrifice 10 days after final injection. To determine the amounts and titers of TM4SF5-specific IgG, 96-well immunoplates (Nalgen Nunc International) were coated with 5 μg/ml of TM4SF5 peptide [21] and then blocked for 2 h with 0.05% of Tween-20 in PBS (PBST) containing 1% BSA. After the blocking solution was removed, serially diluted sera was added, incubated for 2 h at room temperature, washed with PBST, and then incubated for 2 h with detecting antibodies (anti-IgG, anti-IgG1, and anti-IgG2a) conjugated with horseradish peroxidase. A colorimetric assay was developed with a TMB substrate solution, and the absorbance at 450 nm was measured using a Spectra Max 250 microplate reader.

2.6. Colon cancer mouse model

Four-week-old BALB/c mice were injected intraperitoneally with a complex of TM4SF5 peptide (50 μg/mouse) and Lipoplex(O) three times at 10 day intervals as previously described [21]. Ten days after the third immunization, the mice were inoculated subcutaneously in the dorsal right flank with 5×10^6 of CT-26 cells in a 50% matrigel solution (HBSS/Matrigel, 1:1 v/v, BD Biosciences), as previously described [25]. Size of the tumor was measured at 2 day intervals with calipers in three dimensions, and tumor volumes were calculated as width^2 × length/2. The mice were sacrificed 20 days after cancer cell implantation, and the tumors were surgically excised and weighed. Mice were sacrificed when the tumor size reached 2000 mm^3 or the mice lost >20% of initial body weight in accordance with the Guide for the Care and Use of Laboratory Animals of the National Veterinary Research & Quarantine Service of Korea to minimize suffering from a large tumor burden. After the vaccination, the survival rate was recorded for 30 days.

2.7. Immunohistochemistry

To identify the expression of TM4SF5, the specimens were stained with anti-TM4SF5 monoclonal antibody using standard procedures. The specimens were cut into 5 μm thick sections. After deparaffinization by xylene and rehydration in ethanol, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide and 10% methanol in PBS, followed by preincubation in PBS containing 0.2% Triton X-100 (PBST) and 3% bovine serum albumin for 2 h. The sections were then incubated overnight in PBST containing anti-TM4SF5 monoclonal antibody (1:400) at 4 °C, followed by incubation with biotinylated secondary antibody. They were first reacted with streptavidin–biotin peroxidase and subsequently with 3′,3′-diaminobenzinidine (0.5 mg/ml) and hydrogen peroxide, followed by counterstaining with H&E. After rinsing, the sections were mounted, dehydrated, and covered with cover slips. All images were examined using a Nikon Eclipse E-200 microscope (Nikon).

3. Results

3.1. Expression of TM4SF5 and functional effect of anti-TM4SF5 antibody on colon cancer cells in vitro

To validate the presence of TM4SF5 as a specific target in colon cancer, we first checked expression of TM4SF5 in the mouse colon cancer cell line CT-26 as a model. As shown in Fig. 1A, expression of TM4SF5 at the mRNA level was detected in CT-26 cells but not in normal mouse colon tissue. We also confirmed the expression of TM4SF5 at the protein level by a FACS analysis using Cy5.5-conju-

