Original Article
Novel antibody against TMX2 and its effects on breast cancer cells

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Abstract: Although monoclonal antibodies are promising, a truly fully human antibody is yet to be produced. Current human antibodies have the human sequence, but are produced in either transgenic animals or in phages. The aim of this paper was to produce a truly human antibody directed against an epitope of our choice, secreted by human plasma cells. The target protein was TMX2 one of the least studied disulfide isomerases. IgG and anti-TMX2 antibody were determined by both Elisa and western blot. TMX2 KD was evaluated by Surface Plasmon Resonance. TMX2 localization was determined by flow cytometry in MCF-7 cells. Efficiency was evaluated by MTT. Gene expression was evaluated by PCR. We have managed to produce two fully human antibodies directed against TMX2 protein. TMX2 protein was found both in the cytoplasm and cell membrane of breast cancer cells. RGCC TMX2 antibody recognizing an extracellular epitope increased cell proliferation. RGCC TMX2 antibody recognizing an intracellular epitope decreased cell proliferation and gene expression related to cancer survival, differentiation and metastasis. These findings suggest this platform is very promising for novel personalized therapies. TMX2 could be a novel target for cancer treatment.

Keywords: Antibody, human, TMX2, proliferation, cancer

Introduction

Monoclonal antibody therapy for cancer treatment has been increasingly used over the last years. Due to their target specificity and their increased half-life they are considered a very promising type of therapy. Antibodies can be used to apply a direct hit on cancer cells-such as growth signal inhibition or apoptosis initiation-but can also mediate immune effector function [1]. First generation antibodies were produced in mice and therefore could induced allergic responses frequently [2]. Second generation antibodies were chimeric, where murine variable domains were fused to the human constant domains at a ratio of 30%-70% respectively [3]. Advances in antibody engineering lead to humanized antibodies where the human percentage increased to 80%-90%. Most of the approved therapeutic monoclonal antibodies on the market today are either chimeric or humanized and stimulate the human immune system to produce anti-drug antibodies (ADA) [4] . Human antibodies are considered to be the new generation. However, this term is misleading since the human part refers to the sequence and not the host. They are produced either using phage technology or transgenic animals. Phage display technology however, can introduce post-translational modifications that do not occur in humans and therefore can cause immunogenicity [5]. Transgenic animal technology on the other hand, is limited to the production of antibodies that the immune system of the animal is able to recognize. Therefore, there is a high demand on the field for better antibody producing methods. In Research Genetic Cancer Centre (RGCC) we have developed a new platform for the production of truly human antibodies produced from autologous blood cells of the patients. This platform was tested for the production of a fully human antibody against thioredoxin related transmembrane protein 2 (TMX2), using human whole blood cells *ex-vivo*.

TMX2 is a member of the disulfide isomerase (PDI) family and thioredoxin (Trx) superfamily of

a group of proteins that are found in the endoplasmic reticulum (ER) of cells. As such, it is considered to be cytoplasmic. Their role is to catalyze protein folding and thiol-disulfide interchange reactions through redox regulation. Disulfide bond formation is critical for correct protein folding [6] and the PDI family of proteins is responsible for their formation and rearrangement. TMX2 protein consists of an N-terminal ER-signal sequence, a transmembrane domain, a Myb DNA-binding domain repeat signature, a catalytically active thioredoxin domain, and a C-terminal ER-retention sequence [7]. Among the numerous transmembrane-bound proteins of this family, TMX2 is poorly characterized, and the lack of a CXXC active site sequence indicates that TMX2 does not play a role as a thiol-disulfide oxidoreductase [8]. However, further formation about the biological role of TMX2 *in vivo* is scarce. Previously our group has found that TMX2 is overexpressed in breast cancer samples, both patient-derived and commercial cell lines [9].

The aim of this paper is to evaluate the efficiency of our platform by producing an antibody against TMX2 protein. At the same time, using this RGCC TMX2 antibody we tried to gain some information about TMX2 protein *in vivo*. The description of the platform is patent-pending and as such is subjected to patent laws and is not in the scope of this article.

Materials and methods

Antibody production method

The antibodies against TMX2 protein were produced from human whole blood cell isolation. The detailed method is applied for a patent and therefore cannot be disclosed yet. However, the inflammatory environment *in vivo* was mimicked and whole blood cells were pulsed with an 9 mer peptide corresponding to a) a TMX2 extracellular epitope that produced an antibody that will be called for convenience RGCC extra-TMX2 and b) a TMX2 intracellular epitope that produced an antibody called RGCC intra-TMX2. After the experiment, cell culture supernatants containing the antibodies were collected and stored at -20°C until further use.

