INTRODUCTION

Immunotherapies in cancer are often referred to as “Therapeutic cancer vaccines”. Many such therapies based on monoclonal antibodies and immune targeting drugs are currently being developed to treat already diagnosed cancers as opposed to prevent cancer from occurring.

Most approaches to cancer vaccines target various cancer specific antigens (known as Tumor Associated antigens or TAA) to activate or enhance the body’s own cytotoxic T cell responses against cancer cells. While immune stimulation is seen by such an approach, overall clinical response in terms of patient survival has been limited. One drawback with this approach is that such cancer vaccines must be developed individually against specific tumor antigens. This greatly increases the costs of development and vaccine efficacy is uncertain since cancer cells can also evade T cell responses.

We implemented a different approach to cancer vaccines by targeting the universal mechanism that all cancer cells use in order to grow, invade their surroundings and metastasize, which is enzymatic
digestion of extracellular matrix. The enzymes, associated within this process and most related to malignancy are zinc-metalloproteinases (MMPs), of which MMP-2 and MMP-9 have been associated with the progression and aggressiveness of cancer4-6. Our earlier studies indicated that the immunization of mice with specific oligopeptides containing MMP-9 and MMP-2 sequences from humans resulted in a significant inhibition of melanoma tumor growth and was not associated with significant peptide immune-related toxicity7. In addition, we also demonstrated significant inhibition of melanoma tumors in mice immunized with MMP-9 oligopeptides containing rat and mice MMP-9 sequences - a syngeneic approach7.

In the present study, we investigated the mechanistic aspects of antibodies raised against murine MMP-9 oligopeptides and whether tumor-growth inhibition observed in our in vivo studies could be associated with curbing cancer cell invading ability. Therefore, we raised polyclonal antibodies against MMP-9 in rabbits by immunizing them with three oligopeptides from rat MMP-9 and two MMP-9 oligopeptides from mice and subsequently used these purified antibodies in conducting in vitro testing of their efficacy in inhibiting Matrigel invasion of different cancer cell lines. In addition, we tested the antibodies binding to the corresponding MMPs sequences by Western blot.

**MATERIALS AND METHODS**

**Reagents**

All reagents were of analytical grade. All solutions were prepared using pyrogen free MilliQ grade water.

Solutions of peptides were prepared into 3 ml glass vials ISO 8362-1 2R-CL-1 (Medical Glass, Bratislava, Slovakia) or PP Costar Microcentrifuge Tube (Catalog No. 3621, Corning Inc., Corning, NY, USA) depending on solution volume.

Incomplete Freund’s Adjuvant (Catalog No. F5506, Sigma-Aldrich, St. Louis, MO, USA), 2 ml syringe 22Gx11/2” (BKMI, R. Korea), Vortex Vibrofix VF1 (IKA-Werk, Germany), GP Centrifuge (Beckman, Indianapolis, IN, USA), pH/ORP Meter HI 2211 (Hanna Instruments, Smithfield, RI, USA), spectrophotometer Unico 2800 (United Products & Instruments, Inc., Dayton, NJ, USA).

Reagents for enzyme-linked immunosorbent assay (ELISA) assay: sample diluent (10 mM sodium phosphate, 500 mM NaCl, 0.5 % Bovine Serum Albumin (BSA), 0.05 % Tween-20, pH 7.4), substrate buffer (50 mM sodium citrate and hydrogen peroxide, pH 5.0), TMB solution (3,3',5,5'-tetramethylbenzidine), stop solution (2 M sulphuric acid) were homemade. Streptavidin from Streptomyces avidinii (MP Biomedicals LLC, Irvine, CA, USA), Plate washer ZLE201 (Amersham Inc., Amersham, UK), incubator at 37°C - Imperial II (Lab-Line Instruments Inc. Thermo Scientific, Waltham, MA, USA), ELISA plate reader - Luminometer-Photometer LM01A (Immunotech, Beckman Coulter Company, Indianapolis, IN, USA). MMP inhibitor GM6001 was obtained from Sigma-Aldrich (St. Louis, MO, USA). Affinity sorbent columns XK 16/20 were from GE Healthcare (Catalog No. 18-8773-01. Chicago, IL, USA), peristaltic pump P-1 from GE Healthcare (Catalog No. 18-1110-91. Chicago, IL, USA), and SuperFrac Fract Collector was obtained from GE Healthcare (Catalog No. 18-1010-19. Chicago, IL, USA).

