PRODUCTION OF POLYCLONAL ANTIBODIES FOR DETECTING HUMAN TYPE 2 TRANSGLUTAMINASE VARIANTS IN COLON CANCER

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Abstract. Type 2 transglutaminase (TGM2) is a multifunctional ubiquitous protein, involving in protein cross-linking, apoptosis, and cell differentiation. Recently, some reports have suggested that TGM2 expression is a potential prognostic marker, and often associates with advanced stages of disease, metastatic spread, as well as drug resistance in many cancer cell lines although its primary function is unknown. To elucidate the role of TGM2 in cancer, the expression profile of the TGM2 need to be examined. In this study, new polyclonal antibodies detecting four TGM2 variants encoding protein from ENSEMBL database are produced and their specificities are confirmed by western blot analysis with *E.coli* overexpressing TGM2-002 protein, HEK293T cells overexpressing TGM2-S protein and human colon cancer samples. Western blot data showed that these antibodies could detect not only TGM2-002 in *E. coli* overexpressing TGM2-002 but also smaller molecular weight protein (about 62 kDa) in HEK293T overexpressing TGM2-S cells which was further confirmed by MALDI-TOF analysis. We found that both TGM2-1 and TGM2-4 antibodies could detect the full length TGM2-002 protein in colon cancer samples. Furthermore, in normal sample, we found that majority of TGM2-002 protein existed in membrane fraction but not in total lysate whereas TGM2-002 protein was found in both total lysate and membrane fraction in colon cancer samples.

Keywords. antibody, colon cancer, HEK293T, type 2 transglutaminase

1. INTRODUCTION

Transglutaminase is a family of enzymes that catalyze a calcium dependent cross-linking reaction between γ-carboxamide group of a polypeptidebound glutamine residue and the ε-amino group of polypeptide-bound lysine residue to form a ε-(γ-glutamyl)lysine isopeptide bond or catalyze the incorporation of primary amines at selected peptide-bound glutamine residues to form a (γ-glutamyl)polyamine bond. In mammals, eight distinct transglutaminase isoenzymes have been identified in genomic level but only six isoenzymes have been isolated and characterized in protein level: factor XIII-A subunit, which has the role in stabilize the fibrin clot, keratinocyte TG (named as type 1 transglutaminase) and epidermal/hair follicle transglutaminase (type 3 transglutaminase), both of which are involved in terminal differentiation of the keratinocytes, type 2 transglutaminase (named as TGM2 or tissue transglutaminase), prostatic secretory transglutaminase (type 4 transglutaminase), which related in fertility in rodents and type 5 transglutaminase.

Among six isoenzymes, TGM2 is the most ubiquitous member of transglutaminase family. Due to transglutaminase activity, extracellular TGM2 binds and cross-links numerous components of extracellular matrix (ECM) (1), some evidences suggest that TGM2 cross-linking renders the ECM resistance to mechanical and proteolytic degradation and stabilizes cell-ECM interaction and wound healing. Furthermore, TGM2 acts as a G protein in trans-membrane signaling pathway (2, 3) and as a cell surface adhesion/cell migration mediator that is delineated from cultured cell studies (4, 5, 6). TGM2 involvement in a large number of human diseases, such as neurodegenerative disease, autoimmune condition (coeliac disease), inflammation, tissue fibrosis and cancer might be fruitfully consulted from other reviews (7, 8). Furthermore, recent studies have reported that TGM2 highly expresses in many cancer cell lines and exerts antiapoptotic as well as anti-anoikis pro-survival effects (9, 10). TGM2 antiapoptotic activities can be explained that TGM2 serves as an inhibitor of apoptosis by cross-linking the caspase 3 (11) or by activating of some survival pathways, such as NF-κB pathway (12). Moreover, another mechanism can be explained that TGM2 protects retinoblastoma (Rb) protein, a well-known

regulatory of G1/S checkpoint of cell cycle, from degradation (13), or enhances the association of fibronectin and integrin on EMC and activates cell survival pathways. Finally, TGM2 expression also involves in inducing the epithelial-to-mesenchymal transition process which is associated with tumor aggressiveness and metastatic potency (14). Thus TGM2 expression has been considered as a potential prognostic marker and associated with advanced stages of disease, metastatic spread and drug resistance in breast cancer cells (15, 16) or lung cancer cells (17), in ovarian cancer cells (18, 19), in glioblastoma (20).