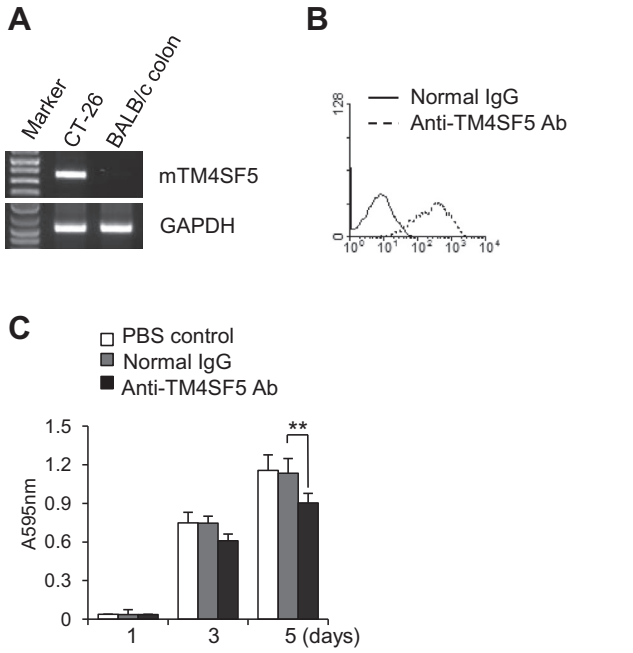

Fig. 1. Expression of TM4SF5 and the effect of anti-TM4SF5 antibody treatment in CT-26 cells. (A) Expression of TM4SF5 mRNA in CT-26 cells and normal mouse colon tissue was determined by a RT-PCR analysis. (B) Expression of TM4SF5 protein in colon cancer cells was determined by a FACS analysis using Cy5.5-conjugated anti-TM4SF5 antibody. Normal IgG was used as a control. (C) CT-26 cells were treated with PBS, normal IgG, or anti-TM4SF5 antibody for the indicated period and the cell growth was examined by a MTT assay. Each bar is expressed as the mean ± standard deviation of three different experiments performed in triplicate. $^{**}P < 0.01$.

gated monoclonal antibody to TM4SF5 (Fig. 1B). To investigate the effect of anti-TM4SF5 monoclonal antibody on colon cancer cell growth, we performed a MTT assay after treatment with PBS, normal IgG or anti-TM4SF5 antibody. The anti-TM4SF5 antibody delayed the growth of CT-26 cells (about 20%), but normal IgG had no effect (Fig. 1C). Therefore, we can conclude that TM4SF5 is expressed in CT-26 cells and that the anti-TM4SF5 antibody has a suppressive effect on the growth of CT-26 cells.

3.2. Production of anti-TM4SF5 antibody after colon cancer cell implantation in mice immunized with a TM4SF5 peptide and Lipoplex(O) complex

To evaluate the prophylactic efficacy of the vaccine containing the complex of TM4SF5 peptide and Lipoplex(O) against colon cancer development, we immunized BALB/c mice with the vaccine three times and then implanted CT-26 cells into the mice 10 days after final immunization (Fig. 2). Two groups of mice were immunized with PBS or TM4SF5 peptide encapsulated in DOPE:CHEMS and then implanted with CT-26 cells as a negative control. When the sera from the tested mice were analyzed by ELISA, significant production of the TM4SF5-specific antibody in response to implantation of CT-26 cells was revealed in the mice immunized with the complex of TM4SF5 peptide and Lipoplex(O) (Fig. 2A). Further analysis was performed to characterize the isotype of the induced antibodies and IgG2a was proved to be the major type (Fig. 2B and C). Immunization with DOPE:CHEMS and TM4SF5 peptide induced production of TM4SF5-specific antibody at a lower level compared to the results obtained by the complex of TM4SF5 peptide and Lipoplex(O), suggesting limited adjuvant activity without CpG-DNA (Fig. 2A and C).