**Test Peptides**

Five peptides mimicking mice and rat MMP-9 sequences were synthesized at GenScript (Piscataway, NJ, USA). Each peptide was synthesized in two forms, one covalently conjugated to keyhole limpet hemocyanin (KLH) protein for immunization, the other conjugated to biotin for IgG ELISA. The both forms of peptides were dissolved in Phosphate Buffered Saline (PBS) at concentration 1.0 mg/ml and aliquots stored at -20°C until experimentation.

Peptide abbreviations and sequences are presented in Table I.

**Preparation of peptide emulsion for immunization**

On the day 147 of re-boosting immunization, 0.5 mL of the solution peptide covalently conjugated to KLH was gradually added to 0.5 mL of Incomplete Freund’s adjuvant (IFA) and simultaneously vortexed thoroughly to get a uniform emulsion.

<table>
<thead>
<tr>
<th>Peptide abbreviation</th>
<th>Peptide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat: R#7</td>
<td>-D-T-D-R-K-Y-G-F</td>
</tr>
<tr>
<td>Rat: R#</td>
<td>-D-H-F-P-F-T-F-E-G-R-S-Y-L-S-C</td>
</tr>
<tr>
<td>Mouse: M#11</td>
<td>-D-K-D-G-K-F-G-F</td>
</tr>
<tr>
<td>Mouse: M#19</td>
<td>-D-Q-D-K-L-Y-G-F-C-P-T-R-V-D-A</td>
</tr>
<tr>
<td>N IgG</td>
<td>Nonspecific rabbit IgG</td>
</tr>
</tbody>
</table>
**Immunization and bleeding procedures**

On day 147, the animals were injected 1000 µL of the mixture individual test solution emulsified withIncomplete Freund’s adjuvant (IFA) subcutaneously on 4 occasions. On day 157 and 164 blood samples were withdrawn from the marginal ear vein into PP 50 mL centrifuge tubes (Catalog No. 4558, Corning Inc. Corning, NY, USA).

**Immune response assay**

The immune response was measured in serum samples with an indirect ELISA with streptavidin on solid-phase. (Enzyme immoabsorbent) EIA plates were coated by adding to well 100 µl of streptavidin dissolved 5 mg/ml in 50 mM carbonate buffer, pH 9.5 and incubated for 20 h at 20°C. The plates were washed 4 times with wash fluid. Biotinylated peptides 10 mg/ml, 100 ml/well in sample diluent were incubated 60 min at 37°C. Control-wells with streptavidin. Subsequently the plates were washed 4 times with wash fluid. Serum from each rabbit was diluted 1:1000 and 1:10000 in sample diluent and added to the wells, coated with corresponding peptide (100 µl per well) and incubated for 1 h at 37°C. Control - wells with sample diluent. The plates were washed 4 times with wash fluid.

Conjugates of sheep anti-rabbit IgG antibody with HRPO (dilution of 1:3000 in conjugate diluent) were added to the wells (100 µl per well). The plates were incubated for 0.5 h at 37°C and washed 4 times as specified above. 100 µl of freshly prepared substrate solution (1 volume (v) 3,3’,5,5’-tetramethylbenzidine [TMB] solution + 7 v substrate buffer) were added to each well, and the plates were left at room temperature for 15 minutes in dark place after which 100 µl of stop solution was added to each well in the same sequence as the addition of substrate solution. The absorbance at 450 nm was completed within 5 minutes.

**Antibody generation**

All experimental procedures in the study were approved by the Institutional Animal Ethics Committee (IAEC) of Russian Cardiocenter. Male Schinenschilla rabbits of 9 - 10-week-old were procured from Nursery of Lab Animals (Stolbovaya, Russia) and were acclimatized for a period of two weeks prior to the study. Animals were maintained on a standard rodent diet ad libitum with free access to water under controlled conditions of temperature on a natural light-dark cycle.
Preparation of antibodies for cell invasion assays

Rabbit polyclonal antibodies against mouse and rat oligopeptides prepared as suspensions of serum IgG fraction in ammonium sulfate (described above) were thoroughly mixed and placed in 6x Slide-A-Lyzer MINI Dialysis units from Thermo Scientific (Catalog No. 88403, Waltham, MA, USA). 500 mcl of each antibody suspension was run against a total volume 4 x 45 ml Dulbecco’s PBS with stirring at 4°C. Antibody solutions were collected and sterilized. Pierce Protein micro BCA kit was used to determine protein concentrations.