ENSEMBL (http://www.ensembl.org/), the joint project between EMBLEBI and Welcome Trust Sanger Institute develops software system which produces and maintains automatic annotation on selected eukaryotic genomes, records nine tgm2-transcripts in human. Among these transcripts, four transcripts which named as *tgm2-002*, *tgm2-004*, *tgm2-201*, *tgm2-202*, encode polypeptides of 687, 281, 606, 627 amino acids, respectively. The molecular mass of full-length TGM2-002 protein, the product from *tgm2-002* transcript, is about 81 kDa. Its structure consists of 4 domains: N-terminal β- sandwich, core domain that contains active-site triad involving Cys^{277}, His^{335} and Asp^{358}, C-terminal β-barrel 1 and 2. Precursor TGM2 mRNA is also alternatively spiced to generate a short form mRNA that encodes a 548 amino acids polypeptide with an approximate molecular mass of 62 kDa and named as TGM2-S via exon skipping and intron retention (21,22). This truncated form of TGM2 protein shares N-terminal 538 amino acids with full-length TGM2 but it has different 10 amino acids and lacks C-terminal GTP binding regulatory domain (Arg580 residue) and usually detected in Alzheimer's patient (21). TGM2-S protein exerts a different cellular role in the cell with full length TGM2 protein. For an example, full-length TGM2 expression in fibroblasts involves with anti-apoptotic activities but expression of TGM2-S induces an apoptotic response in fibroblasts (21). Additionally, in neuroblastoma, full-length TGM2 protein acts as repressor of cell differentiation but the short form TGM2-S induces cell differentiation (20). TGM2 variants might confer growth and survival advantages, thus these variants might preferentially express in cancer cell lines or the expression of TGM2 variants just simply is a consequence of cellular alternations of cancer cells, but in that case expression of TGM2 variants also acts as a biomarker for diagnostic or therapeutic purposes in cancer (23, 24). A recent study has first time provided evidence to demonstrate that TGM2 is a novel marker for colon cancer (25), the neoplastic disease has greatly increased in recent years (26). Therefore, it is important to survey the expression of these TGM2 variants in colon cancer. Unfortunately, expression of TGM2 variants has never been systematically addressed in colon cancer, at least in protein level because of lacking the tool to detect TGM2 variants. The aim of this study is to producce polyclonal antibodies for detecting TGM2 variants in colon cancer.

2. MATERIALS AND METHODS

2.1 Polyclonal antibody production

The peptide sequences were designed from alignment results and antigenic determinant regions from the Hopp-Woods scales, Kyte-Doolittle hydropathy plots and Emini surface probability algorithms (Gene Runner software version 3.05). For conjugation, peptides were modified by adding cysteine residue at the end of peptides as **C**-FTRANHLNKLAEKEE, **C**-LETNGRDHHTA and **C**- AGTKARFPLRD, except GRDCSRRSSP. Four peptide sequences as C-FTRANHLNKLAEKEE, GRDCSRRSSP, C-LETNGRDHHTA and C-AGTKARFPLRD were purchased from Anygen Company, Korea and subcutanously injected into female BALB/cJ mice for producing the antibodies named as TGM2-1, TGM2-2, TGM2-3, TGM2-4, respectively (Table 1). Peptides (2.5 mg/5 mice) were coupled to 5 mg Keyhole Limpet Hemocyanin (KLH, #77600, Pierce Biotechnology, USA) by incubating overnight at 4^{o}C in the presence of 1.25 mg sulfo-SMCC (#22122, Pierce Biotechnology, USA) in 1.5 mL 1x PBS (Biosesang, Korea), and stored in -80 ^{o}C. KLH conjugated peptides (0.83 mg/5 mice) were mixed with 500 µl Freund complete adjuvant (F5881, Sigma-Aldrich, USA) for 5 hours to produce an emulsion mixture. The emulsion mixture was divided and subcutaneously injected into the mice. After 2 weeks, the mice were booster injected repeatedly 3 times at 1-week interval. KLH conjugated peptides (0.55 mg/5 mice) were freshly mixed with 500 µl Freund incomplete adjuvant (F5506, Sigma-Aldrich, USA) to produce an emulsion mixture for booster injection before one day. One week after last injection, blood was collected into 1.5 mL microcentrifuge tube and stood the tube in room temperature for 1 hour. The serum was

separated from blood cell debris by centrifuging at 4,000 rpm in 4 ^{o}C for 20 minutes. The supernatant was centrifuged at 13,200 rpm in 4^{o}C for 20 minutes, after which serum was collected and stored in -20^{o}C until used.