IgG determination in culture supernatants

The presence of IgG in whole blood culture supernatants was determined qualitatively by western blot against human IgG Fc fragments. Briefly, culture supernatants were subjected to SDS-PAGE according to Laemmli [10] using a Mini Protean Tetra System (BioRad). After electrophoresis, proteins were transferred into a nitrocellulose membrane using a Trans-blot SD, Semi-dry Transfer cell (BioRad) and were blocked with 5% BSA in PBS for 1 hour. Membranes were incubated using goat anti human IgG Fc fragment antibody (Bethyl laboratories, A80-104A) in 5% BSA/PBS overnight. Membranes were then washed 3 times with 0.05% Tween-20 in PBS and incubated with anti-goat-Alkaline phosphatase secondary antibody (Millipore) for one hour. After washing, membranes were incubated with NBT/BCIP (sigma) until signal development. IgG was also determined quantitatively using a human IgG Elisa quantitation kit (Bethyl laboratories, E80-104) according to manufacturer's instructions. The absorbance was read at 450 nm using a uQuant spectrophotometer (MQX200, BIOTEK).

Anti-TMX2 antibody determination

The presence of an anti-TMX2 antibody on whole blood cell culture supernatants was determined qualitatively by western blot, using the supernatants as primary antibody. Briefly, TMX2 protein (Cusabio, CSB-YPO239-51HU) was run in a 15% polyacrylamide gel according to Laemmli method. Membranes treated as stated in the previous IgG section. However, membranes were incubated with cell culture supernatants overnight as a detection antibody and a goat anti-human-alkaline phosphatase (Millipore, AP112A) as a secondary antibody. As a positive control, commercial anti-TMX2 antibody (Cusabio, CSB-PAO23951-LAO1HU) was also used. Antibody against TMX2 was also determined quantitatively using a sandwich Elisa. Briefly, 96 well plates (BRAND, 781722) were coated with 4 ug/ml TMX2 protein (Cusabio, CSB-YPO23951HU) in carbonate/bicarbonate solution and incubated overnight at 4°C. Next day, plates were washed 5 times with 200 ul PBS and blocked with 200 ul blocking buffer (5% BSA in PBS) for 1 hour. Wells were then washed again and incubated with 100 ul cell culture supernatants or a commercial anti-TMX2 antibody (Cusabio, CSB-PA-O23951LAO1HU) overnight at 4°C. Next day, wells were washed again and incubated with either a HRP conjugated goat anti-human IgG-Fc antibody (Bethyl laboratories, A80-104P) or

a goat anti-rabbit-HRP antibody (abcam, ab-20043) for 1 hour. Wells were then washed and incubated with 100 ul TMB solution (ThermoFisher, N301) for 15 minutes. The reaction was stopped with the addition of 100 ul stop buffer (2 M H_2SO_4) and the absorbance was read at 450 nm using a uQuant spectrophotometer (MQX200, BIOTEK).

Anti-TMX2 antibody kinetic evaluation

Antibody and antigen interaction was measured by Surface Plasmon Resonance (SPR). Experiments were performed using SPR-Navi 200 (BioNavis) at 21°C using flow rate 20 μl/min. BioNavis carboxymethyl dextran (CMD 2D) sensor slides were used. Data were analyzed using TraceDrawer for MP-SPR Navi. Goat anti human IgG antibody was immobilized on a sensor surface using amino coupling chemistry. Sensor surfaces were activated using NHS 0.05 M in EDC 0.2 M mixture prior to introduction of the hybridoma supernatant containing our TMX2 antibody. Surface group deactivation was performed using 1 M ethanolamine (pH 8.5). One of the channels was used as a reference without immobilized protein. Eight TMX2 protein concentrations (Cusabio, CSB-YPO23951HU) ranging from 10 nM to 10 uM were diluted in PBS/0.05% Tween 20 running buffer and sequentially run. TMX2 protein interaction with captured anti-TMX2 antibody was measured for 10 minutes. Slide surface was regenerated in order to remove bound TMX2 protein using a 3 minute injection with 10 mM Glycine pH 1.7 between each TMX2 protein concentration.

Cell culture

MCF-7 cancer cells were grown until 80-90% confluency in RPMI with 10% FBS, 2 mM L-glutamine and 1% Non essential amino acids. MCF-7 is considered to be a model for hormone sensitive breast cancer.

TMX2 protein localization

TMX2 localization was determined in MCF-7 cells using flow cytometry using both an intracellular and an extracellular staining protocol. Briefly, cells were grown until 80% confluency in cell culture flasks and detached using a scrapper. 10^5 cells were used for each experiment. For intracellular staining cells were pelleted by centrifugation for 5 minutes at 300 g in FACS tubes and then fixed with the addition of 2 ml ice cold methanol with vigorous vortexing. Cells were then centrifuged for 5 minutes at 500 g. Pellet was washed with PBS, supernatant was discarded and cell pellet was incubated with 5 uL TMX2-FITC (abbexa, abx305754) antibody for 30 minutes. After incubation pellet was washed with PBS to remove unbound antibody and re-suspended in 1 ml PBS. For extracellular staining, no fixation was needed, so cell pellet was incubated with 5 uL TMX2-FITC antibody without prior methanol treatment. An unstained control was also used. Cells were analyzed in a FACSCalibur (BecktonDickinson).