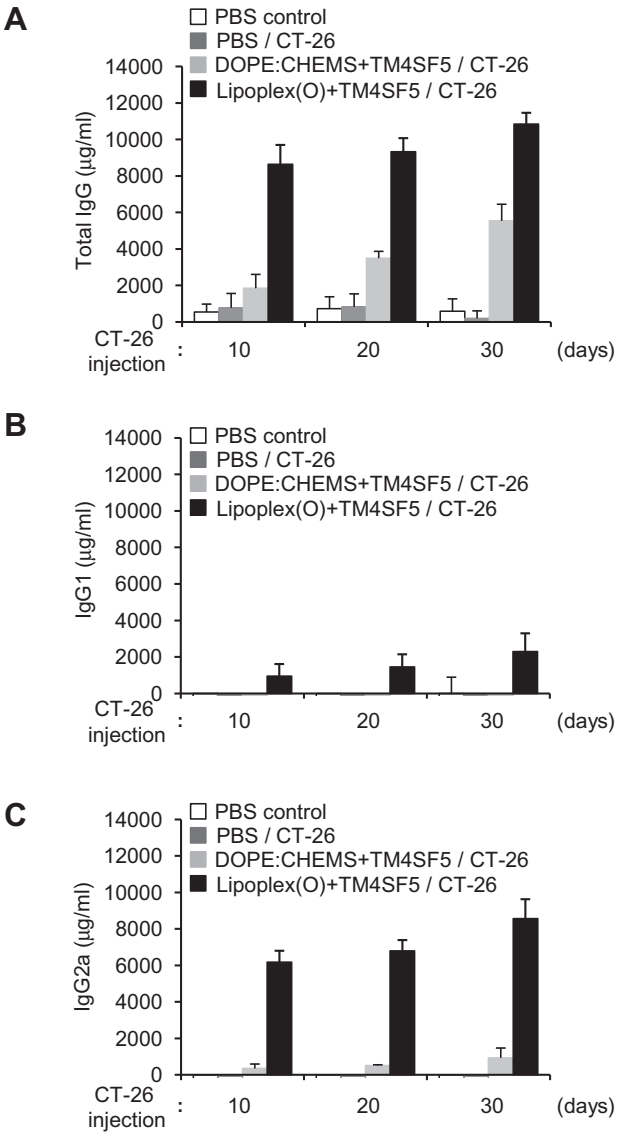

Fig. 2. Induction of a strong serologic response to CT-26 cell implantation in mice immunized with TM4SF5 epitope and Lipoplex(O). BALB/c mice were immunized with PBS control, TM4SF5 peptide and a DOPE:CHEMS complex (DOPE:-CHEMS+TM4SF5), or a complex of TM4SF5 peptide and Lipoplex(O) (Lipoplex(O)+TM4SF5). The immunized mice were implanted with CT-26 cells ($n = 12$ per group; $n = 8$ per PBS control). The sera were collected, and the amounts of TM4SF5 peptide-specific total IgG (A), IgG1 (B), and IgG2a (C) were assayed using an ELISA kit. Each bar is expressed as the mean ± standard deviation of 8 or 12 mice.

3.3. Suppression of tumor cell growth by immunization with a TM4SF5 epitope and Lipoplex(O) complex in colon cancer model

While CT-26 cells implanted into BALB/c mice grew continuously into a tumor mass, tumor development was significantly inhibited in the mice immunized with TM4SF5 peptide and Lipoplex(O) (Fig. 3A–C); tumor volume and tumor weight were significantly reduced in the mice. Immunization with DOPE:CHEMS and TM4SF5 peptide showed no prophylactic activity, which is in agreement with the limited antibody production capacity (Fig. 2A and C), suggesting the essential role of CpG-DNA in anti-cancer activity of the peptide vaccine. Apparently, immunization with the complex of TM4SF5 peptide and Lipoplex(O) does not induce significant side effects as there was no difference in the body weight of the mice during the experiment (Fig. 3D). Analysis of the tumor tissues obtained from the tested mice using immunohis-