Cell cultures

Human Prostate Cancer (DU145), Mouse Breast cancer (4T1) and Human Pancreas Carcinoma (MIA PaCa-2) were obtained from ATCC (American Type Culture Collection, Rockville, MD, USA). All cells were maintained in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL). Cells were incubated at 37°C in a tissue culture incubator equilibrated with 95% air and 5% CO₂.

Invasion assay

Corning BioCoat Matrigel Invasion Chambers were used with Corning Matrigel Matrix cell culture insert in 24-well plates (Corning, NY, USA). Plate was rehydrated for approximately two hours in the cell culture incubator by adding warm (37°C) cell culture medium without FBS to the inserts and to the lower chamber. Cells suspension was mixed with antibodies, as specified, and seeded on the insert in the well. Equal numbers of cells were added to each insert. DMEM with 1% FBS was added to the well below the insert to serve as a chemoattractant. The plates with the inserts were then incubated in a culture incubator equilibrated with 95% air and 5% CO₂ for 24 hours. After incubation, the media/antibodies were discarded from the inserts. The cells on the upper surface of the inserts were gently scrubbed away with cotton swabs. Cells that had penetrated the Matrigel membrane and migrated onto the lower surface of the Matrigel were fixed with Methanol and stained with hematoxylin and eosin. Pictures were taken under the microscope and cells were counted. For the sake of uniformity, pictures were always taken at the center of the insert.
**IMMUNOLOGICAL TARGETING OF MMP-9 BY IN VITRO ANTI-CANCER ANTIBODIES**

**Western Blot**

Whole DU145 cell lysates were prepared using lysis buffer [50 mM Tris-HCl (pH=7.4), 1% TritonX-100, 150 mM NaCl, 1 mM EDTA, 2 mM Na3VO4, and 1X Complete protease inhibitors (Roche Applied Science, Indianapolis, IN, USA)]. The protein concentration was measured by the DC protein assay (Bio-Rad, Hercules, CA, USA). 50 µg of protein per sample was separated by electrophoreses using standard Laemmli method as described and transferred to a PVDF membrane. Proteins were detected with commercially available anti-MMP-9 antibody (Cell Signaling, Danvers, MA, USA) as a reference antibody, and anti-β-actin antibody as a loading control (Cell Signaling Technology, Danvers, MA, USA).

**RESULTS**

**Immune response**

The immune response to individual peptides evaluated by ELISA using complementary antiserum and expressed as an average A450 is presented in Table II. The results of serum titration for two bleedings: on day 157 and 164 tested at serum dilutions 1:1000 and 1:10000 show that test oligopeptides generated immune response of similar intensity in all rabbits (Table II). Based on the immune response, the sera from five bleedings for each rabbit immunized with a particular peptide were pooled and each pool tested for the immune response and subsequently used for the purification of polyclonal antibodies specific for each test peptide.