Anti-TMX2 antibody biological activity

Cell culture supernatants were subjected to affinity chromatography for the isolation of anti-TMX2 antibodies (Epimax, abcam, ab138-915) and then TMX2 positive fragments were also passed through another affinity chromatography column for the isolation of IgG antibodies (MabTrap, GE Healthcare, 17-1128-01). The final fraction contained IgG antibodies specifically against TMX2 protein. MCF-7 cells were plated in 96 well cell culture plates at 20,000 cells per well and left for 24 hr to adhere to plastic. Then, cells were incubated with either RGCC extra-TMX2 Ab, RGCC intra-TMX2 Ab (fragment 5) at various concentrations, a commercial antibody recognizing an extracellular TMX2 fragment (Cusabio, CSB-PAO23951LAO1HU) and a commercial antibody recognizing an intracellular TMX2 fragment (Novus biologicals, NBP1-87305) both at 1 ug/ml. Cells were left incubated with the antibodies for 24 hr. Next day cell confluency was evaluated microscopically and proliferation was determined using the MTT assay. Briefly, cells were incubated with 20 ul MTT (5 mg/ml) for 3 hours at 37°C, 5% CO_2. After incubation, supernatant was carefully removed, formazan crystals were dissolved in 100 ul DMSO per well and absorbance was measured at 570 nm 450 nm using a uQuant spectrophotometer (MQX200, BIOTEK). NAD(P)H-dependent cellular oxidoreductase enzymes are capable of reducing the tetrazolium dye MTT to its insoluble formazan, which has a purple color. The more intense the color, the more dye is reduced and the more proliferative are the cells.

Table 1. Fragments derived from the use of affinity column chromatography for TMX2 and IgG isolation

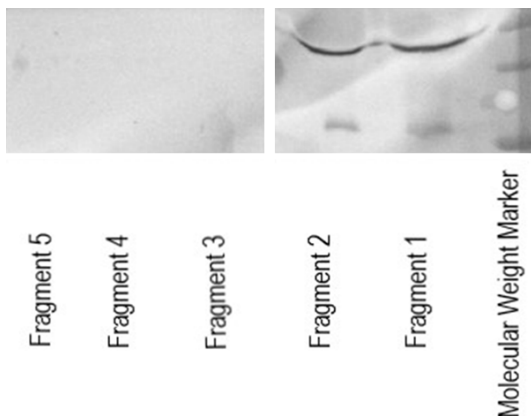| FRAGMENT	PROPERTY
Fragment 1	Initial cell culture supernatant
Fragment 2	TMX2 negative
Fragment 3	TMX2 positive
Fragment 4	TMX2 positive, IgG negative
Fragment 5	TMX2 positive, IgG positive

Figure 1. IgG determination using western blot. Affinity chromatography was used on co-culture supernatants containing the produced antibody for IgG and anti-TMX2 antibody isolation. The chromatography fragments were evaluated for the present of an IgG antibody. Fragments 1 and 2 have high IgG levels compared to fragments 3, 4 and 5 which do not have detectable IgG. From right to left: molecular weight marker, fragment 1, fragment 2, fragment 3, fragment 4 and fragment 5.

Gene expression

The effect of intracellular TMX2 antibody on the expression of genes related to cell proliferation, differentiation and metastasis was also studied. The optimum concentration of 0.05% RGCC intra-TMX2 antibody was chosen for incubation, based on the results of the biological activity experiments. Total RNA from cultured MCF-7 cells with and without RGCC intra-TMX2 Ab addition was extracted using an RNeasy Mini Kit (74105, Qiagen, Hilden, Germany). Total RNA samples were evaluated spectrophotometrically, and 1 µg of each RNA sample was used as a template for cDNA synthesis using a PrimeScript RT Reagent Kit (RR037A, Takara, Beijing, China). Real-time qPCR was then performed using KAPA SYBR Fast Master Mix (2×)

Universal (KK4618, KAPA Biosystems, MA, USA) in a final volume of 20 µl. Specific primers were designed using Beacon Designer. Primer sequences were evaluated by BLAST searching to exclude those that would amplify undesired genes. The PCR program was as follows: initial denaturation at 95°C for 2 min followed by 45 cycles of denaturation at 95°C for 10 s and annealing at 59°C for 30 s. Melting-curve analysis was performed from 65°C to 95°C with 0.5°C increments for 5 s at each step. The qPCR products were run on agarose gels and visualized, to validate the results. ΔCt value was used for analysis of experiments. In all sets of reactions, cDNA from Universal Human Reference RNA (740000-41, Agilent, CA, USA) was used as a positive control. Template-free and negative controls were also used in all experiments. All the reactions were performed in triplicate. ΔCt value was used for analysis of experiments. Finally, relative quantification was performed using the normal samples as the reference group according to Livak [11].

Statistical analysis

For the appropriate experiments, data are presented as Mean Value ± SEM. Tests for significant differences between groups were performed using a two-tailed student's t-test. A minimal value of $P = 0.05$ was chosen as the level of significance.

Results

Chromatography fragments

The initial cell culture supernatant that contains the antibody was passed through an affinity chromatography column for the isolation of antibody that binds specifically to TMX2 and then through another affinity chromatography column for the isolation of IgG only antibody. Therefore, we ended up with 5 different fragments as it can be seen in **Table 1**.

