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RHAMM-target peptides inhibit invasion of breast cancer cells

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Abstract

RHAMM is hyaluronan- receptor with multiple functions in the cell, RHAMM is involved in proliferation, motility, migration, invasion, mitotic spindle formation in tumour cells. Therefore, RHAMM could be a relevant target for molecular targeted therapies against tumors.The role of RHAMM-target peptides in inhibition invasion for preventing breast cancer has not yet been investigated. Base on this, we analyzed the RHAMM-target peptides for their therapeutic activity against breast cancer cells. In the present study, we examined the effect of RHAMM-target peptides on the invasion of breast cancer cells (MDA-MB-231), using confocal microscopy. We shown that RHAMM-target peptides decreased formation of invadopodia of breast cancer cells. The treatment of breast cancer cells by RHAMM –target peptides inhibited the invasion up to 99 %. Additionally, RHAMM-target peptides induced the morphological changes of of breast cancer cells. Therefore, based on these results, we can conclude that RHAMM-target peptides may be potential anti-cancer agents.

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Introduction

Cancer is on the second place after the disease of the cardiovascular system. Every year, 10 million new cases of malignant tumors and more than 5.2 million cancer related deaths are recorded (1). Despite the increase in diagnostic capabilities the proportion of active cases and the proportion of patients with the disease have been identified at an early stage of tumor, remains low (2). Thus, high mortality rates, poor diagnosis of malignant tumors, show the need for the development of new diagnostic and therapeutic methods, and events for medical and diagnostic care to cancer patients. The major scientific problem is the development of new targeted approaches for the diagnosis and treatment of cancer. Lack of specificity of anticancer drugs, remains a major challenge oncology (3).

The fundamental goal of cancer research is identification of therapies that induce selective cancer cell death. Therapeutic peptides are increasingly gaining popularity for medical use in a variety of applications, including tumour vaccines, antimicrobial therapy and nucleic acid delivery (4, 5). Guidelines for using the peptides to treat cancer include the following approaches: the direct use of peptides as drugs (angiogenesis inhibitors), targeted agents, carriers of cytotoxic drugs and radionuclides (targeted therapy and radiation therapy), hormones and vaccines; the use of peptides as biomarkers of cancer and regulators and progression of cancer, since peptides are capable of specifically binding to the various receptors, as well as part of the signal transduction pathways (6-8). The direct use of peptides as therapeutic agents for the treatment of cancer is gaining momentum in recent years. The antitumor activity of different peptides based on the mechanisms of action, limiting growth tumour (9). These mechanisms include inhibition of angiogenesis, inhibition of protein-protein interactions, inhibition of enzymes, proteins, blocking the signal transduction pathways or gene expression (10, 11). Another category of anti-peptides act as a peptide antagonists which preferentially bind to a known receptor. It was shown, that RHAMM-binding peptides specifically bind to RHAMM receptor, block the signal pathway, induce the apoptosis, necrosis of cancer cells (12, 13). Such selective

peptides have great prospects, because their main mechanism of action targeted to a specific molecular target cancer cells that may lead to cell death (14, 15). Another type of peptides is capable to destroy the cell membrane of cancer cells. For example, it is known that membrane-lytic peptides such as cationic antimicrobial peptides able to physically destroy the cytoplasmic and mitochondrial membranes of cancer cells, leading to their death (16-19). Many natural or synthetic "pro-apoptotic" peptides act as therapeutic agents able to induce apoptosis, induce cell death or enhance the effect of chemotherapy drugs. For example, so-called cell-penetrating peptides have been used in the treatment of cancers because they greatly increased efficiency anti-cancer drugs commonly used in chemotherapy (20-23). It is shown that necrotic peptides isolated Australian frogs and toads, or from the stings of insects, as well as various protective peptides derived from various organisms, also have a moderate anti-tumor activity (24-27). Another example is depsipeptides which show a wide range of biological activity. These depsipeptides possess antitumor activity selectively cytotoxic to cancer cells, reduce multi-drug resistance of tumours (28-33). The literature on using peptides, especially for cancer targeting is steadily increasing (34).

Thus, targeted chemotherapy and targeted delivery of drugs based peptides are excellent tools to minimize the problems of traditional chemotherapy. At present time the different approaches exist, including the addition a targeting ligand, increasing the binding affinity or activation of the penetration at the correct site (35-37). However, use of peptides *in vivo* is limited by their low stability, cell permeability and absence of high selectivity to target.

RHAMM/HMRR is a multifunctional extracellular and intracellular protein, a receptor of hyaluronan mediated motility, which use hyaluronan (HA) as a substrate and bind to microtubules, participating in control of mitotic-spindle integrity (38). HA is a polysaccharide that involved in structural and signaling functions of cells, which are important for immunity, tissue organization and homeostasis (39, 40). The function of HA depends from its size, large HA fragments are responsible for structural functions, while the small HA fragments bind and interact with cell receptors such as CD44 and RHAMM. These small HA fragments induce signaling pathways that control proliferation, differentiation, cell-adhesion, motility and invasion cells (41-44). High expression of HA is associated with onset of such diseases as arthritis, diabetes and cancer (45-47). It is known now, that inside of cell, RHAMM is responsible for proliferation, motility, invasion of tumour cells (44, 48-52).

It was demonstrated that RHAMM-expression dramatically increased in many different types of cancer cells, such as myeloid leukemia, colorectal, breast, prostate, ovarian, multiple myeloma, gastric cells. It shown that such overexpression of RHAMM and HA correlates with poor prognostic factor (53-69). In experimental tumour models, RHAMM is an oncogenic protein that promotes both signalling through the RAS-ERK1,2 pathway and mitotic spindle instability leading to aberrant chromosome segregation (61, 62). Carboxyl terminal RHAMM sequence, which contains the HA and microtubule binding regions, is required for its oncogenic effects in these cancer models (61). Therefore, RHAMM could be a relevant target for molecular targeted therapies against tumors. The RHAMM-target peptides were design and characterized, and it was showed, that they were the most specific and selective ligands for RHAMM, they were stable in serum and they easily penetrate to cells (63). These peptides also demonstrated the selective cellular uptake by breast and prostate cancer cells MDA-MB-231, they inhibited proliferation and induced apoptosis in prostate cancer cells (63). Based on this, we analyzed the therapeutic potential of RHAMM-target peptides for treatment breast cancer.

The goal of this research is investigation of the effect RHAMM-target peptides on invasion breast cancer cells.

Materials and Methods

Abbreviations: Fetal bovine albumin (FBS), phenyl-nitroanilide (pNA), Dulbecco's modified Eagle's medium (DMEM), Enzyme-linked immunosorbent assay (ELISA), EEDFGEEAEEEA (p.#35), VEGEGEEGEEY (p.#37), FTEAESNMNDLV (p.#40), 3,3′,5,5′-tetramethylbenzidine (TMB)

Materials

DMEM and FBS were purchased from Multicell (Canada), HEPES was purchased from Sigma (USA), trypsin was purchased from Gibco BRL (USA), Triton X-100 was purchased from Bioshop (Canada), plastic tissue culture dishes were purchased from BD Falcon (USA), QCM™ Gelatin Invadopodia assay (red) kit was purchased from Millipore (USA), Quick Start Bradford Protein Assay (Bio-RAD, USA).

Cell lines and cell culture

Human breast carcinoma cell lines (MDA-MB-231, MCF-7), 10T ½ cells and RHAMM ($^{-/-}$) Mefs cells were obtained from American Type Culture Collection (Manassas, VA) and were cultured in DMEM supplemented with 10 % (v/v) heat-inactivated FBS and 10 mM HEPES at pH 7.2. All cultures were incubated in a humidified atmosphere of 5 % CO_2 at 37^0 C.

Protein detection

Protein has been measured by Quick start Bradford protein assay using standard procedure.

Fluorescent gelatin degradation assay for investigation of invadopodia formation

To study effect of RHAMM-target peptides on invadopodia formation, the QCM™ Gelatin Invadopodia assay (red) kit was used. All procedures carried out according to the manual protocol.