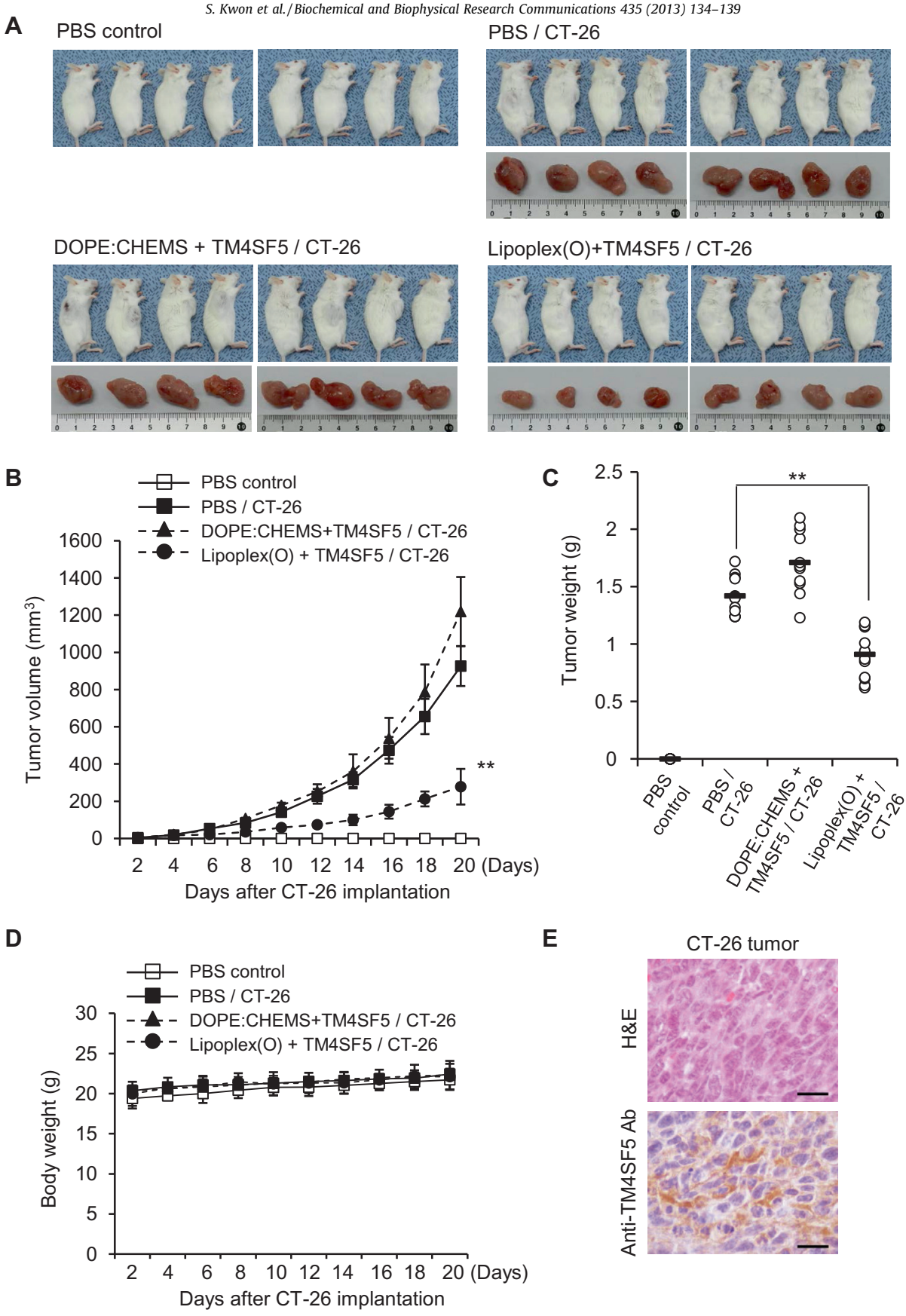

Fig. 3. Prophylactic efficacy of a vaccine containing TM4SF5 peptide and Lipoplex(O) complex in colon cancer implanted mice. (A–D) Tumor development in mice implanted with CT-26 cells was inhibited by immunization with the TM4SF5 peptide and Lipoplex(O) complex (n = 12 per group; n = 8 per PBS control). (A) Macroscopic appearance of HCC tumor tissues. Eight of 12 tested mice are shown. (B) Tumor growth was measured by tumor volume. Tumor volumes were calculated as (length × width^2)/2. (C) Tumor growth was measured by tumor weight. (D) Body weights were measured at the indicated time intervals. (E) Histology of tumor tissue derived from CT-26 cell-implanted mice was observed by staining with hematoxylin and eosin (H&E). An immunohistochemical analysis (IHC) was performed with anti-TM4SF5 monoclonal antibody. TM4SF5 positive area was expressed as brown color. Scale bars, 50 μm. $^{**}P < 0.01$.

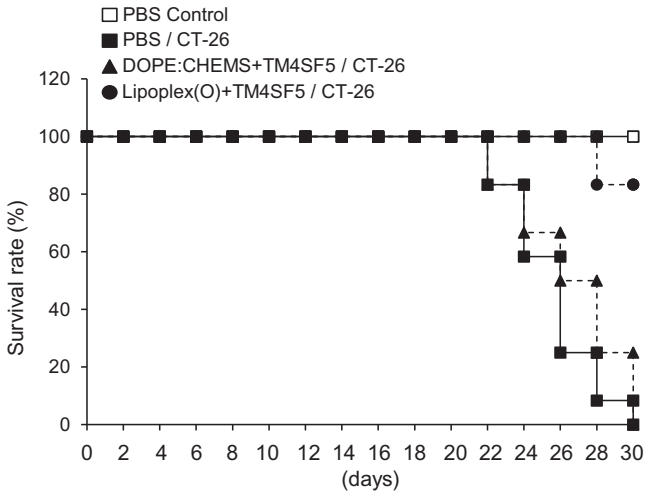

Fig. 4. Survival rate of tumor-bearing mice. BALB/c mice immunized with the TM4SF5 peptide and Lipoplex(O) complex or indicated combinations were implanted with CT-26 cells, and the survival rate was monitored until 30 days after implantation (n = 12 per group; n = 8 per PBS control).

tochemistry with anti-TM4SF5 antibody revealed robust expression of TM4SF5 in the tumor tissues, confirming that the tumors derived from the implanted CT-26 cells (Fig. 3E).