2.2 Overexpression of TGM2-002 protein in *E. coli*

The TGM2-002-C277S/pET28b (NCBI Reference Sequence: NM_004613) was offered by Dr. Kim Soo Youl, National Cancer Center, Korea. The TGM2- 002-C277S/pET28b was transformed into *Escherichia coli* (*E. coli*) BL21 strain via heat shock method. After induction with 1 mM isopropyl β-D-1- thiogalactopyranoside (IPTG, I100B, Biosesang, Korea), induced *E. coli* was incubated at 37 ^{o}C in shaking incubator for 3 hours. Subsequently, 1 mL *E. coli* cultured liquid was centrifuged and pellet was collected into 1.5 mL microcentrifuge tube. 200 µL lysis buffer (50 mM Tris, pH 8.0, 1 mM EDTA) supplemented with protein inhibitors (0.1 mM AEBSF, 2 µg/mL leupeptin, 1 µg/mL aprotinin, 1 µg/mL pepstatin A and 0.1 mM PMSF) was added into the tube and the samples were sonicated on ice. Cell lysate from non-induced E.coli (without IPTG) was used for control. Cell lysate from induced *E. coli* was separated into soluble and insoluble fractions by centrifugation. Protein concentrations of cell lysate, soluble and insoluble fraction were determined by BCA Protein assay kit (#23227, Pierce Biotechnology, USA).

Table 1. The TGM2 variants polyclonal antibodies

Antibody name / Peptide / variant	TGM2-1 CFTRANHLN KLAEKEE	TGM2-2 GRDCSRRSSP	TGM2-3 CLETNGRDH HTA	TGM2-4 CAGTKARFP LRD	Predictive size (kDa)
TGM2-002	O	O	O	O	81
TGM2-202	O	O	-	O	70
TGM2-201	O	O	O	-	68
TGM2-004	-	O	O	O	30
TGM2-S	O	O	O	O	62

The aim of this study focuses in producing antibodies that named as TGM2-1, TGM2-2, TGM2-3, TGM2-4 antibodies to detect protein coding TGM2 variants, except for TGM2-003 protein. Blank region means that this TGM2 variant cannot be detected by upper peptide recognized antibody, the character "O" means predictive positive detection.

2.3 Overexpression of TGM2-S protein in HEK293T cells

The TGM2-S/pCR3.1 (NCBI Reference Sequence: BC003551) was a kindly gift from Dr. Lee Tae Hoon, School of Dentistry, Chonnam National University, Korea. HEK293T cells were maintained in Dulbecco Modified Eagle media (Gendepot, USA) supplemented with 10% fetal bovine serum (Gendepot, USA) and 1% penicillin-streptomycin (Gendepot, USA). Media were changed twice or three times per week. Once the cells reached 70-80% confluence, *tgm2-S*/pCR3.1 or pCR3.1 control vector was transfected into the cells by lipofectamine 2000 reagent (#11668019, Invitrogen, USA). At 48 hours after transfection, the cells were washed twice with 1x PBS (Biosesang, Korea) and collected by adding RIPA buffer (50 mM Tris, pH 8.0 with 150 mM sodium chloride, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate) supplemented with protease inhibitors. After that, samples were sonicated on ice and followed by centrifuging at 13,200 rpm and 4^{o}C for 30 minutes. The supernatant was collected and transferred into new 1.5 mL microcentrifuge tube, Protein concentrations of cell lysate was determined by BCA Protein assay kit (Pierce Biotechnology, USA)

2.4 Protein idetification using MALDI-TOF analysis

For verification of TGM2-S variant, gel was excised and digested with trypsin (V5280, Promega, USA) in α-cyano-4-hydroxycinnamic acid in 50% acetonitrile /0.1% trifloroacetic acid, and then subjected to MALDITOF analysis (Ettan MALDI-TOF Pro, GE Healthcare, USA). Spectra were collected from 350 shots per spectrum over an *m/z* range of 600-3000 and calibrated by two-point internal calibration using auto-digestion peak (*m/z* 842.5099, 2211.1046). Peak list was generated using the Ettan MALDI-TOF Pro Evaluation Module (version 2.0.16). The threshold used for peak-picking was as follows: 5000 for minimum resolution of monoisotopic mass, 2.5 for *S/N* of all proteins in the NCBI database using the MASCOT search program (version 2.3), developed by The Matrixscience (http://www.matrixscience.com/). The following parameters were used for the database search: trypsin as the cleaving enzyme, a maximum of one missed cleavage, iodoacetamide (Cys) as a fixed modification, oxidation (Met) as a variable modification, monoisotopic masses, and a mass tolerance of ± 0.1Da. Protein score is -10*Log (*p*), where *p* is the probability that the observed match is a random event, and greater than 75 is significant ($p<0.05$).

2.5 Protein extraction of colon cancer samples

Colon cancer samples were obtained with informed consent from the Chonnam National University Hwasun Hospital–National Biobank of Korea. Briefly, the tissue samples from healthy individuals and from stage II as well as stage III tumor were obtained from resected specimens within 30 minutes after surgical resection, snap frozen in liquid nitrogen, and stored at -80°C. All tissue samples were from unique patients. Numeric specimen codes were used to protect the identity of the patients. Detailed clinicopathologic information for all tissue specimens are summarized in Table 2.