Cell lines used

MDA-MB-231 human breast adenocarcinoma cells), 10T ½ cells and RHAMM ($^{-/-}$)

Mefs cells were cultured to 70-80 % confluence in tissue culture plates. Cells were detached using 0.25 % trypsin-EDTA, then pelleted, followed re-suspension in growth medium to a concentration of 1, 000 cells/ml. Cells were seeded in a volume of 500 µl/well and cultured for the desired duration of degradation for 24 hrs.

Preparation of substrate and seeding of cells

Briefly, 24-wells glass chamber slides were first coated with 250 µl/well of dilute poly-L-lysine. Then the poly-L-lysine was removed. Next, 250 µl of dilute glutaraldehyde in DPBS was added to each well for activation of the poly-L-lysine surface. Then each well was again washed three times with 500 µl of DPBS. Finally, 200 µl of gelatin mixed at a 1:5 ratio of fluorescently-labeled: unlabeled gelatin, was coated onto each well for 10 min. All steps were performed in the dark to protect the glass slides from photobleaching. The gelatin substrates were disinfected, using 500 µl/well of 70 % ethanol for 30 min at RT. After this step, free aldehydes were quenched by the addition of 500 µl/well of amino-acid-containing growth medium. Cell types of interest were detached and seeded as described in the previous section and incubated at 37^0C in 5 % CO_2 for 24 hrs. For the experiments, involving a modulation of invadopodia formation, the RHAMM-target peptides (40 µg/ml final concentration) in DMEM were added and incubated at 37^0C in 5 % CO_2 for 40 hrs.

Sample fixation and staining

After 40 hrs incubation with RHAMM-target peptides, the samples were fixed for 30 min at RT with 250 µl/well of 3.7 % formaldehyde in DPBS. Samples were then washed three times with 500 µl/well of fluorescent staining buffer. After this step, samples were incubated during 1 hr at RT with FITC-conjugated phalloidin (2 µg/ml) and DAPI (1µg/ml) in staining buffer. After staining, the cells were mounted on a glass slide and immediately analyzed under a laser confocal microscope, using excitation wave at 358, 494 and 550 nm. We performed all experiments were in triplicate and repeated three times.

Confocal microscopy

Confocal images were captured, using a laser-scanning confocal microscope (Fluoview FV-1000; Olympus) at x20 objective magnification for quantification studies (5 fields of view per well) with a 1.0 zoom factor or 60X PlanApo/1.45 objective magnification (oil immersion) with a 2.0 zoom factor and then images were analyzed with Fluoview software (FV10-ASW version 01.07: Olympus).

Image analysis

"Percent degradation area of total cell area" was quantified using ImageJ analysis software, kindly distributed by the National Institute of Health (NIH). A high intensity threshold was used for positive DAPI signal, then analyzed as "particles" for determination of a nuclear (cells) count. Similarly, setting a high intensity threshold for FITC-phalloidin signal enabled measurement of total cell area per field of view. Conversely, a low intensity threshold was set for diminished fluorescent gelatin signal to enable quantification of total degradation area per field of view.

Statistical analysis

Prism 4 software (GraphPad Software, Inc.) was used to perform tests for statistical significance. For statistical comparison, one-way analysis of variance (ANOVA) was used with $P<0.05$ considered significant.

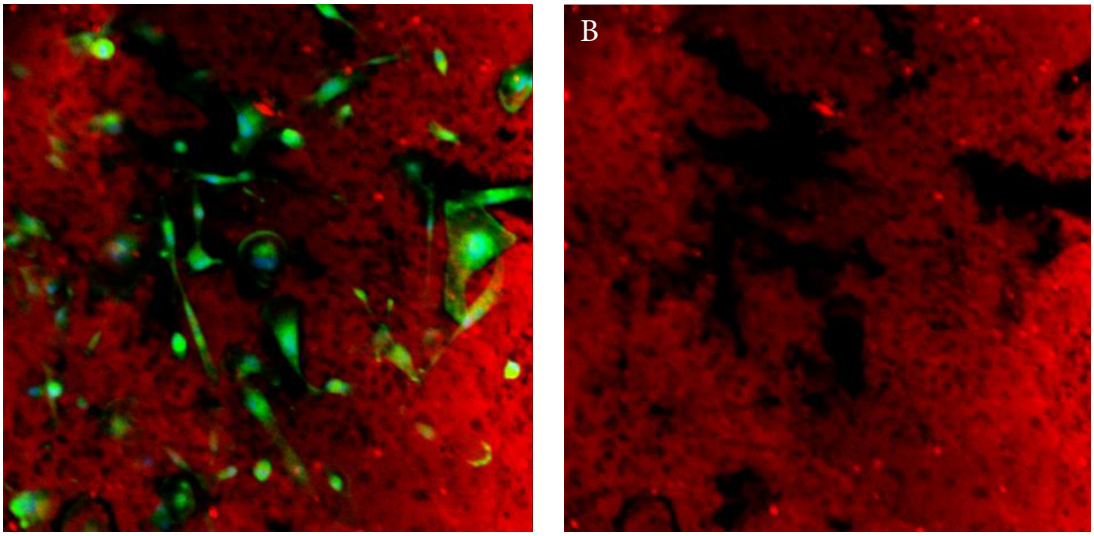


Figure 1. Fluorescent gelatin degradation and phalloidin/DAPI staining of MDA-MB-231 cells. Fluorescein-gelatin matrices (red) were coated onto 24-well glass chamber slides. MDA-MB-231 cells were plated onto the gelatin substrates at 1, 000 cells/well for the culture duration of 40 hrs. F-actin and nuclei were stain, respectively, with FITC-phalloidin (green) and DAPI (blue). (A) Cells were imaged at 20x objective magnification (bar=100 µm) at 5 fields of view per well. (B) Area of fluorescent gelatin degradation by MDA-MB-231 cells.

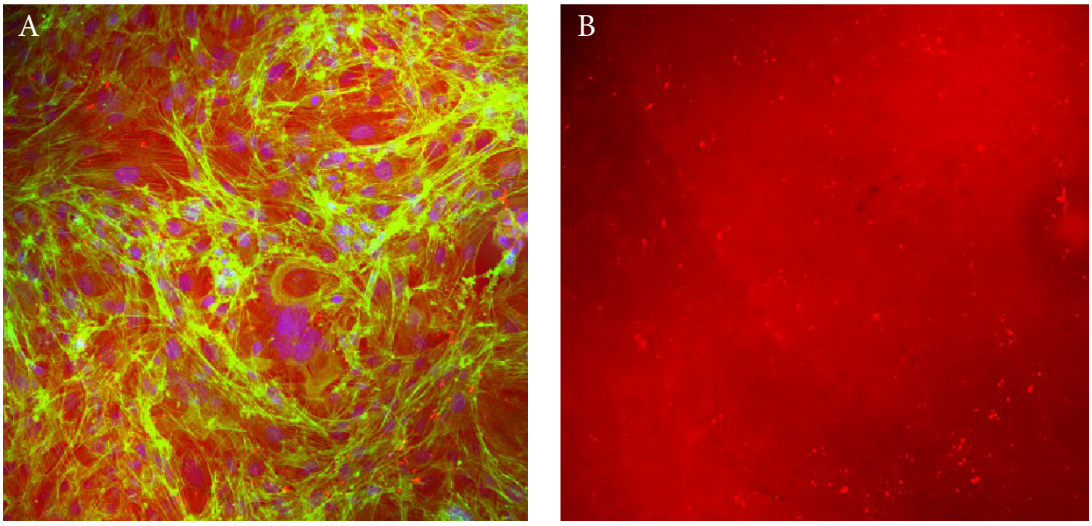


Figure 2. Fluorescent gelatin degradation and phalloidin/DAPI staining of 10 T ½. Fluorescein-gelatin matrices (red) were coated onto 24-well glass chamber slides. 10 T ½ cells (A) were plated onto the gelatin substrates at 1, 000 cells/well for the culture duration of 40 hrs. F-actin and nuclei were stain, respectively, with FITC-phalloidin (green) and DAPI (blue). Cells were imaged at 20x objective magnification (bar=100 µm) at 5 fields of view per well. (B) Area of fluorescent gelatin degradation by 10 T ½ cells.

Results

RHAMM-target Peptides Inhibited the Invasion of MDA-MB-231 Cells.