3.4. Increased survival of the mice immunized with a TM4SF5 peptide and Lipoplex(O) after CT-26 cell implantation

To further confirm the efficacy of the peptide vaccine in suppression of tumor growth in the CT-26 cell implanted mice, survival of the tested mice was examined until 30 days after implantation. Control mice implanted with CT-26 cells without pre-immunization started to die after 22 days and all of the mice died after 30 days. The mice immunized with DOPE:CHEMS and TM4SF5 peptide died after CT-26 cell transplantation with a milder but similar pattern. However, only two among 12 mice immunized with a complex of TM4SF5 peptide and Lipoplex(O) died within 28 days after CT-26 cell transplantation (Fig. 4). Therefore, we can conclude that a complex of TM4SF5 peptide and Lipoplex(O) efficiently induced a protective immune response and inhibited growth of colon cancer cells *in vivo*.

4. Discussion

Finding the molecular signatures of cancers has important implications not only for understanding the progression of cancers but also for the design of cancer-specific prophylaxis or therapeutics [26]. As many of the molecular targets identified in tumors are located inside of the cells and are widely expressed in normal cells as well, targeted anti-tumor therapy has shortcomings to overcome, such as target accessibility and side effects. Therefore, targeting surface proteins that are specifically expressed in tumors but not in normal tissues is a strategy of choice to prevent or treat tumors with high efficacy and minimal side effects. Here, we validated a membrane protein TM4SF5 as a specific target for vaccination to prevent colon cancers in a mouse model.

Previously, we reported that vaccination with a complex of TM4SF5 peptide and Lipoplex(O) induces production of TM4SF5-specific antibodies and inhibits growth of HCC *in vivo* [21]. As mRNA of TM4SF5 is expressed in HCC as well as in colon cancers, we postulated that the same strategy can work in a colon cancer model. In this study, we found that TM4SF5 protein is expressed in the mouse colon cancer cell line CT-26 (Fig. 1). We also confirmed that the pre-immunization with a complex of TM4SF5 peptide and Lipoplex(O) induces production of TM4SF5-specific antibodies in response to colon cancer cell implantation (Fig. 2). Immunization induced a protective immune response against colon cancer progression, which resulted in reduced tumor growth and enhanced survival of mice after colon cancer cell implantation (Figs. 3 and 4). Therefore, TM4SF5 can be an efficacious target for anti-cancer therapy against HCC as well as colon cancers. Considering that colon cancers frequently metastasize to the liver [27], the peptide vaccine targeting TM4SF5 may have dual functions to control both primary and liver-metastasized colon cancers.

For peptide vaccines, carrier proteins are commonly used to provide general T cell epitopes and to enhance immune responses. However, we used a B cell epitope of TM4SF5 to target TM4SF5 protein *in vivo* without a conventional carrier. Instead, we used Lipoplex(O), consisting of natural phosphodiester bond CpG-DNA and liposome complex, for enhanced immunostimulatory activity and efficacious delivery into the cells [21,24,28]. Although we previously confirmed that antibody production by a peptide vaccine is dependent on the MHC, CD4, TLR9, and Th1 response [21,24], the mechanism of the vaccine to induce the antibody production must still be clarified. The antibodies produced by vaccination and CT-26 cell implantation may contribute to anti-cancer activity through functional disruption of TM4SF5 proteins in the colon cancer cells, as previously suggested for antibody-based cancer immnuotherapy targeting the tetraspanin CD151 [15].

Vaccination induces a humoral immune response as well as a cellular immune response, and the combined effects of these can induce protection against non-self or altered-self antigens [29]. Therefore, in addition to the TM4SF5-specific antibodies that we confirmed in this study, enhanced cellular immunity induced by the peptide vaccine may be involved in the prophylactic effects of the vaccine. Further study on the immune cell profiles may lead to a better understanding of the mechanisms involved in the vaccination effects.

Here, we examined the short-term protection effect of the vaccination from colon cancer cells implanted in mice. A memory function is an essential property of vaccines; a much faster and stronger immune response is induced by antigen challenge long after vaccination [29]. Especially for cancer vaccines, a memory function is required to prevent recurrence. In order to check whether the peptide vaccine can induce a suitable memory function, investigation of the long-term effects of the vaccine in terms of prophylaxis against colon cancer in a mouse model is necessary.