**TABLE 2.** Immune response of rabbit antiserum to complementary antigens.

<table>
<thead>
<tr>
<th>Rabbit #</th>
<th>Oligopeptide</th>
<th>Bleed on Day 157</th>
<th></th>
<th></th>
<th>Bleed on Day 164</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sera dilution</td>
<td>1:1000</td>
<td>1:10000</td>
<td>1:1000</td>
<td>1:10000</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>R#7</td>
<td>&gt;4</td>
<td>1.370</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>1.250</td>
<td></td>
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<tr>
<td>2</td>
<td>R#7</td>
<td>&gt;4</td>
<td>2.129</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>1.949</td>
<td></td>
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<tr>
<td>3</td>
<td>R#7</td>
<td>3.666</td>
<td>0.577</td>
<td>2.762</td>
<td>&gt;4</td>
<td>0.378</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>R#12</td>
<td>&gt;4</td>
<td>3.804</td>
<td>&gt;4</td>
<td>3.843</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>R#12</td>
<td>&gt;4</td>
<td>3.939</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>3.593</td>
<td></td>
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<tr>
<td>6</td>
<td>R#12</td>
<td>&gt;4</td>
<td>3.736</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>3.593</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>R#18</td>
<td>&gt;4</td>
<td>3.330</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>3.326</td>
<td></td>
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<tr>
<td>8</td>
<td>R#18</td>
<td>&gt;4</td>
<td>1.368</td>
<td>&gt;4</td>
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<td>1.349</td>
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<tr>
<td>9</td>
<td>R#18</td>
<td>&gt;4</td>
<td>2.662</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>2.489</td>
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<tr>
<td>10</td>
<td>M#11</td>
<td>&gt;4</td>
<td>2.931</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>2.964</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M#11</td>
<td>3.707</td>
<td>0.853</td>
<td>3.911</td>
<td>&gt;4</td>
<td>0.911</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M#11</td>
<td>&gt;4</td>
<td>2.368</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>2.550</td>
<td></td>
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<td>13</td>
<td>M#19</td>
<td>&gt;4</td>
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<tr>
<td>14</td>
<td>M#19</td>
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<td>&gt;4</td>
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<tr>
<td>15</td>
<td>M#19</td>
<td>&gt;4</td>
<td>3.028</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td>2.131</td>
<td></td>
</tr>
</tbody>
</table>

The immune response in pooled rabbit sera immunized with specific antigens containing MMP-9 sequences from rat and mice is presented in Figure 1. The pooled sera for each test oligopeptide were tested at two-fold dilutions ranging from 1:10,000 to 1:640,000 and immune response to the specific antigen expressed as A450.

The results show that the sera from rabbits immunized with rat peptides (A1 through A3) had higher antibody titer than the sera obtained from the immunization with mouse A1 and A2 peptides.

Characterization of polyclonal antibodies against rat and mice MMP-9 sequences

Characterization of polyclonal antibodies to specific MMP-9 peptides after their subsequent purification on affinity columns are presented in Table III and Figure 2. Each antibody was tested at two-folds dilutions from 100 ng/ml to 0.78125 ng/ml and the affinity to corresponding peptide sequences expressed as A450nm.

The results show approximately equal specificity and affinity of the antibodies for all antigens except Mouse peptide #11, which was two times weaker.

Inhibition of cancer cell invasion by anti-MMP-9 antibodies

The affinity purified polyclonal antibodies against individual oligopeptides mimicking specific sequences of MMP-9 in rat and mice were tested for their ability to inhibit extracellular matrix invasion of cancer cells. The results presented in Figure 3 and 4 show that all test antibodies generated against rat and mice MMP-9 oligopeptides were effective in inhibiting Matrigel invasion of both mouse breast cancer cells (4T1) and human prostate cancer cells (Du145).
In an effort to enhance the inhibitory effect of antibodies on cancer cells migration through ECM we applied them in various combinations. In order to correct for different protein content in various antibody mixtures we added a non-specific rabbit IgG to equalize the protein content in each test sample. We also controlled total protein concentration by adding the same amount of PBS-BSA in all Control samples. That way, antibody protein concentrations were kept constant for every tested combination.

The results (Figure 5) show that the most effective in inhibiting mouse breast cancer cells invasion was a combination of all five antibodies. The mixtures containing two and three antibodies against rat MMP-9 either without or with antibody M#11 could inhibit 4T1 cell invasion by about 60%. The

As such, Figure 3 shows that the highest inhibition of breast cancer cells invasion could be obtained with antibody against mouse M#19 oligopeptide (44% inhibition) and rat R#18 oligopeptide (33 % inhibition). The antibodies directed towards these murine MMP-9s were also effective in inhibiting extracellular matrix invasion of human prostate cancer cells. As presented in Figure 4, the highest inhibition of invasion could be obtained with antibodies against mouse MMP-9 oligopeptides: M#11 (40% inhibition) and M#19 (96% inhibition), demonstrating interspecies efficacy of these antibodies (Figure 4). In general, the antisera against mouse oligopeptides were more effective in inhibiting extracellular matrix (ECM) invasion of cancer cells from human prostate than the murine 4T1 breast cancer cells.

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**TABLE 3.** Immune affinity of antibodies to their complementary antigens.