Over 100 cells per condition were analyzed to obtain the "percent degradation area of total cell area" data depicted in **Figures 1**. It should be point out, that although the theoretical maximum of "percent degradation area of total cell area" consists 100 %, we observed here the higher amounts of degradation, because of cellular movement during proteolysis. Because of higher level of degradation gelatin matrix was observed after culture cells for 40 hrs, in our next experiments we used this time to examine effect RHAMM-target peptides on invasion cancer cells.

To analyze whether RHAMM-target peptides can inhibit the invasion of breast cancer cells, we seeded MDA-MB-231 cells onto fluorescein-gelatin matrices and then treated with RHAMM-target peptides or a DMEM control for 40 hrs. Then we visualized and quantified the degradation of fluorescein gelatin matrix by MDA-MB-231 cells. It was shown, that untreated MDA-MB-231cells (DMEM control) demonstrated gelatin proteolysis and degradation patterns, which are attributed to invadopodia or podosome formation. These areas devoid of fluorescein-gelatin fluorescence (**Fig. 1**). Our results shown that MDA-MB-231 cells (DMEM control) degraded about 70 % of total cell area, whereas no degradation by (non-forming cancer) 10 T1/2 cell types (**Fig. 2**) or non-invasive RHAMM ($^{-/-}$) cells was observed (**Fig. 3**).

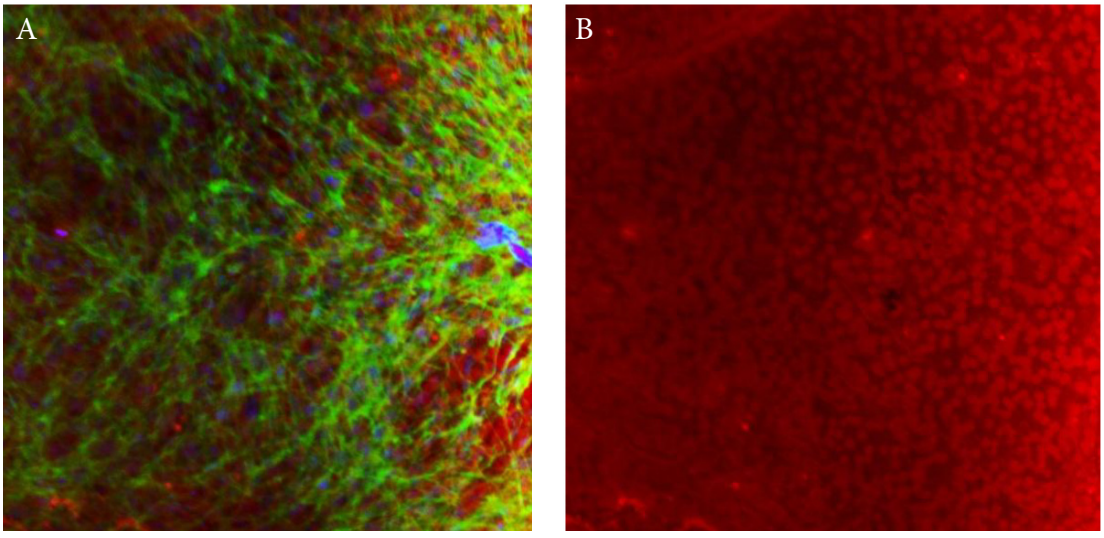


Figure 3. Fluorescent gelatin degradation and phalloidin/DAPI staining of RHAMM (-/-) cells. Fluorescein-gelatin matrices (red) were coated onto 24-well glass chamber slides. (A) RHAMM (-/-) cells were plated onto the gelatin substrates at 1, 000 cells/well for the culture duration of 40 hrs. F-actin and nuclei were stain, respectively, with FITC-phalloidin (green) and DAPI (blue). Cells were imaged at 20x objective magnification (bar=100 µm) at 5 fields of view per well. (B) Area of fluorescent gelatin degradation by RHAMM (-/-) cells.

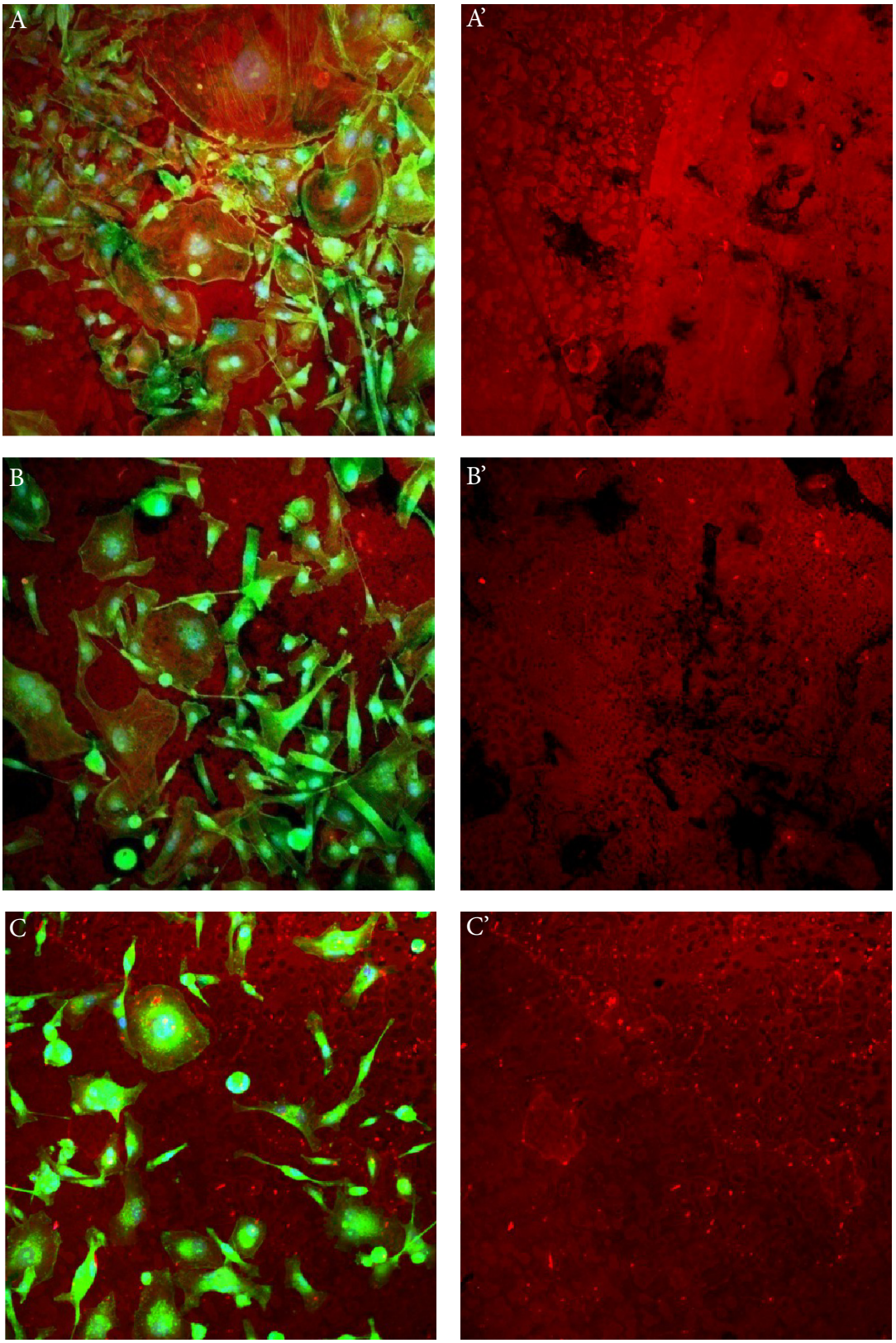


Figure 4. (A, B, C). Inhibition of gelatin degradation by RHAMM-target peptides in MDA-MB-231 cells. Fluorescein-gelatin matrices (red) were seeded with MDA-MB-231 cells and simultaneously treated with RHAMM-target peptides (40 µg/ml, 8x10-7 M): (A) EEDFGEEAEEEA, (B) VEGEGEEGEEY, (C) FTEAESNMNDLV or a DMEM control. Following 40 hrs. treatment, cells were fixed and stained for F-actin and nuclei with FITC-phalloidin (green) and DAPI (blue). Samples were imaged at 20x objective magnification (bar=100 µm) at 5 fields of view per well. (A', B', C') Areas of fluorescent gelatin degradation by MDA-MB-231 cells. "Percent degradation area of total cell area" was quantified using ImageJ analysis software.