IgG determination in fragments

First, all chromatography fragments were analyzed for IgG presence. As it can be seen from **Figure 1**, fragments 1 and 2 had detectable levels of IgG in contrast with the rest of them. When a more sensitive technique like Elisa was used for the detection of IgG levels, it was found that fragments 3, 4 and 5 had 18 ng/ml,
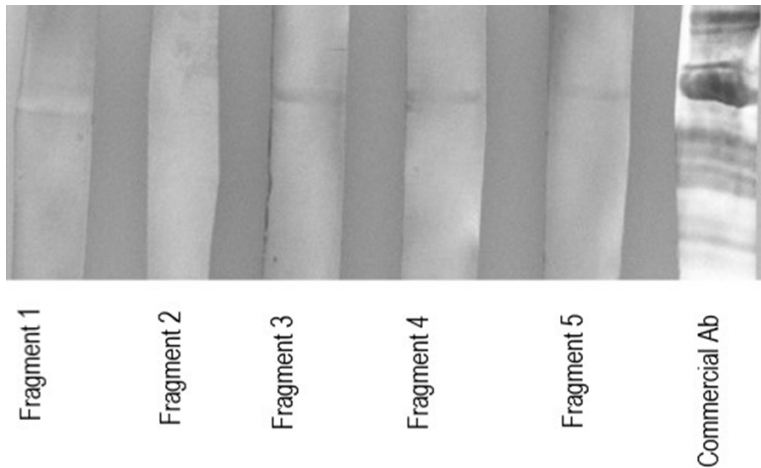
Figure 2. TMX2 determination using chromatography fragments as primary antibodies in western blot. Affinity chromatography was used on co-culture supernatants containing the produced antibody for IgG and anti-TMX2 antibody isolation. The binding efficiency of the chromatography fragments was evaluated with western blot. Secondary antibodies were anti-human alkaline phosphates conjugates. From right to left: commercial TMX2 antibody, fragment 5, fragment 4, fragment 3, fragment 2 and fragment 1.
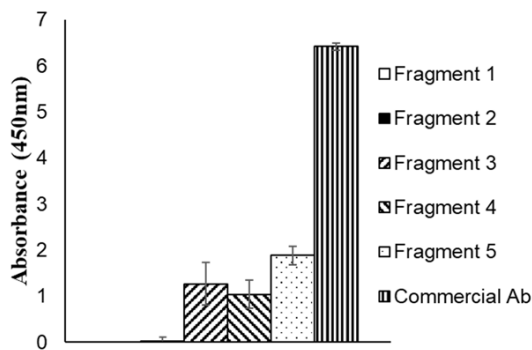

Figure 3. Absorbance for TMX2 determination by Elisa, using chromatography fragments as detection antibodies. Affinity chromatography was used on co-culture supernatants containing the produced antibody for IgG and anti-TMX2 antibody isolation. The binding efficiency of the chromatography fragments was evaluated with Elisa. Positive control was a commercial TMX2 antibody. Bars represent means ± SEM.

100 ng/ml and 40 ng/ml IgG respectively. It should be noted that the fragments had different dilution factors and therefore their IgG concentration cannot be compared.

anti-TMX2 determination in fragments

Then, all fragments were tested as a primary antibody against TMX2 protein in western blot experiments. In was found that fragment 4 and

5 gave the most intense signal (**Figure 2**). Once more, it should be noted that the fragments have different dilution factors and cannot be compared. The ability of our fragments to recognize TMX2 protein was tested with a more sensitive technique as well, sandwich Elisa. **Figure 3** shows the absorbance of each sample as well as of a commercial TMX2 antibody used as positive control.

TMX2 kinetic evaluation

Bivalent interaction model was used for calculation which is typical for antibody protein interaction because an antibody contains two equal binding sites. Steady state affinity (KD) was found to be 34 nM. Kinetics evaluation graph for anti-TMX2 antibody can be found in **Figure 4**. TMX2 protein was successfully removed from TMX2 antibody with short glycine injection and anti-TMX2 activity remained after regeneration based on repetition sample.

TMX2 localization

MCF-7 cells were analyzed for the presence of TMX2 both intracellularly and extracellularly. It was found that the majority of TMX2 occurs in the cytoplasm (99.4%). However, there is a considerable percentage of TMX2 found on MCF-7 cell surface (11.4%) (**Figure 5**).