For total lysate extraction, frozen tissue samples were homogenized in 1 mL of pre-chilled lysis buffer containing 20 mM Tris, pH 7.5, 150 mM sodium chloride, 1 mM EDTA, 1 mM EGTA, 1% Triton-100, 2.5 mM Sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin, 1mM PMSF and circulated for overnight at 4°C. Samples were sonicated on ice and centrifuged for 10 minutes in 13,000 rpm at 4°C.

For membrane fraction extraction, frozen tissue samples were homogenized in 800 µL of pre-chilled buffer (1x PBS, pH 7.4) supplement with protein inhibitor mixture and incubated for 30 minutes at 4°C. To remove the aqueous fractions containing lipids and soluble proteins, the homogenized samples were centrifuged for 30 minutes at 5,000 rpm and 4°C. The pellet was then washed 5 times with pre-chilled 1x PBS and centrifuged for 10 minutes at 13,000 rpm at 4°C, after which the pellet was re-suspended in 200 µL urea buffer (10 mM Tris–HCl, pH 7.4, 7 M urea, 2 M thiourea, 0.49% CHAPS) supplemented with protease inhibitors and samples were sonicated on ice through 5 cycles in 10% amplitude, each cycle consisting of 5 seconds of sonication followed by a 5 seconds break. The samples were then centrifuged at 13,000 rpm for 30 minutes and 4 °C, the supernatant was collected, and the protein concentration of each sample was determined using Bradford assay (Bio-Rad Laboratories, USA).

2.6 Western blot analysis

The same amount of proteins (50µg) were resolved by polyacrylamide gel electrophoresis and transferred onto PVDF membrane (Bio-Rad Laboratories, USA). Subsequently, membranes were blocked with 5% skim milk (BD Bioscience, USA) or 5% bovine serum albumin (Biosesang, Korea), and washed with washing buffer (25 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.4) for 3 times. For first antibody binding, membranes were gently shaken in laboratory made antibodies (TGM2-1, TGM2-2, TGM2-3, and TGM2-4 antibodies, dilution ratio 1:1000 in blocking solution) or commercial TGM2 antibody (TGM2-C antibody, MS-300-P1, Labvision, USA, dilution ratio 1:400 in 5% skim milk), beta actin antibody (A5441, dilution ratio 1:5000 in 5% skim milk) at 4 °C for overnight, followed by washing for 3 times. After that, the membranes were gently shaken with anti-mouse immunoglobulin G horseradish peroxidase linked antibody (#7076, Cell Signaling, USA, dilution ratio 1:2000 in 5% skim milk), and followed by washing for 3 times. The membranes were incubated with West-one solution (#16033, Intron Biotechnology, Korea) for 1 minute before observation by ImageQuant LAS-4000 machine (GE Healthcare, UK).

3. RESULTS AND DISCUSSION

3.1 Confirmation antibodies specificities by western blot analysis in *E. coli* BL21 overexpressing TGM2-002

The method for expressing hexa-histidine-tagged human tissue transglutaminase in *E. coli* BL21 with pET system was described in previous report (27) In this study, the TGM2 protein was produced in a high yield but majority of protein was found in the insoluble fraction of the *E. coli* lysate, as judged by SDS–PAGE (Figure 1A). This result was similar to previous report (27), over 90% induced human TGM2 protein exists in inclusion body. Based on this result, insoluble fraction of TGM2 overexpressed *E.coli* was chosen as positive sample for western blot analysis, whereas total lysate of non-induced sample was chosen as negative control.

To confirm the laboratory-made antibodies specificities, 5 μg of positive or negative control was loaded on SDS gel, and then transferred onto PDVF membrane. The membranes were blocked by 5% skim milk and incubated with TGM2-1, TGM2-2, TGM2-3, TGM2-4. Western analysis results showed that all antibodies could detect the TGM2-002 protein (~81 kDa) with different ability. In standard resolution mode, TGM2-1 and TGM2-4 antibodies could detect the TGM2-002 protein band, but TGM2-3 antibody could only detect that band in high resolution mode and TGM2-3antibody only can detect that in super resolution mode (Figure 1B, C, D, E).