As we confirmed that the peptide vaccine has a significant prophylactic effect against colon cancer in this study, it is necessary to check its therapeutic effects in tumor-bearing mice in the near future. Furthermore, application of the TM4SF5-specific monoclonal antibody to treatment of colon cancers in a mouse model will yield more information for future therapeutics in humans.

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References

[1] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, CA Cancer J. Clin. 55 (2005) (2002) 74–108.

[2] V. Korinek, N. Barker, P.J. Morin, D. van Wichen, R. de Weger, K.W. Kinzler, B. Vogelstein, H. Clevers, Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC−/− colon carcinoma, Science 275 (1997) 1784–1787.

[3] N.P. Khan, A.A. Pandith, M.U. Hussain, A. Yousuf, M.S. Khan, K.A. Wani, S. Mudassar, Novelty of Axin 2 and lack of Axin 1 gene mutation in colorectal cancer: a study in Kashmiri population, Mol. Cell Biochem. 355 (2011) 149–155.
[4] A.R. Folsom, J.S. Pankow, J.M. Peacock, S.J. Bielinski, G. Heiss, E. Boerwinkle, Variation in TCF7L2 and increased risk of colon cancer: the Atherosclerosis Risk in Communities (ARIC) study, Diabetes Care 31 (2008) 905–909.
[5] J. Guo, T. Cagatay, G. Zhou, C.C. Chan, S. Blythe, K. Suyama, L. Zheng, K. Pan, C. Qian, R. Hamelin, S.N. Thibodeau, P.S. Klein, K.A. Wharton, W. Liu, Mutations in the human naked cuticle homolog NKD1 found in colorectal cancer alter Wnt/Dvl/beta-catenin signaling, PLoS One 4 (2009) e7982.
[6] W.S. Samowitz, J.A. Holden, K. Curtin, S.L. Edwards, A.R. Walker, H.A. Lin, M.A. Robertson, M.F. Nichols, K.M. Gruenthal, B.J. Lynch, M.F. Leppert, M.L. Slattery, Inverse relationship between microsatellite instability and K-ras and p53 gene alterations in colon cancer, Am. J. Pathol. 158 (2001) 1517–1524.
[7] S. Markowitz, J. Wang, L. Myeroff, R. Parsons, L. Sun, J. Lutterbaugh, R.S. Fan, E. Zborowska, K.W. Kinzler, B. Vogelstein, et al., Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability, Science 268 (1995) 1336–1338.
[8] O. Korchynskyi, M. Landstrom, R. Stoika, K. Funa, C.H. Heldin, P. ten Dijke, S. Souchelnytskyi, Expression of Smad proteins in human colorectal cancer, Int. J. Cancer 82 (1999) 197–202.
[9] K. Forrester, C. Almoguera, K. Han, W.E. Grizzle, M. Perucho, Detection of high incidence of K-ras oncogenes during human colon tumorigenesis, Nature 327 (1987) 298–303.
[10] P.G. Rychahou, L.N. Jackson, S.R. Silva, S. Rajaraman, B.M. Evers, Targeted molecular therapy of the PI3K pathway: therapeutic significance of PI3K subunit targeting in colorectal carcinoma, Ann. Surg. 243 (2006) 833–842 (discussion 843–834).
[11] M.M. Richardson, L.K. Jennings, X.A. Zhang, Tetraspanins and tumor progression, Clin. Exp. Metastasis 28 (2011) 261–270.
[12] K.R. Anderson, R.A. Singer, D.A. Balderes, L. Hernandez-Lagunas, C.W. Johnson, K.B. Artinger, L. Sussel, The L6 domain tetraspanin Tm4sf4 regulates endocrine pancreas differentiation and directed cell migration, Development 138 (2011) 3213–3224.
[13] T. Lekishvili, E. Fromm, M. Mujoomdar, F. Berditchevski, The tumour-associated antigen L6 (L6-Ag) is recruited to the tetraspanin-enriched microdomains: implication for tumour cell motility, J. Cell Sci. 121 (2008) 685–694.
[14] M. Zoller, Tetraspanins: push and pull in suppressing and promoting metastasis, Nat. Rev. Cancer 9 (2009) 40–55.