Fragment 5 biologic activity

Then the effect of RGCC extra-TMX2 Ab fragment 5 on cell proliferation was tested using MTT. Fragment 5 is the fragment which is positive for TMX2 and IgG, so it is the sample that contains IgG against TMX2 protein. Fragment 5 was tested on MCF-7 breast cancer cell line for 24 hr. As it can be seen in **Figure 6A**, fragment 5 increases MCF-7 proliferation significantly (0.5% antibody *vs* control: 105.9 *vs* 100, *p-value* = 0.0007; 10% antibody *vs* control: 114.7 *vs* 100, *p-value* = 0.0001). These results were unexpected and we sought to examine the phenomenon further. We tested 2 commercial anti-
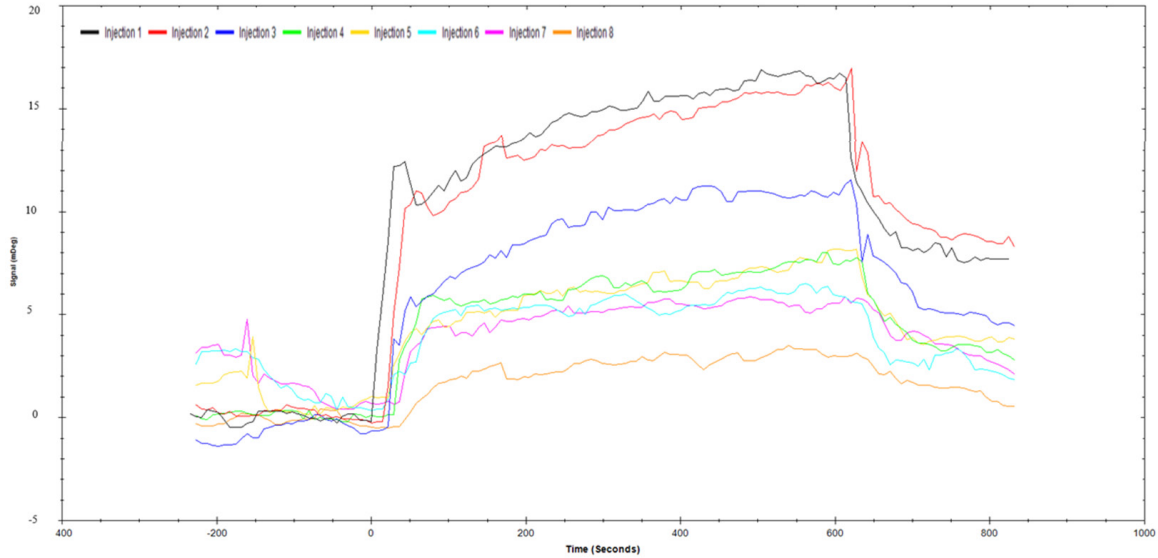
Figure 4. TMX2 kinetics. Binding kinetics of TMX2 antibody to TMX2 protein were evaluated using SPR. Evaluation of KD was determined using TraceDrawer software. Injections used for the determination of KD were: Injection 1 = 10 uM, Injection 2 = 5 uM, Injection 3 = 1 uM, Injection 4 = 500 nM, Injection 5 = 200 nM, Injection 6 = 100 nM, Injection 7 = 50 nM, Injection 8 = 10 nM of TMX2 protein.
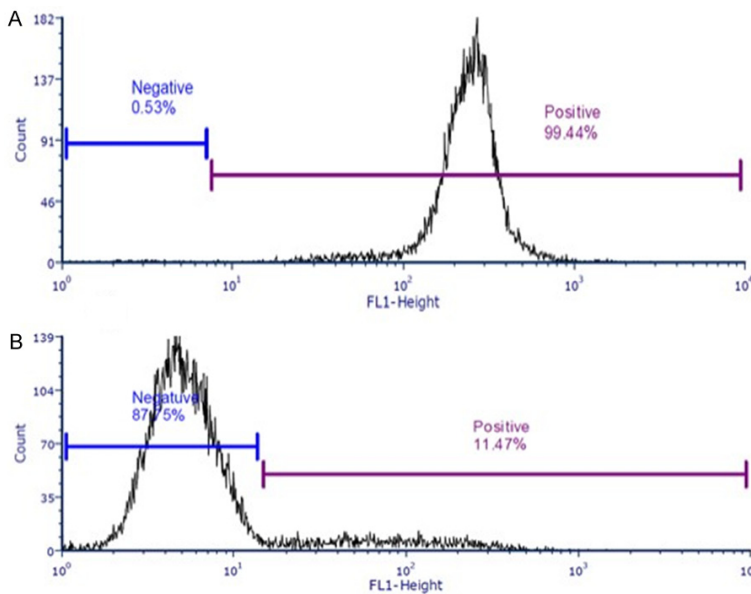

Figure 5. TMX2 localization. TMX2 protein was detected in MCF-7 cell line, both in the cytoplasm (up) and cell surface (down) using flow cytometry.

cellular Ab: 100 vs 116, *p*-value = 0.05) (**Figure 6C**). Based on these results we produced a second antibody recognizing an intracellular TMX2 epitope. It was found that RGCC intra-TMX2 Ab fragment 5 decreased MCF-7 proliferation significantly (Control *vs* TMX 0.05%: 100 *vs* 92.7*, p-value = 0.06*; Control *vs* TMX 0.1%: 100 *vs* 96.6*, p-value = NS*; Control *vs* TMX 0.5%: 100 *vs* 103.2, *p-value* = NS; Control vs TMX 1%: 100 vs 99.8, *p-value = NS*; Control *vs* TMX 10%: 100 *vs* 117*, p-value =* 0.0003) (**Figure 6B**). MCF-7 cell confluency after 24 hr incubation with RGCC intra-TMX2 antibody was also evaluated microscopically (**Figure 7**).