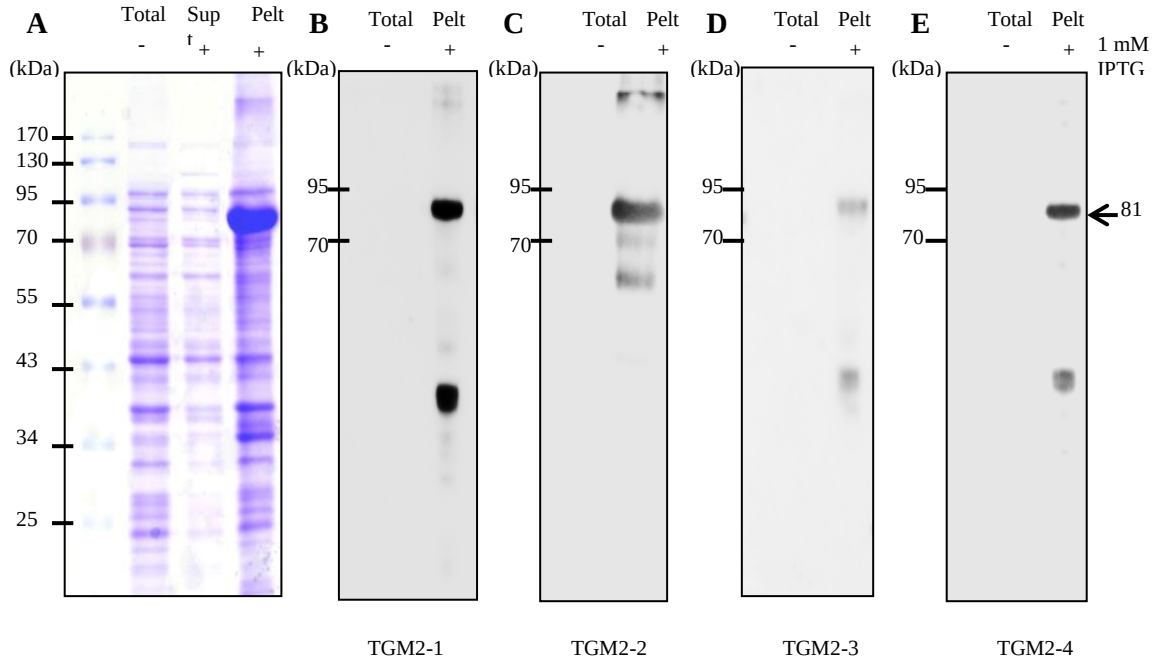


Figure 1. Overexpression and detection human TGM2-002 protein in TGM2-002 transfected E.coli samples. Human TGM2-002 C277S protein was successfully overexpressed in E. coli and existed as a dark band in 81 kDa when the gel was stained with Coomassie blue R250, majority of TGM2-002 protein formed inclusion body (A). To confirm laboratory made TGM2 antibodies, insoluble fraction of overexpressed TGM2 E. coli lysate was used as positive control. The membranes were detected via western blot analysis using TGM2-1 (B),TGM2-2 (C),TGM2-3 (D), TGM2-4 antibodies(E). These results showed that all antibodies could detect the TGM2-002 protein. Arrow indicates the predicted molecules (81 kDa). Total lysate (total), supernatant (supt), pellet (pelt).

3.2 Confirmation antibodies specificities by western blot analysis in HEK293T cells overexpressing TGM2-S

To confirm the laboratory-made antibodies specificities, 50 μg of TGM2 overexpressed HEK 293T cell lysate or pCR3.1 vector transfected HEK 293T cell lysate was loaded on SDS gel, followed by transferring onto PDVF membrane. The membranes were blocked by 5% skim milk and incubated with TGM2-1, TGM2-2, TGM2-3,-TGM2-4 antibodies. These results showed detective abilities of antibodies

were different. Among 4 antibodies, TGM2-1 and TGM2-4 antibodies detected a very strong band in 62 kDa, whereas TGM2-2 and TGM2-3 antibodies could not detect that band (Figure 2). To confirm that TGM2 was overexpressed in HEK 293T cell line, TGM2 commercial antibody was used to detect the band. TGM2 commercial antibody showed that TGM2 was overexpressed in transfected HEK 293T cell and the TGM2 band in same molecular weight with the band detected by our laboratory-made antibodies. The membranes were re-probed with beta-actin to confirm the same amount of protein was loaded.