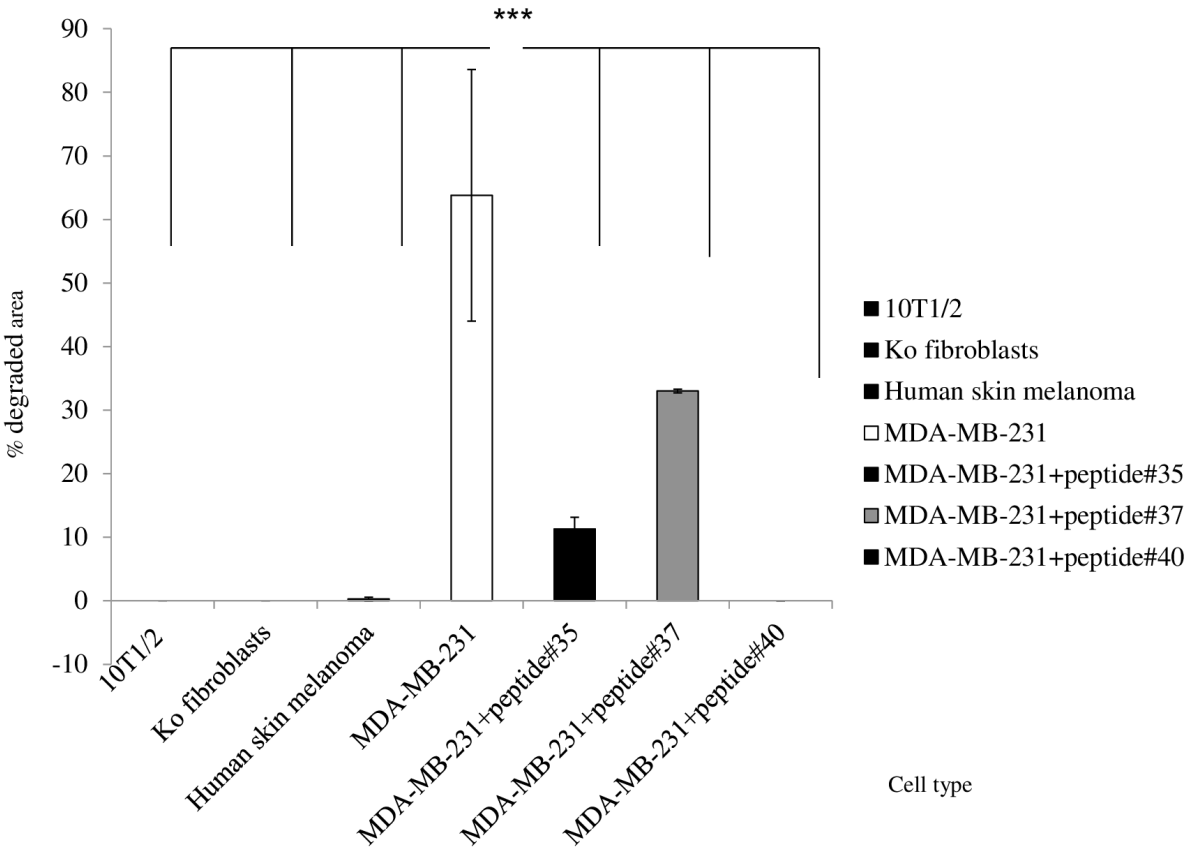


Figure 5. Percent degradation area of total cell area for different types of cells and treatment.

RHAMM-target peptides inhibited the invasion MDA-MB-231 cells during the 40 hrs treatment (**Fig. 4 row A, row B, row C**). For example, cells-treated with peptide #35 (Figure 4 row A) degraded only 12 % of total cell area, which indicates, that the invasion MDA-MB-231 cells was decreased on 87 %. The cells-treated with peptide #37 (**Fig. 4 row B**) degraded the 33 % of total cell area, so the inhibition of invasion was 67 %. The cells–treated with peptide #40 demonstrated the highest-level inhibition of invasion almost on 100 % (**Fig. 4 row C**).

Quantification analysis of degradation area normalized to number of MDA-MB-231 cells also indicated that all three peptides decreased significantly the degradation area of MDA-MB-231 cells (**Fig. 5**). These data demonstrate that RHAMM-target peptides inhibit the invadopodia formation and invasion of MDA-MB-231 cells.

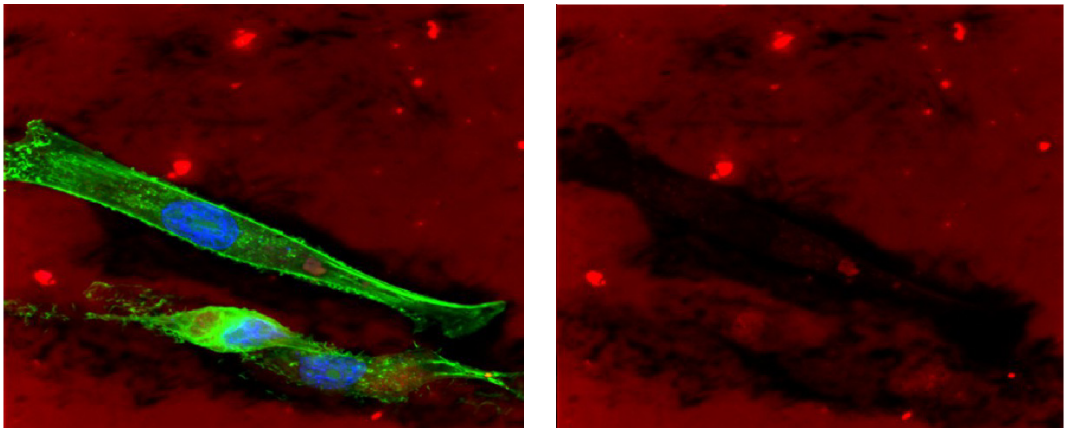
Treatment with RHAMM-target peptides changes the morphology breast cancer cells during invasion process

We analyzed the population of cells in invasion assay, using images with high magnification x60. Using invasion assay, we find out, that after treatment cells with peptides there are strong morphological (and, probably, biochemical) differences inside population MDA-MB-231 cells during the process invasion. We noticed the appearance senescent cells in population, that perfectly correlates with the start invasion process (**Fig. 4A, B, C**). These data indicate, that these senescent cells somehow involved in invasion process. These cells had a different morphology, big-size (their size is in approximately > 10 times bigger compared to regular cells), big nucleus, round shape morphology, absence invadopodia (**Fig. 6C**). Additionally, the senescent cells contain many nucleus inside, their amount varies from 2-, 4- up to 6 nuclei per cell. The function of these senescent cells is not known yet. However, we noticed, that they interact, associate with of cancer cells, regular size (**Fig. 4A, B, C; 6C**), they were always surrounded by these cells, which were invasive and co-localized with area degradation of matrix. These regular cancer cells have typical malignant cell morphology, elliptical shape, long-size of invadopodia, polarized cell morphology (**Fig. 6A, B**).