[15] J.F. Haeuw, L. Goetsch, C. Bailly, N. Corvaia, Tetraspanin CD151 as a target for antibody-based cancer immunotherapy, Biochem. Soc. Trans. 39 (2011) 553–558.
[16] F. Muller-Pillasch, C. Wallrapp, U. Lacher, H. Friess, M. Buchler, G. Adler, T.M. Gress, Identification of a new tumour-associated antigen TM4SF5 and its expression in human cancer, Gene 208 (1998) 25–30.
[17] S.A. Lee, S.Y. Lee, I.H. Cho, M.A. Oh, E.S. Kang, Y.B. Kim, W.D. Seo, S. Choi, J.O. Nam, M. Tamamori-Adachi, S. Kitajima, S.K. Ye, S. Kim, Y.J. Hwang, I.S. Kim, K.H. Park, J.W. Lee, Tetraspanin TM4SF5 mediates loss of contact inhibition through epithelial-mesenchymal transition in human hepatocarcinoma, J. Clin. Invest. 118 (2008) 1354–1366.
[18] S.A. Lee, Y.M. Kim, T.K. Kwak, H.J. Kim, S. Kim, W. Ko, S.H. Kim, K.H. Park, M. Cho, J.W. Lee, The extracellular loop 2 of TM4SF5 inhibits integrin alpha2 on hepatocytes under collagen type I environment, Carcinogenesis 30 (2009) 1872–1879.
[19] S.A. Lee, H.W. Ryu, Y.M. Kim, S. Choi, M.J. Lee, T.K. Kwak, H.J. Kim, M. Cho, K.H. Park, J.W. Lee, Blockade of four-transmembrane L6 family member 5 (TM4SF5)-mediated tumorigenicity in hepatocytes by a synthetic chalcone derivative, Hepatology 49 (2009) 1316–1325.
[20] S.A. Lee, K.H. Park, J.W. Lee, Modulation of signaling between TM4SF5 and integrins in tumor microenvironment, Front Biosci. 16 (2011) 1752–1758.
[21] S. Kwon, D. Kim, B.K. Park, S. Cho, K.D. Kim, Y.E. Kim, C.S. Park, H.J. Ahn, J.N. Seo, K.C. Choi, D.S. Kim, Y. Lee, H.J. Kwon, Prevention and therapy of hepatocellular carcinoma by vaccination with TM4SF5 epitope-CpG-DNA-liposome complex without carriers, PLoS One 7 (2012) e33121.
[22] S. Kwon, D. Kim, B.K. Park, G. Wu, M.C. Park, Y.W. Ha, H.J. Kwon, Y. Lee, Induction of immunological memory response by vaccination with TM4SF5 epitope-CpG-DNA-liposome complex in a mouse hepatocellular carcinoma model, Oncol. Rep. 29 (2013) 735–740.
[23] D. Kim, J.W. Rhee, S. Kwon, Y.E. Kim, S.Y. Choi, J. Park, Y. Lee, H.J. Kwon, Enhancement of immunomodulatory activity by liposome-encapsulated natural phosphodiester bond CpG-DNA in a human B cell line, BMB Rep. 43 (2010) 250–256.
[24] D. Kim, S. Kwon, J.W. Rhee, K.D. Kim, Y.E. Kim, C.S. Park, M.J. Choi, J.G. Suh, D.S. Kim, Y. Lee, H.J. Kwon, Production of antibodies with peptide-CpG-DNA-liposome complex without carriers, BMC Immunol. 12 (2011) 29.
[25] H. Yoshiji, S. Kuriyama, M. Kawata, J. Yoshii, Y. Ikenaka, R. Noguchi, T. Nakatani, H. Tsujinoue, H. Fukui, The angiotensin-I-converting enzyme inhibitor perindopril suppresses tumor growth and angiogenesis: possible role of the vascular endothelial growth factor, Clin. Cancer Res. 7 (2001) 1073–1078.
[26] M.D. Bacolod, F. Barany, Molecular profiling of colon tumors: the search for clinically relevant biomarkers of progression, prognosis, therapeutics, and predisposition, Ann. Surg. Oncol. 18 (2011) 3694–3700.
[27] D.J. Gallagher, N. Kemeny, Metastatic colorectal cancer: from improved survival to potential cure, Oncology 78 (2010) 237–248.
[28] D. Kim, H.J. Kwon, Y. Lee, Activation of Toll-like receptor 9 and production of epitope specific antibody by liposome-encapsulated CpG-DNA, BMB Rep. 44 (2011) 607–612.
[29] A. Abbas, A. Lichtman, S. Pillai, Cellular and Molecular Immunology, sixth ed., Saunders, Elsevier, Philadelphia, 2007.