bodies for their effect on the proliferation of MCF-7. One antibody recognizes and intracellular part and the other recognizes and extracellular part. In MCF-7 cells, the intracellular antibody decreased significantly cell proliferation (untreated vs intracellular Ab: 100 vs 85, *p-value* = 0.007) whereas the extracellular antibody increased cell proliferation (untreated vs extra-

Gene expression

Finally, the effect of RGCC intra-TMX2 Ab on gene expression was studied. The genes selected were involved in cancer progression, differentiation, and metastasis (BAX, FAS, N-CADH, VIMENTIN, B-CNT, EPCAM, CD44, HGFR, RhoA). It was found that the addition of RGCC intra-
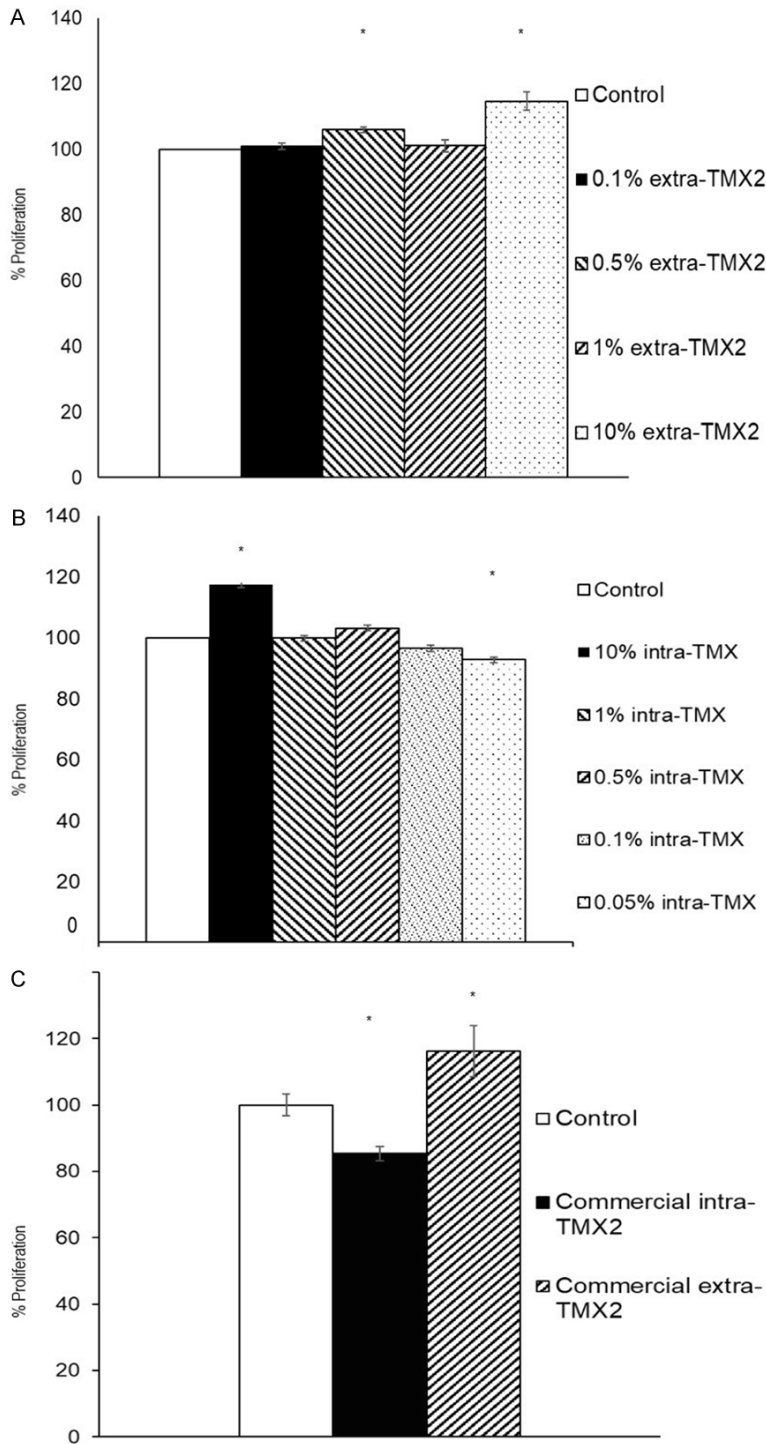
Figure 6. The effect of fragment 5 on MCF-7 cell proliferation. Chromatography fragment 5 which contains the IgG anti-TMX2 antibody was evaluated for biologic efficiency. MCF-7 cancer cells were incubated for 24 hr with various concentrations of fragment 5 antibody, specific for both intracellular and extracellular epitopes. Commercial TMX2 antibodies were also used. A. Cells were incubated for 24 hr with a range on RGCC TMX2 extracellular antibody concentrations. Bars represent means ± SEM. Control vs TMX 0.5% P = 0.0007; Control vs TMX 10% P = 0.0001; B. Cells were incubated for 24 hr with a range on RGCC TMX2 intracellular antibody concentrations. Bars represent means ± SEM. Control vs TMX 0.05% P = 0.06; Control vs

TMX 10% P = 0.0003. C. Cells were incubated for 24 hr with a commercial antibody recognizing an intracellular TMX2 part and an antibody recognizing an extracellular TMX2 part. Bars represent means ± SEM. Control vs TMX2 Intracellular: 100 vs 85, p-value = 0.007; Control vs TMX2 Extracellular: 100 vs 116, p-value = 0.05.