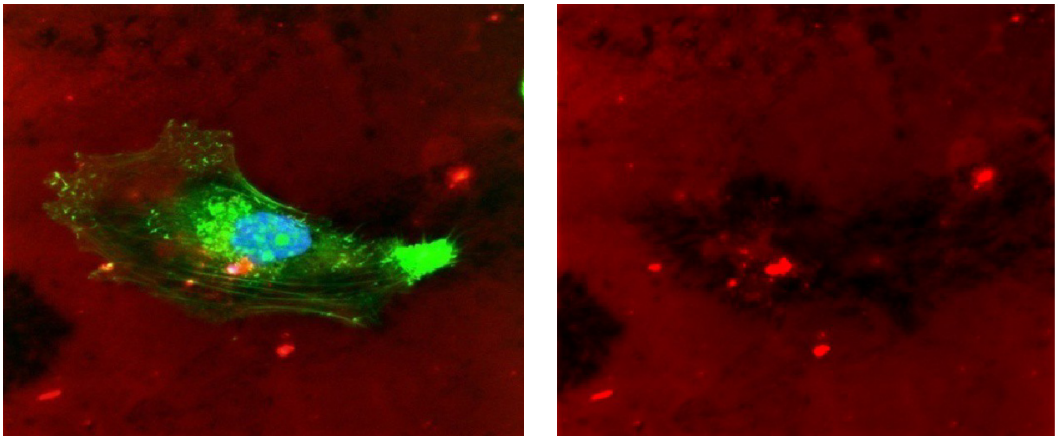
The actin was localized at the edges of cells and extensively accumulated at the ends of invadopodia, which allows them to degrade matrix and invade inside. The actin was organized, no aberrant stress fibers, shape of cells perfectly correlated with area degradation (**Fig. 6B**). Cell-treated with RHAMM-target peptides showed the senescent and regular cancer cells in population (**Fig. 7, 8**). The senescent cells demonstrated uncharacteristically round morphology, showed aberrant actin stress fibers in cytoplasm, actin was not accumulated at the edges of cells, the cells did not have any invadopodia (**Fig. 7**).

Additionally, the invasive cells (treated with RHAMM-target peptides showed smaller amount of invadopodia, more short size invadopodia, more round shape and they did not accumulate of actin at the invadopodia edge (Figure 8).

A) Cancer cells



B) Cancer cell



C) Senescent cell associated with regular cancer cell

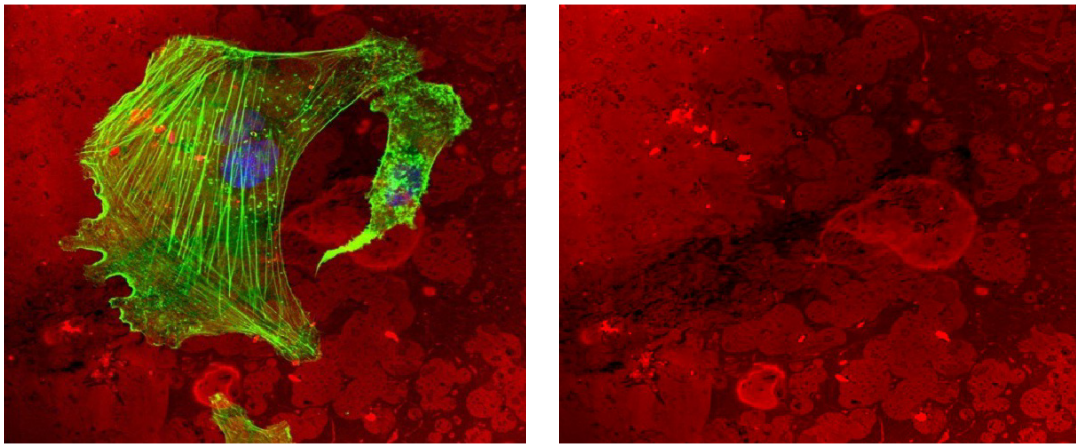


Figure 6. Analysis of morphology senescent and regular MDA-MB-231 cells. Fluorescein-gelatin matrices (red) were coated onto 24-well glass chamber slides. MDA-MB-231 cells were plated onto the gelatin substrates at 1, 000 cells/well for the culture duration of 40 hrs. F-actin and nuclei were stain, respectively, with FITC-phalloidin (green) and DAPI (blue). (A, B) Cells were imaged at 60x objective magnification (bar=10 µm) at 5 fields of view per well. (C) Senescent cells in MDA-MB-231 cells treated with RHAMM-target peptides.

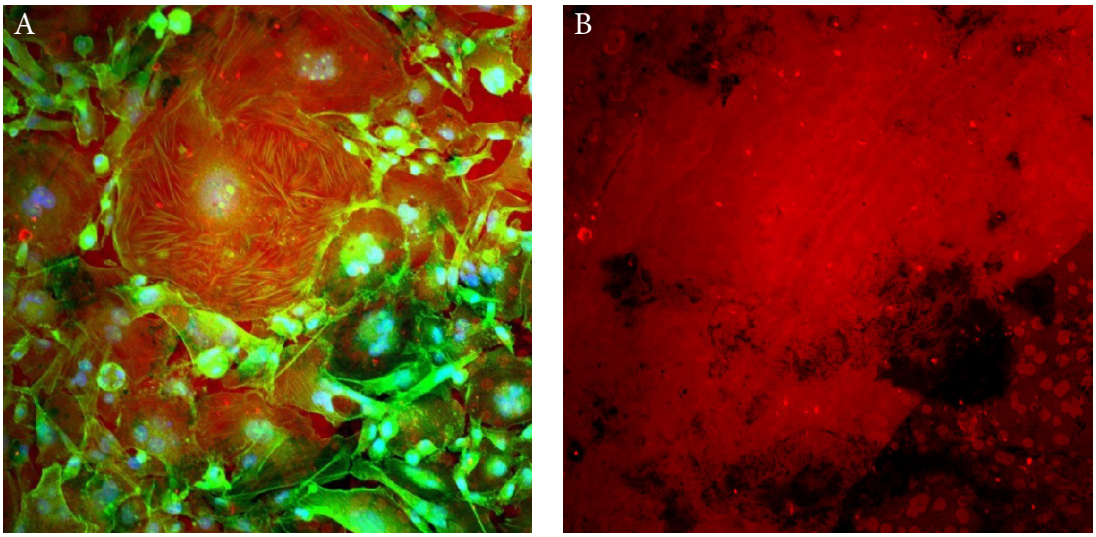


Figure 7. Aberrant actin stress fibers disassembly in senescent cells treated with RHAMM-target peptide. (A) Fluorescein-gelatin matrices (red) were seeded with MDA-MB-231 cells and simultaneously treated with RHAMM-target peptide (40 µg/ml, 8x10-7 M): VEGEGEEGEEY or a DMEM control. Following 40 hrs. treatment, cells were fixed and stained for F-actin and nuclei with FITC-phalloidin (green) and DAPI (blue). Samples were imaged at 20x objective magnification (bar=100 µm) at 5 fields of view per well. (B) Area of fluorescent gelatin degradation.

Discussion

Surgery and chemotherapy are usually used for treatment cancer. However, in many cases the anti-cancer drugs are very toxic to the whole organism and destroy the healthy cells of body (70), and produce side effects. Therefore, we need a development the novel anticancer drugs with higher specificity, low toxicity, selective targeting cancer cells and without side effects.

RHAMM is ideal molecular target for anti-cancer therapeutic agents, because it is hyper expressed in tumour tissues, such as breast cancer (60, 61, 71), leukemia (72), colon cancer (73), oral squamous cell carcinoma (74), aggressive fibromatosis (75), endometrial carcinoma (76, 77) as well as multiple myeloma (58).