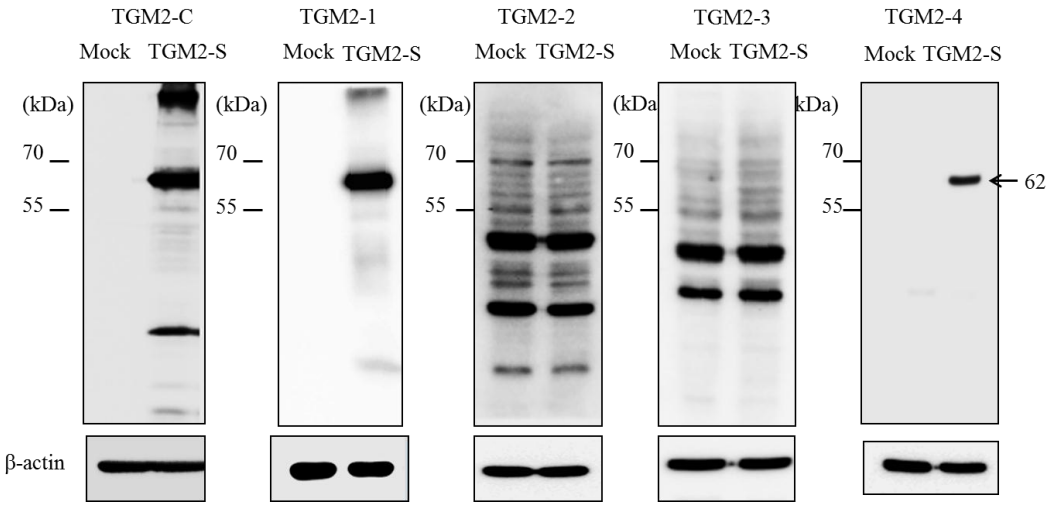
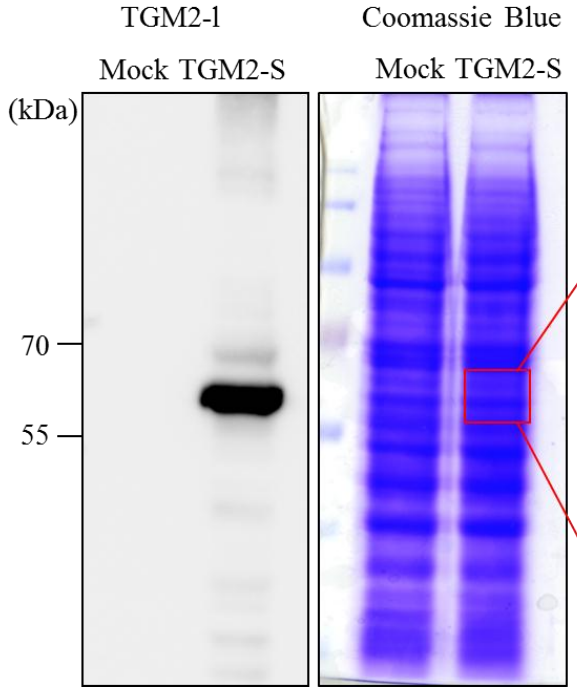


Figure 2. Overexpression and detection human TGM2-S variant in HEK293T cells overexpressing TGM2-S. The samples (mock and human TGM2-S overexpressing cell lysate) were detected by western blot analysis using TGM2-C (TGM2 commercial antibody), TGM2-1, TGM2-2, TGM2-3, and TGM2-4 antibodies. Human TGM2-S was successfully overexpressed in HEK293T cells and appeared as a band in 62 kDa, this result was confirmed by western blot analysis using TGM2 commercial antibody. TGM2 transfected HEK293T cell lysate was used as positive control. Among 5 antibodies, TGM2-C, TGM2-1 and TGM2-4 antibodies strongly detected the TGM2-S protein which has molecular weight about 62 kDa, whereas TGM2-2 and TGM2-3 antibodies could not detect that protein . Arrow indicates the predicted molecules (62 kDa)

3.3 Protein identification by MALDI-TOF analysis

For verification of the detected band by laboratory-made antibodies, 200 μg of protein from transfected HEK293T cell lysate was loaded into SDS-polyacrylamide gel. The experiment was done in duplicate, one gel for western blotting and one gel for the protein identification. Using western blotting analysis for band detection, the correspondent region in gel was collected, chopped into small pieces, followed by extracting and recovering protein, an then performed MALDI-TOF analysis. There are total 57 queries matched with TGM2-S protein sequence, protein scored 365 and 8 queries scored over 37, probability based Mowse scores (Figure 3). Due to this result, we could confirm that the band detected by TGM2-1 and TGM2-4 antibodies was TGM2-S protein.