<table>
<thead>
<tr>
<th>Antibody ng/ml</th>
<th>R_#7</th>
<th>R_#12</th>
<th>R_#18</th>
<th>M#11</th>
<th>M#19</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.726</td>
<td>2.399</td>
<td>2.286</td>
<td>1.135</td>
<td>2.505</td>
</tr>
<tr>
<td>50</td>
<td>1.823</td>
<td>1.388</td>
<td>1.28</td>
<td>0.569</td>
<td>1.51</td>
</tr>
<tr>
<td>25</td>
<td>0.966</td>
<td>0.815</td>
<td>0.664</td>
<td>0.315</td>
<td>0.794</td>
</tr>
<tr>
<td>12.5</td>
<td>0.494</td>
<td>0.428</td>
<td>0.34</td>
<td>0.167</td>
<td>0.405</td>
</tr>
<tr>
<td>6.25</td>
<td>0.251</td>
<td>0.223</td>
<td>0.171</td>
<td>0.09</td>
<td>0.207</td>
</tr>
<tr>
<td>3.125</td>
<td>0.127</td>
<td>0.115</td>
<td>0.088</td>
<td>0.049</td>
<td>0.107</td>
</tr>
<tr>
<td>1.5625</td>
<td>0.068</td>
<td>0.058</td>
<td>0.049</td>
<td>0.032</td>
<td>0.059</td>
</tr>
<tr>
<td>0.78125</td>
<td>0.038</td>
<td>0.038</td>
<td>0.032</td>
<td>0.027</td>
<td>0.039</td>
</tr>
</tbody>
</table>
This confirms that antibodies against murine MMP-9 enzymes are effective in inhibiting ECM invasion of cancer cells originating from different organs in mice and humans.

We also tested whether these combinations of antibodies were effective in inhibiting Matrigel migration of human pancreatic cancer cells MIA PaCa-2. The results show that the ability to digest connective tissue and invade extracellular matrix gradually decreased with increasing the number of different antibodies in a combination (Figure 6). Here too, five antibodies applied together were most effective resulting in nearly 95% inhibition of ECM invasion. This confirms that antibodies against murine MMP-9 enzymes are effective in inhibiting ECM invasion of cancer cells originating from different organs in mice and humans.

The representative images of the Matrigel invading MIA PaCa-2 cells in the presence of various anti-MMP-9 antibody combinations are presented on Figure 7. The photographed inserts correspond to numerical data for each antibody combinations presented on Figure 6. All cell invasion experiments were performed in triplicates.

**Fig. 2.** Immune response of affinity purified antibodies to complementary antigens. Specific antibodies were isolated from pooled immune sera by two consecutive steps: total serum immunoglobulin precipitation with ammonium sulfate followed specific antibodies purification by affinity chromatography on Sepharose 4 Fast Flow columns loaded with individual synthetic peptides. Immune response of affinity purified antibodies was evaluated by immune response assay as described in the legend to Figure 1. See Materials and Methods for experimental details.

**Fig. 3.** Efficacy of antibodies against specific rat and mice MMP-9 oligopeptides in inhibiting murine breast cancer cells invasion through Matrigel. Inhibition is presented as the percentage of invaded 4T1 cells mixed with specified individual antibodies. Control cells were mixed with non-specific antibody at the same concentration as test peptides.
Fig. 4. Efficacy of antibodies against specific rat and mice MMP-9 oligopeptides in inhibiting human prostate cancer cells invasion through Matrigel™. The results present percentage of invaded DU145 (Human Prostate cancer) cells when mixed with specified individual antibodies as described in the Material and Methods.

Fig. 5. Inhibition of 4T1 cells invasion through Matrigel in the presence of various combinations of antibodies generated against rat and mouse MMP-9 oligopeptides. The results show the percentage of invading cells where the Control sample is set to 100%.

Fig. 6. Inhibition of pancreatic cancer cells (MIA PaCa-2) invasion through Matrigel in the presence of various combinations of antibodies generated against rat and mouse MMP-9 oligopeptides. The results show the percentage of invading cells where the Control sample is set to 100%.
Halting the spread of cancer in tissue and its metastasis to different organs has been a ‘holy grail’ of cancer therapies. Current modalities used in cancer therapy are not effective against metastasis, which is responsible for over 90% death from cancer. Among different anti-cancer approaches it appears that vaccines present the biggest opportunity to succeed. In particular, when the antigen of choice can target universal mechanism involved in cancer, especially its metastasis.