Previously, we demonstrated that RHAMM-target peptides interact with HA binding domain of RHAMM with high selectivity and specificity (12, 13). These RHAMM-target peptides have a small size (M.w. 4 kDa), analogous distribution of negative charges, such as seen in HA, and they interact with cell surface RHAMM and easily penetrate inside of cancer cells. We demonstrated that these peptides were stable in serum and they blocked RHAMM/HA interactions in various *in vitro* as-

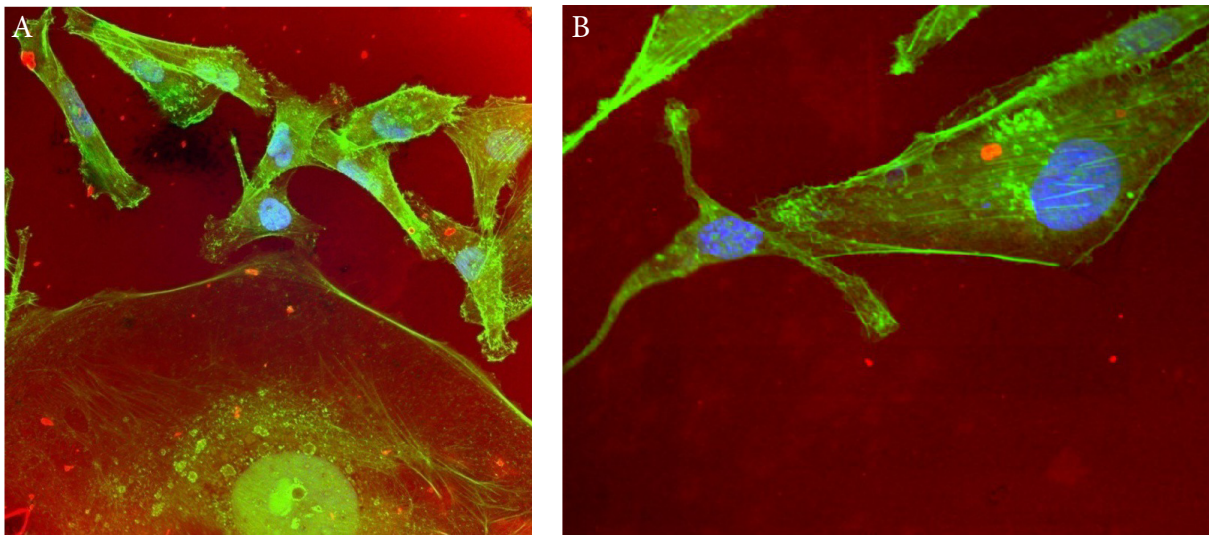


Figure 8. Changes in morphology MDA-MB-231 cells treated with RHAMM-target peptides during invasion process. Fluorescein-gelatin matrices (red) were seeded with MDA-MB-231 cells and simultaneously treated with RHAMM-target peptide (40 µg/ml, 8x10-7 M): EEDFGEEAEEEA or a DMEM control. Following 40 hrs. treatment, cells were fixed and stained for F-actin and nuclei with FITC-phalloidin (green) and DAPI (blue). Samples were imaged at 60x objective magnification (bar=10 µm) at 5 fields of view per well: (A) senescent and regular of MDA-MB-231 cells; (B) regular of MDA-MB-231 cells.

says. Probably, these peptides also bind to MAPs and motor proteins, because their molecules possessing similar degree of negative charges, such as seen in HA. In our previous study, we showed that these peptides induced apoptosis and inhibited cell viability of prostate cancer cells (11, 14). These results indicated that these peptides may be used as therapeutic agents for treatment cancers in which RHAMM and HA hyper-expression or accumulation is linked to poor outcome (43, 44, 57-60).

The invasion of cells is very important for movement, motility and migration of cells. The invasion of cancer cells is crucial step in tumor metastasis. The process of invasion is very complex event and includes many steps, such as adhesion cell to the matrix, activation of proteases enzymes, degradation of proteins of extra-cellular matrix, formation of invadopodia, accumulation actin at the edges of filaments, and movement of the cell body through the hole in the extra-cellular matrix (78). Many different proteins participate in each of these steps, such as actin, tubulin, integrins, proteases, kinases and cytoskeleton proteins. But the most important feature of invasion process is the formation of invadopodia or podosomes, which have a high proteolytic activity. Additionally, many other molecules involved in the formation and function of invadopodia. It was shown that many events are involved in this process, such as Src phosphorylation of scaffolding protein Tks5 (79), N-WASP activation, cortactin regulation of the Arp2/3 complex, actin polymerization (80, 81), generation of reactive oxygen species by NADH oxidases (82), overexpression of matrix metalloproteinases (83).

Here we demonstrated the proteolytic time-course degradation gelatin matrix with MDA-MB-231 cells and modulation invadopodia formation by RHAMM-target peptides on breast cancer cells. Our results showed that untreated MDA-MB-231 cells degraded almost completely (up to 75 %) total cell area, however, the non-cancer cells (10 T ½) or RHAMM ($^{-/-}$) cells lacked the degradation areas. Using confocal analysis, we demonstrated that RHAMM-target peptides significantly (up to 100 %) inhibited the invasion and invadopodia formation of MDA-MB-231 cells. At the same time, they did not affect on invasion of non-cancer cells or RHAMM ($^{-/-}$) cells. Quantification analysis showed that RHAMM-target peptides effectively inhibited the invasion of MDA-MD-231 cells compare with untreated cells. Interestingly, that we observed two different types of MDA-MB-231cells in degradation area: regular and senescent cells. The senescent cells showed round morphology, big nucleus or polynucleus (containing 2-, 4- or 6 small nucleus), they don't have almost invadopodia or long-size invadopodia. Senescent – RHAMM-target peptides treated cells demonstrated aberrant actin structure, stress fibers, chaotic distribution actin inside of cells. The function of senescent cells is unknown yet. The localization of senescent cells was not correlated with degradation area, however, these cells co-localized, associated, interact with regular cancer cells, which are invasive type. In contrast to senescent cells, regular cells have a smaller size, small nucleus, tight connections with each other's, many long-size invadopodia and intensive actin expression at the end of invadopodia. Our results indicate that invasive cells extend small and long, narrow at the edges, localized invadopodia that preferentially degrade the matrix. Localization and shape of regular cancer cells exclusively correlates with form and localization of degradation areas. In general, our results indicate that *in vitro* exposure of MDA-MB-231 cells to RHAMM-target peptides induces striking changes of cell morphology and behaviour patterns.

These results indicate that RHAMM-target peptides can be considered as ant-metastatic agents for prevention of metastasis in breast cancer.

References

1. World Cancer Report 2014. World Health Organization. 2014. pp. Chapter 5.2.
2. Siu, Albert L. Screening for Breast Cancer: U.S. Preventive Services Task Force Recommendation Statement. Annals of Internal Medicine. 2016, 164: 279–96.
3. Kakde D., Jain D., Shrivastava V., Kakde R. and Patil A.T. Cancer therapeutics—opportunities, challenges and advances in drug delivery. Journal of Applied Pharmaceutical Science. 2011, 1 (9): 1–10.
4. Zheleva D. I., Fischer P.M., Zhelev N. Z., Melville J. E., Gavine A-L., and Lane D. P. Design of cell-permeable peptides inhibitors of cyclin D1/ CDK4 complex. M. Nature Biotech. Sh.R.2000, 11: 25-30.
5. Eldar-Finkelman H. and Eisenstein M. Peptide inhibitors targeting protein kinases. Current Pharmaceutical Design. 2009, 15 (21): 2463–2470.
6. Rosca E.V., Koskimaki J.E., Rivera C.G., Pandey N.B., Tamiz A.P. and Popel A.S. Antiangiogenic peptides for cancer therapeutics. Current Pharmaceutical Biotechnology.2011, 12 (8): 1101–1116.
7. McClue S.J., Blake D., Clarke R., Cowan A., Cummings L., Fischer P.M., MacKenzie M., Melville J., Stewart K., Wang S., Zhelev N., Zheleva D., Lane D.P. In vitro and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine). Int J Cancer. 2002, 102 (5):463-470.
8. Mochly-Rosen D. and Qvit N. Peptide inhibitors of protein-protein interactions: from rational design to the clinic. Chimica Oggi. 2010, 28 (1): 14–16.
9. Smolarczyk R., Cichon T., Graja K., Hucz J., Sochanik A., and Szala S. Antitumor effect of RGD- 4C-GG-D (KLAKLAK) 2 peptide in mouse B16(F10) melanoma model. Acta Biochimica Polonica. 2006, 53 (4): 801–805.
10. Liang J.F. and Yang V.C. Synthesis of doxorubicin-peptide conjugate with multidrug resistant tumor cell killing activity. Bioorg. Med. Chem. Lett. 2005.15: 5071-5075.
11. Akenteva N.P, Shushanov S.S, Kotelnikov A.I. Effects of RHAMM/HMMR-Selective Peptides on Survival of Breast Cancer Cells. Bulletin of Experimental Biology and Medicine (Russia). 2015, 159 (5): 618-621.
12. Luyt L.G., Turley E.A., Esguerra K.V. Rhamm binding peptides. International Patent WO2011/150495. London Health Sciences Centre Research Inc. 2011.
13. Esguerra K.V., Tolg C., Akentieva N., Price M., Cho C.F., Lewis J.D., McCarthy J.B., Turley E.A., Luyt L.G. Identification, design and synthesis of tubulin-derived peptides as novel hyaluronan mimetic ligands for the receptor for hyaluronan-mediated motility (RHAMM/HMMR). Integr. Biol. (Camb).2015, 7 (12): 1547-1560.
14. Akentieva N.P., Shushanov S.S. RHAMM (receptor hyaluronan-mediated motility)-target peptides induce apoptosis in prostate cancer cells. Problems in oncology. 2016, 62 (3):512-518.
15. Aina O.H., Sroka T.C., Chen M.L. and Lam K.S. Therapeutic cancer