Query	Score	Expect
SLIVGLK	39	1.3e-04
SLIVGLK	38	1.6e-04
NEFGEIQGDK	42	6.7e-05
SEGTYCCGPGPVPVR	40	1.0e-04
YLLNLNLEPFSGK	63	4.8e-07
YLLNLNLEPFSGK	57	2.2e-06
YLLNLNLEPFSGK	49	1.4e-05
VVTNYNSAHDQNSNL LIEYFR	37	2.2e-04

Figure 3. Confirmation TGM2-S in HEK293T overexpressing TGM2-S samples. To confirm the band detected by TGM2-1 and TGM2-4 antibodies is the TGM2-S variant, 200µg total protein was loaded into 12% SDS gel, experiment was duplicated, one set to detect by western blotting, one set for detect by staining with Coomassie blue (R250), the correspond region of the band detected by TGM2-1 antibody was excised and send to MALDI-TOF analysis. Right box indicates the highly matched queries ($p<0.05$), probability based Mowse score. Mowse scores in query are more than 35: homology. more than 40: identity, whereas in protein if Mowse scores are over 75: 95% significant identification. There are total 57 queries matched with TGM2-S protein sequence, protein scored 365 and 8 queries scored over 37, probability based Mowse scores. Due to this result, we confirmed that the band detected by TGM2-1 and TGM2-4 antibodies was TGM2-S variant

3.4 Primary evaluation TGM2 variants in colon cancer samples

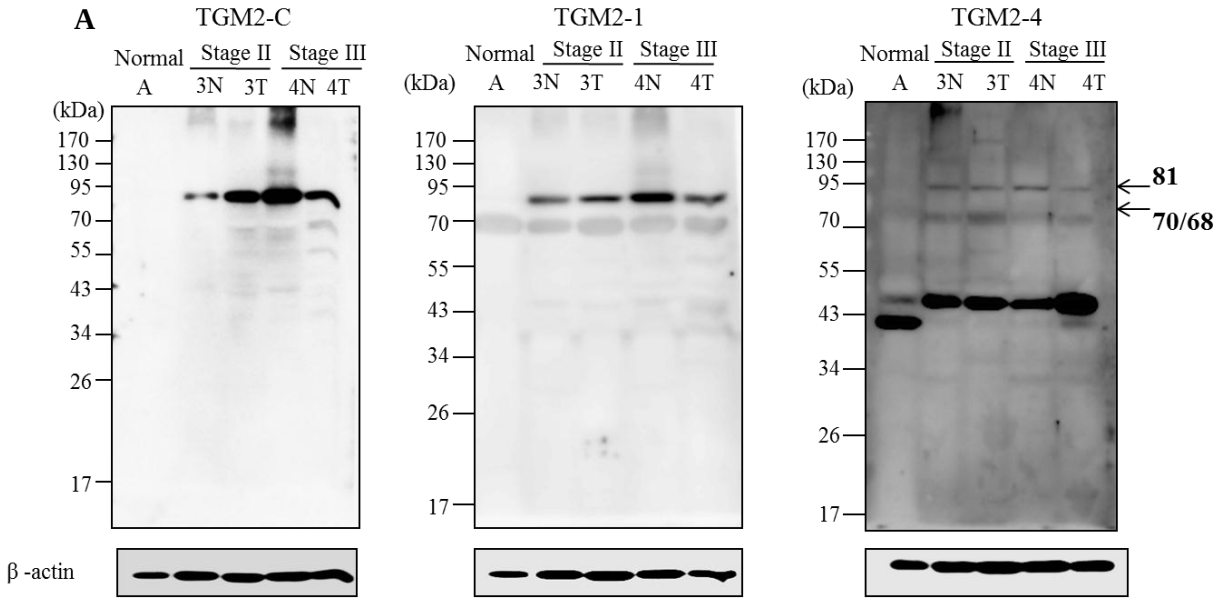
In previous study, TGM2-002 protein (molecular weight ~81 kDa) was strongly expressed in colon cancer (25). The western blot results showed that TGM2-002 expression in normal samples was lower than cancer ones, and TGM2-002 protein could only be detected in membrane fraction, not in total lysate fraction of normal tissue. On the contrary, colon cancer sample gave a clear band in both total lysate fraction and membrane fraction. In addition, TGM2-002 expression also related with cancer progress, TGM2-002 protein expression in adjacent tissue from stage III cancer patients was higher in adjacent tissue from stage II cancer patients in both total lysate and membrane fraction

Both of TGM2-1 and TGM2-4 antibodies could detect one band which had same molecular weight with the full length TGM2-002 protein. In theory, TGM2-1 antibody could detect more two bands of TGM2-201 variant and TGM2-202 variant which have molecular weight about 68 and 70 kDa, respectively. However, TGM2-1 antibody only detect one band which has molecular weight about 68 ~70 kDa in total lysate fraction of colon cancer samples. In total lysate fraction of colon cancer samples, TGM2-4 could detect the band in 30 kDa, the predicted molecular weight of TGM2-004 variant and the band in 70 kDa the predicted molecular weight of TGM2-202 variant. Interestingly, the 70kDa protein did not exist in both total lysate and membrane fraction of normal samples. These results suggested the 70kDa might be a potential marker for colon cancer, but this molecule should be studied further to identify, determine prevelance in larger number of patients, and the function of this molecule in cellular process.