TMX2 antibody decreased the expression of all the above genes (**Figure 8**).

Discussion

In this study antibodies against TMX2 protein were produced using a novel platform by culturing human cells ex-vivo. TMX2 antibodies were used to study their specificity for TMX2 protein and biological effects on MCF-7 breast cancer cell line.

Monoclonal antibody therapies are an ever-evolving market for various conditions, with oncology being the most demanding [12]. First generation antibodies were produced in mice, and therefore due to the human anti-mouse response, side effects were frequent and severe [13]. Recent technological advances resulted in the production of human antibodies just within a few years of research. The term human however, is misleading because it does not refer to the host but to the sequence. Human antibodies originate either from phages or transgenic animals or B cell transformation. These techniques do not come without problems. Low transformation efficiency [14], limited antigenic repertoire and residual antigenicity [15] are some of the hurdles in antibody production. At Research Genetic
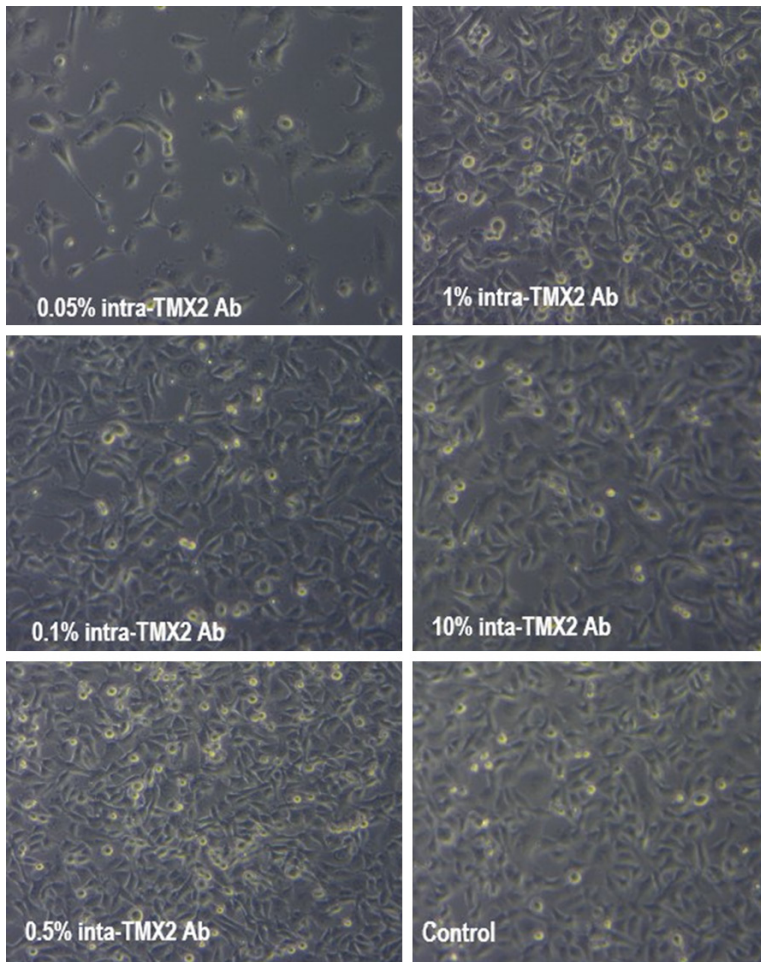
Figure 7. Microscopic evaluation of MCF-7 incubated with various concentrations of RGCC TMX2 intracellular antibody.
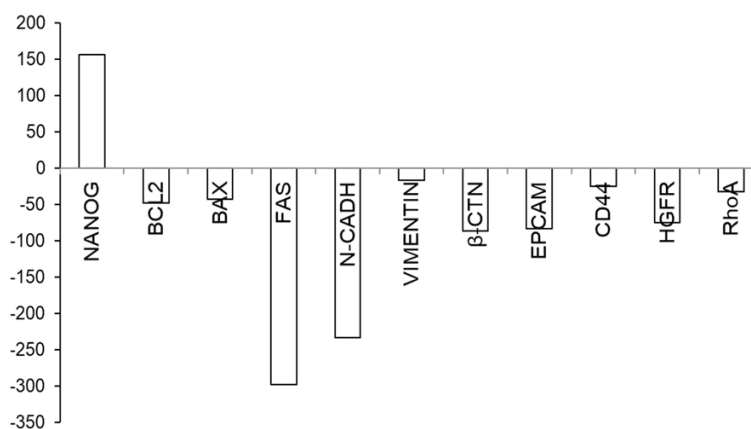

Figure 8. The effect of RGCC TMX2 intracellular Ab on MCF7 gene expression. Optimal concentration of RGCC intracellular TMX2 antibody, as determined by viability experiments, was evaluated for its effect on cancer cell gene expression. MCF-7 cells were incubated with 0.05% TMX2 for 24 hr. Genes that are involved in cancer survival, differentiation and metastasis were studied. TMX2 Ab addition decreased all genes studied.