**Antibody binding to their corresponding MMP-9 peptides**

The results presented in Figure 8 show the binding of specific antibodies to their corresponding MMP-9 peptides as identified by Western Blot. The R#12 showed the strongest binding to its corresponding peptide and among all tested antibodies only M#11 was found not to bind to its corresponding MMP-9 peptide sequence.

**DISCUSSION**

Halting the spread of cancer in tissue and its metastasis to different organs has been a ‘holy grail’ of cancer therapies. Current modalities used in cancer therapy are not effective against metastasis, which is responsible for over 90% death from cancer. Among different anti-cancer approaches it appears that vaccines present the biggest opportunity to succeed. In particular, when the antigen of choice can target universal mechanism involved in cancer, especially its metastasis.

*Fig. 7. Microscopic images of Matrigel invading MIA PaCa-2 cells mixed with an individual antibody (R#12) and various combinations of antibodies generated against different MMP-9 mice and rat sequences. Images taken from the center of each insert after cells were fixed and stained with Hematoxylin and Eosin.*
MMPs appear to be an ideal target for the development of anti-cancer vaccine. They are disease-associated and involved in cancer cell tissue invasion, angiogenesis and metastasis. Earlier attempts that aimed at the MMPs in cancer by early generation of pharmaceutical MMP inhibitors were not successful due to unacceptable side effects or insufficient efficacy of these drugs. However, our earlier studies indicate that inhibiting MMP activity by vaccination is a promising therapeutic approach.

As such our earlier in vivo studies using specific oligopeptides containing MMP-2 and MMP-9 sequences from human, and MMP-9 peptides from rat and mice demonstrated significant inhibition of tumor growth in mice. This in vitro study evaluated some mechanistic aspects of tumor growth inhibition by investigating the efficacy of polyclonal antibodies against oligopeptides from rat and mouse MMP-9 on inhibition of cancer cell invasion through extracellular matrix (Matrigel™).

The results obtained here provide another support for further development of effective anti-cancer vaccine. They confirm that MMP-9 activity is a viable target of anti-cancer strategy and a suitable antigen for the vaccine aiming at the universal mechanism essential in all types of cancer and associated with its ECM invasive activities. Moreover, they document universal -non-species related - efficacy of anti-MMP-9 antibodies. It is an extension of our earlier in vivo studies, which showed that specific oligopeptides containing human MMP sequences can generate an immune response in mice, but also oligopeptides from rat and mice MMPs can generate an immune response in a syngeneic animal model. In both cases the immunization was associated with significant anti-tumor efficacy.

Our Matrigel invasion study shows that antibodies raised against rodent MMP-9 are effective in inhibiting the invasion of cancer cells originating from different organs in both mice and humans, specifically the breast cancer (mice) and prostate cancer (human). We have shown that anti-invasive efficacy of these antibodies can be enhanced by using them in various combinations and as such targeting different regions of the MMP protein. This effect is plausible given that individual antibodies may target different epitopes of MMP-9 and show more efficacy when they are applied together. Specificity of various antibodies towards individual MMPs has been confirmed by their specific binding identified by immunoblot. Interestingly antibody R#12 showed the strongest binding capacity and was also associated with most significant inhibition of breast cancer and prostate cancer cells Matrigel invasion. Combination of R#12 with 2 or 3 other antibodies did not enhance its anti-invasive capacity further. The Western blot did not detect the M#11 antibody binding to its MMP-9, which may relate to its weakest binding capacity and other specificity aspects. Interestingly, this antibody was not very effective in inhibiting extracellular matrix invasion of breast cancer cells; however, it gave good results with human prostate cancer cells. This again, stresses the need to use a combination of antibodies to target cancer cell invasion for maximal effects.

CONCLUSIONS

This and our previous in vivo results further reinforce the urgent need for conducting specific targeted investigations towards the development of universal and effective anti-cancer vaccine.

ACKNOWLEDGEMENTS:
We thank Dr. M. Waheed Roomi for providing valuable advice in cancer cells invasion experiments.

CONFLICT OF INTEREST:
Authors declare no conflict of interest.
REFERENCES