Table 2. Clinical information of the sample from colon cancer patients

No	Age	Gender*	Colon site	Tumor stage	Pathologic grade	TNM**
1	64	M	Hepatic flexure	II	Poor	T3N0M0
2	86	F	Hepatic flexure	II	Poor	T3N0M0
3	66	M	Sigmoid	II	Well	T3N0M0
4	64	M	Rectum	III	Moderate	T3N2M0
5	76	M	Sigmoid	III	Well	T3N1M0
6	64	M	Sigmoid	III	Moderate	T3N1M0

**Gender: M: Male, F: Female.*
***TNM: In TNM system (Tumor, node, metastasis) system, cancer is evaluated and given a score based on the extent of the tumor, the extent of spread to the lymph nodes, and the presence of metastasis. In briefly, cancer is given the TNM score with T1–T4 (depended on size and extent of the primary tumor), N0 (no regional lymph node involvement) or N1-N3 (involved with regional lymph nodes), M0 (no distant metastasis) or M1 (distant metastasis).*



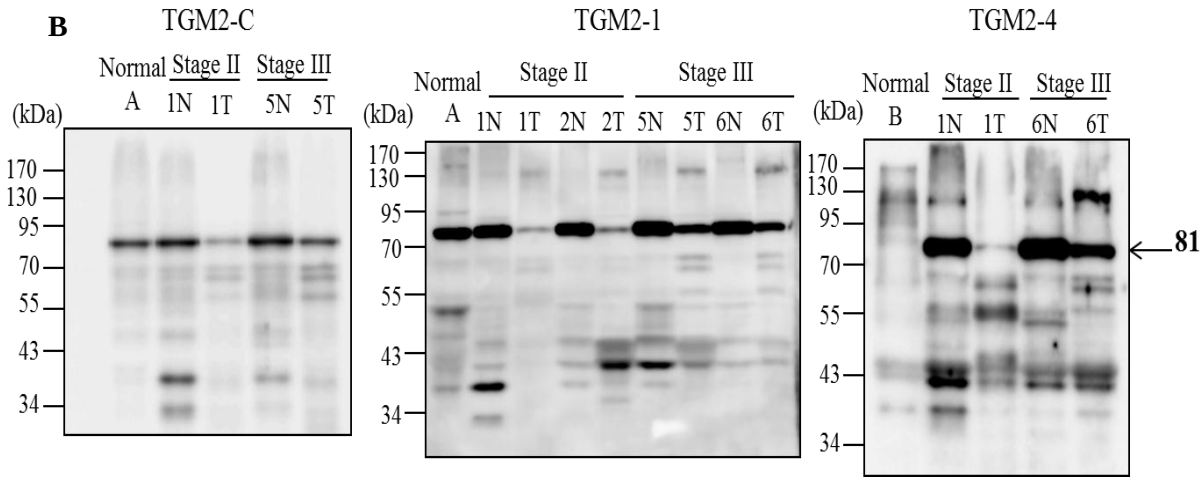


Figure 4. Preliminary evaluation of TGM2 variants expression in total lysate (A) and membrane fractions (B) of colon cancer samples by Western blot analysis. TGM2-002 protein is strongly expressed in colon cancer and also increases in cancer progress. TGM2-C, TGM2-1 and TGM2-4 antibodies detected one band which has molecular weight about 81 kDa , predictive size of TGM2-002 protein in both total lysate (A) and membrane fraction (B) of colon cancer samples. α-TGM2-4 can detect one band in 70 kDa, expecting size of TGM2-202 variant in total lysate fraction of cancer samples, which does not exist in total lysate (A) and membrane fraction (B) in normal samples. In normal case, TGM2-002 expression is lower than cancer and TGM2-002 protein only exists in membrane fraction (B). Arrow indicates the predicted molecules (81, 70, 68 kDa). Normal samples were collected from colons of 2 healthy individuals, named as normal A and normal B. Cancer samples were collected form colon cancer patient, named as No-N or No T. The numbers was used to protect the identity of the patients, the character "N" or "T" indicates that the samples were collected from adjacent normal tissues or tumor tissues, respectively.

4. CONCLUSIONS

In this study, TGM2 polyclonal antibodies for detecting human TGM2 variants are successfully produced. TGM2-1 and TGM2-4 antibodies can detect the full-length TGM2-002 protein in colon cancer samples. Furthermore, TGM2-002 is strongly expressed in colon cancer sample in both total lysate and membrane fraction and is related with cancer progress. TGM2 polyclonal antibodies need to further investigation for determine TGM2 variants expression as potential biomarker of colon cancer patients.

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