targeting peptides. Biopolymers. 2002, 66 (3): 184–199.
16. Zhasloff, M. Antimicrobial peptides of multicellular organisms. Nature. 2002, 415: 389-395.
17. Brogden K.A. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? Nat. Rev.Microbiol. 2005, 3: 238-250.
18. Mader J.S., Hoskin, D.W. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin. Investig. Drugs. 2006, 15: 933-946.
19. Kim Y.J., Varki, A. Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glycoconj.J. 1997, 14: 569-576.
20. Lindgren M., Rosenthal-Aizman K., Saar, K., Eiriksdottir, E., Jiang, Y., Sassian, M., Ostlund, P., Hallbrink, M., and Langel, U. Overcoming methotrexate resistance in breast cancer tumour cells by the use of a new cell-penetrating peptide. Biochem. Pharmacol. 2006, 71: 416-425.
21. Liang, J.F., and Yang, V.C. Synthesis of doxorubicin-peptide conjugate with multidrug resistant tumor cell killing activity. Bioorg. Med. Chem. Lett. 2005, 15: 5071-5075.
22. Steiner H, Hultmark D., Engstrom A, Bennich H, Boman HG. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature. 1981; 292 (5820):246-248.
23. Lehrer Rl. Primate defensins. Nat. Rev. Microbiol. 2004, 2 (9): 727-738.
24. Hancock REW. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect. Dis. 2001, 1 (3):156-164.
25. Koczulla AR, Bals R. Antimicrobial peptides: current status and therapeutic potential. Drugs. 2003, 63 (4):389-406.
26. Otvos L. Jr. Antibacterail paptides and proteins with multiple cellular targets. J. Pept. Sci. 2005, 11 (11): 697-706.
27. Powers J-PS., Hanckock REW. The relationship between peptide structure and antibacterial activity. Peptides 2003, 24 (11):1681-1691.
28. Nicolaou KC, Murphy F., Barluenga S., Ohshima T., Wei H, Xu J., Gray DLF, Baudoin O. Total synthesis of the novel immunosuppressant sanglifehrin A, J.Am.Chem.Soc. 2000, 122: 3830-3838.
29. White J.D., Hong J., Robarge LA. Total synthesis of cryptophycins-1, -3, -4 and -24 (arenastatin A0 and -29, cytotoxic depsipeptides from cyanobacteria of the nostocacae. J. Org. Chem. 1999, 64: 6206-6216.
30. Xia Z., Smith CD. Total synthesis of dendroamide A, a novel cyclic peptide that reverses multiple drug resistance. J. Org. Chem. 2001, 66:3459-3466.
31. Boger DL, Zhou J., Borzilleri RM., Nukii S., Castle SL. Synthesis of (9R, 12 S)- and (9S, 12 S)-cycloisodityrosine and their N-methyl derivatives. J. Org. Chem. 1997, 62:2054-2069.
32. Inoue T., Inaba T., Umezawa I., Yuasa M., Itokawa H., Ogura K., Komatsu K, Hara H, Hoshino O. Reglose-lective synthesis of 14-membered blaryl ethers. Total synthesis of RA V11 and deoxybouvardin. Chem. Pharm. Bull. 1995, 43: 1325-1335.
33. Davies J.S. REW. The cyclization of peptides and depsipeptides. J. Peptide Sci. 2003, 9:471-501.
34. Vives, E., Schmidt, J., and Pelegrin, A. Cell-penetrating and cell-targeting peptides in drug delivery. Biochim. Biophys. Acta. 2008, 1786: 126-38.
35. Myrberg, H., Zhang, L., Mae, M., and Langel, U. Design of a tumor-homing cell-penetrating peptide. Bioconjug.Chem. 2008, 19: 70-75.
36. Jiang, T., Olson, E.S., Nguyen, Q.T., Roy, M., Jennings, P.A., and Tsien, R.Y. Tumor imaging by means of proteolytic activation of cell-penetrating peptides. Proc. Natl. Acad. Sci. USA. 2004, 101:17867-72.
37. Pipkorn, R., Waldeck, W., Spring, H., Jenne, J.W., and Braun, K. Delivery of substances and their target-specific topical activation. Biochim. Biophys. Acta. 2006, 1758: 606-10.
38. Telmer, P. G.; Tolg, C.; McCarthy, J. B.; and Turley, E. A. How does protein with dual mitotic spindle and extracellular matrix receptor functions affect tumor susceptibility and progression? Commun. Integr. Biol. 2011, 4(2): 182-185.
39. Laurent, T. C., and Fraser, J. R. E. Hyaluronan. FASEB J. 1992, 6: 2397-2404.
40. Laurent, T. C. Biochemistry of Hyaluronan. Acta Otolaryngol Suppl.1987, 442: 7-24.
41. Lee, J. Y.; and Spicer, A. P. Hyaluronan: a multifunctional, megaDalton, stealth molecule. Curr. Opin. Cell Biol. 2000, 12(5): 581-586.
42. Tzircotis G, Thorne RF, Isacke CM. Chemotaxis towards hyaluronan is dependent on CD44 expression and modulated by cell type variation in CD44-hyaluronan binding. J Cell Sci. 2005, 118:5119–5128.
43. Udabage L, Brownlee GR, Nilsson SK, Brown TJ. The over-expression of HAS2, Hyal-2 and CD44 is implicated in the invasiveness of breast cancer. Exp Cell Res. 2005, 310:205–217.
44. Bourguignon LY, Singleton PA, Zhu H, Zhou B. Hyaluronan promotes signaling interaction between CD44 and the transforming growth factor beta receptor I in metastatic breast tumor cells. J. Biol. Chem. 2002, 277:39703–39712.
45. Naot, D., Sionov, R.V., and Ish-Shalom, D. CD44: Structure, function, and association with the malignant process. Adv. Cancer Res.1997, 71: 241-319.
46. Turley E.A., Noble P.W., Bourguignon L.Y. Signaling properties of hyaluronan receptors. J. Biol. Chem. 2002, 277: 4589–4592.
47. Adamia S., Maxwell C.A., Pilarski L.M. Hyaluronan and hyaluronan synthases: potential therapeutic targets in cancer. Curr. Drug Targets Cardiovasc. Haematol. Disord. 2005, 5: 3–14
48. Hardwick C., Hoare K., Owens R., Hohn H.P., Hook M., Moore D., Cripps V., Austen L., Nance D.M., Turley E.A. Molecular cloning of a novel hyaluronan receptor that mediates tumor cell motility. J. Cell. Biol. 1992, 117:1343–1350.
49. Hall C.L., Yang B., Yang X., Zhang S., Turley M., Samuel S., Lange L.A., Wang C., Curpen G.D., Savani R.C., Greenberg A.H., Turley E.A. Overexpression of the hyaluronan receptor RHAMM is transforming and is also required for H-ras transformation. Cell. 1995, 82:19–26.
50. Maxwell C.A., Keats J.J., Crainie M., Sun X., Yen T., Shibuya E., Hendzel M., Chan G., Pilarski L.M. RHAMM is a centrosomal protein that interacts with dynein and maintains spindle pole stability. Mol. Biol. Cell. 2003,14:2262–2276.
51. Assmann V., Jenkinson D., Marshall J.F., Hart I.R. The intracellular hyaluronan receptor RHAMM/IHABP interacts with microtubule and actin filaments. J. Cell. Sci. 1999, 112 (Pt 22):3943–3954.
52. Joukov V., Groen A.C., Prokhorova T., Gerson R., White E., Rodriguez A., Walter J.C., Livingston D.M. The BRCA1/BARD1 heterodimer modulates ran-dependent mitotic spindle assembly. Cell. 2006, 127:539–552.
53. Gust K. M., Hofer M. D., Perner S.R., Chinnaiyan A. M., Varambally S., Moller P., Rinnab L., Rubin M. A., Greiner J., Schmitt M., Kuefer R., and Ringhoffer M. RHAMM (CD168) is overexpressed at the protein level and may constitute an immunogenic antigen in advanced prostate cancer disease. Neoplasia. 2009, 11(9): 956-963.
54. Wang C., Thor A.D., Moore D.H., Zhao Y., Kerschmann R., Stern R., Watson P.H., and Turley E. A. The overexpression of RHAMM, a hyaluronan-binding protein that regulates ras signalling, correlates with overexpression of mitogen-activated protein kinase and is a significant parameter in breast cancer progression. Clin. Cancer Res.1998, 4(3): 567-576.
55. Greiner J., Ringhoffer M., Taniguchi M., Schmitt A., Kirchner D., Krähn G., Heilmann V., Gschwend J., Bergmann L., Döhner H., and Schmitt M. Receptor for hyaluronan acid-mediated motility (RHAMM) is a new immunogenic leukemia-associated antigen in acute and chromic myeloid leukemia. Exp. Hematol. 2002, 30(9): 1029-1035.
56. Assmann V., Marshall J. F., Fieber C., Hofmann M., and Hart I.R. The