Cancer Center, we have developed a novel method for the production of truly fully human monoclonal antibodies against specific antigens of our choice using isolated human blood cells. The method of antibody production is patent-pending and cannot be discussed yet, however the scope of this article is not about the method but the product itself.

After a successful cancer therapy, a small and very distinctive cell population, known as cancer stem cells can remain in hiding until they can be re-activated by specific factors resulting in cancer relapse and metastasis. Using microarray analysis RGCC group has previously found that breast cancer stem cells as well as breast cancer cell lines, over-express TMX2 among other genes [16]. Based on this work we decided to use RGCC's novel platform to produce an antibody against TMX2 and study its effects on breast cancer cell lines.

In this study, a novel human anti-human TMX2 antibody that recognizes an extracellular fragment of TMX2 protein and one recognizing an intracellular one were successfully produced. The novelty of this study is that not only do the antibodies recognize a new epitope, but they are produced by human blood cells *ex vivo*. Furthermore, the antibody that is specific for the intracellular epitope decreases breast cancer cell proliferation indicating a possible role for TMX2 protein as a therapeutic target. The production of TMX2 antibody by

blood cells was verified both by IgG determination by Elisa and also by using RGCC antibody as the detection antibody in a TMX2 determination assay by Elisa. The specificity of RGCC antibody for TMX2 protein was also confirmed by western blot, were it was used as a primary antibody. TMX2 binding affinity was found to be 34 nM which indicates a very good binding between RGCC TMX2 antibody and TMX2 protein.

Next, efficacy of RGCC TMX2 antibody was evaluated. MCF-7 cells were incubated with various TMX2 antibody concentrations for 24 hr and cell proliferation was determined. It was found that RGCC extracellular TMX2 antibody increased MCF-7 proliferation. Seeking to explain this finding, the same experiment was performed using 2 commercially available antibodies. It was found that the antibody that recognizes the intracellular domain of TMX2 protein caused a decrease in MCF-7 proliferation and the respective antibody for the extracellular domain, increased MCF-7 proliferation. Based on this finding a novel TMX2 antibody was produced, this time recognizing an intracellular epitope. It was found that this antibody decreased cell proliferation significantly as well as the expression of genes related to cell survival, differentiation and metastasis. TMX2 location was also examined. Flow cytometry experiments suggested that although the majority of TMX2 resides intracellularly, there is also a percentage found on the cell surface.

TMX2 is a fairly newly identified protein and the information concerning its role *in vivo* is scarce. TMX2 belongs to the PDI family of proteins. Although PDI plays a crucial role in redox protein folding and guards the cell from general protein misfolding, it also supports the survival and progression of various cancer types [17]. On the other hand is was also found that inhibitors of PDI suppress apoptosis [18]. Sodium butyrate induces apoptosis in colorectal cancer cells via PDI upregulation [19]. Therefore, TMX2 antibody proliferative effect could be a result of blocking TMX2-induced apoptosis. However, it is dangerous for such generalizations to be made on all PDI members since different PDIs can have different effects. In a study for the role of thioredoxin domain-containing protein family in hepatocellular carcinoma, it was found that patients with elevated TXNDC1 (TMX1)/7/9/14 (TMX2) mRNA level had poor overall survival and those with high TXNDC11

mRNA level had better overall survival [20] indicating that different members of the same family have different biological effects. RGCC extracellular TMX2 antibody increased cell proliferation possibly by activating the protein. On the other hand, RGCC intracellular TMX2 antibody decreased proliferation possibly by interfering with the binding of TMX2 substrates. It also decreased the expression of genes that are important for cell proliferation, differentiation and metastasis. RGCC's TMX2 antibodies could either be internalized and bound to intracellular TMX2 or could act on TMX2 protein found on the cell surface, since TMX2 protein was also detected on the plasma membrane.

In this study the production a truly fully human antibody using human blood cells *ex-vivo* was demonstrated. Specificity of RGCC antibodies was confirmed by western blot and Elisa and their effect on breast cancer cells was evaluated. The protein that was chosen as a target was TMX2, the least studied member of the PDI family. RGCC group has demonstrated that TMX2 could play a role in cancer cell stemness, differentiation and metastasis and therefore TMX2 can be an attractive candidate for targeted therapy. The results of the biological effect of RGCC extracellular TMX2 antibody on MCF-7 were not anticipated since cell proliferation was increased. However, changing the target to an intracellular domain decreased cell proliferation and gene expression related to cell proliferation, differentiation and metastasis. Future experiment include the determination of TMX2 signaling pathway using knock out models in order to elucidate its biological role.

Conclusion

In conclusion, production a real human antibody against an epitope of choice was achieved. The antibodies produced are autologous, secreted from patient's own cells opening the road to novel personalized therapies. RGCC intracellular TMX2 antibody decreased cell proliferation and gene expression that regulate cancer survival and metastasis indicating that TMX2 protein could be a novel target for cancer therapies.

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Disclosure of conflict of interest

None.

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