human hyaluronan receptor RHAMM is expressed as an intracellular protein in breast cancer. J. Cell Sci. 1998, 111: 1685-1694.
57. Maxwell C. A., Rasmussen E., Zhan F., Keats J. J., Adarnia S., Strachan E., Crainie M., Walker R., Belch A. R., Pilarski L. M., Barlogie B., Shaughnessy J. Jr., and Reiman T. RHAMM expression and isoform balance predict aggressive disease and poor survival in multiple myeloma. Blood. 2004, 104(4): 1151-1158.
58. Crainie M., Belch A. R., Mant M. J., and Pilarski L. M. Overexpression of the receptor for hyaluronan-mediated motility (RHAMM) characterizes the malignant clone in multiple myeloma: identification of three distinct RHAMM variants. Blood. 1999, 93(5): 1684-1696.
59. Wang C., Thor A. D., Moore D. H., Zhao Y., Kerschmann R., Stern R., Watson P. H., Turley E. A. The overexpression of RHAMM, a hyaluronan binding protein that regulates ras signalling, correlates with overexpression of mitogen-activated protein kinase and is a significant parameter in breast cancer progression. Clin. Cancer Res. 1998, 4(3): 567-576.
60. Assman V., Gillett C. E., Poulsom R., Ryder K., Hart I. R., and Hanby A. M. The pattern of expression of the microtubule-binding protein RHAMM/IHAMP im mammary carcinoma suggests a role in the invasive behaviour of tumour cells. J. Pathol. 2001, 195(2): 191-196.
61. Tolg C., Hamilton S. J., Morningstar L., Zhang J., Esguerra K. V., Telmer P. G., Luyt L. G., Harrison R., McCarthy J. B., Turley E. A. RHAMM promotes interphase microtubule instability and mitotic spindle integrity trough MEK1/ERK1, 2 activity. J Biol. Chem., 2010, 285: 26461-26474.
62. Kurokawa H., Kazuto N., Fukumoto H., Tomonari A., Suzuki T., Saijo N. Alteration of caspase-3 (CPP32/Yama/apopaim) in wild-type MCF-7 breast cancer cells. Oncology Rep.1999, 6: 33-37.
63. Rizzardi A.E., Vogel R.I., Koopmeiners J.S., Forster C.L., Marston L.O., Rosener N.K., Akentieva N., Price M.A., Metzger G.J., Warlick C.A., Henriksen J.C., Turley E.A., McCarthy J.B., Schmechel S.C. 2014. Elevated hyaluronan and hyaluronan-mediated motility receptor are associated with biochemical failure in patients with intermediate-grade prostate tumors. Cancer. 120 (12), 1800-1809.
64. Sliva D., Rizzo M.T., English D. Phosphatidylinositol 3-kinase and NF-κB regulate motility of invasive MDA-MB-231 human breast cancer cells by the secretion of urokinase-type plasminogen activator. J. Biol. Chem. 2002, 277:3150-7.
65. Brunet A., Bonni A., Zigmond M.J., et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999, 96:857-68.
66. Byeong-Chel Lee, Tae-Hee Lee, Shalom Avraham and Hava Karsenty Avraham. Involvement of the Chemokine Receptor CXCR4 and Its Ligand Stromal Cell-Derived Factor 1α in Breast Cancer Cell Migration Through Human Brain Microvascular Endothelial Cells. Mol. Cancer. Res. 2004, 2: 327-337.
67. Muller A., Homey B., Soto H., et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001, 410:50-6.
68. Soule H.D., Vazguez J., Long, A., Albert S. and Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst. 1973, 51:1409-1416.
69. Levenson A.S., Jordan V.C. MCF-7: the first hormone-responsive breast cancer cell line. Cancer Res. 1997, 57: 3071-3078.
70. Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum-based anti-cancer chemotherapy. Cancer Treat. Rev. 1998, 24: 331-344.
71. Maxwell C.A., McCarthy J., Turley E. Cell-surface and mitotic-spindle Rhamm: moonlighting or dual oncogenic functions? J. Cell. Sci. 2008, 121: 925-932.
72. Hus I., Kawiak J., Tabarkiewicz J., Radej S., Hoser G., Bojarska-Junak A., Schmitt M., Giannopoulos K., Dmoszynska A., Rolinski J. Immunotherapy with irradiated autologous leukemic cells in patients with B-CLL in early stages. Oncol Rep. 2008, 20(2):443-51.
73. Zlobec I., Terraciano L., Tornullo L., Gunhert U., Vuong T., Jass J.R., Lugli A. Role of Rhamm within the hierarchy of well-established prognostic factors in colorectal cancer. Gur. 2008.
74. Yamano Y., Uzawa K., Shinozuka K., Fushimi K., Ishigami T., Nomura H., Ogawara K., Shiiba M., Yokoe H., Tanzawa H. Hyaluronan-mediated motility: a target in oral squamous cell carcinoma. Int. J. Oncol. 2008, 32: 10001-9.
75. Tolg C., Poon R., Fodde R., Turley E.A., Alman B.A. Genetic deletion of receptor for hyaluronan-mediated motility (Rhamm) attenuates the formation of aggressive fibromatosis (desmoids tumor). Oncogene. 2003, 22: 6873-82.
76. Rein D.T., Roehrig K., Schondorf T., Lazar A., Fleisch M., Niederacher D., Bender H.G., Dall P. Expression of the hyaluronan receptor Rhamm in endometrial carcinoma suggests a role in tumor progression and metastasis. J. Cancer Res. Clin. Oncol. 2003, 129: 161-4.
77. Sohr S. and Engeland K. Rhamm is differentially expressed in the cell cycle and downregulated by the tumor suppressor p53. Cell Cycle. 2008, 7 (21): 3448-3460.
78. Lippman M.E. and Bolan G. Oestrogen-responsive human breast cancer in long-term tissue culture. Nature. 1975, 256:592-593.
79. Seals D.F. et al. The adaptor protein Tks5/Fish is required for podosome formation and function, and for the protease-driven invasion of cancer cells. Cancer Cell. 2005, 7:155-165.
80. Yamaguchi H. et al. Molecular mechanisms of invadopodium formation: the role of the N-WASP-Arp2/3 complex pathway and cofilin. J. Cell. Biol. 2005, 168: 441-452.
81. Weaver A.M. Invadopodia : specialized cell structures for cancer invasion. Clin. Exp. Metastasis. 2006, 23: 97-105.
82. Diaz B. et al. Tks5-dependent, Nox-mediated generation of reactive oxygen species is necessary for invadopodia formation. Sci. Signal. 2009, 2:ra53.
83. Clark E.S. and Weaver A.M. A new role for cortactin in invadopodia: regulation of protease secretion. Eur. J. Cell Biol. 2008, 87:581-590.