Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Article
Volume 11, Issue 4, 2021, 12252 - 12266
https://doi.org/10.33263/BRIAC114.1225212266

RHAMM-Target Peptides Inhibit Proliferation and Viability of Cancer Cells

Natalia P. Akentieva [1,*], Alexey F. Topunov [2]

[1] Department Kinetics of Chemical and Biological Processes, Institute of Problems of Chemical Physics Russian Academy of Sciences, Academician Semenov Avenue 1, City Chernogolovka, Moscow Region142432, Russia; a_aken@icp.ac.ru (N.P.A.);
[2] Bach Institute of Biochemistry, Federal Research Center of Biotechnology, Russian Academy of Sciences, Leninsky prospect 33, Moscow 119071, Russia; aftopunov@yandex.ru (A.F.T.);
* Correspondence: na_aken@icp.ac.ru;

Scopus Author ID 56370037400
Received: 4.12.2020; Revised: 30.12.2020; Accepted: 1.01.2021; Published: 3.01.2021

Abstract: The incidence of cancer in the world is growing exponentially. Therefore, the search for targeted cancer therapy methods is the most urgent and actively developing the biomedicine field. This work is devoted to studying RHAMM-target peptides' effect on the proliferation and viability of ovarian cancer, prostate cancer, breast carcinoma, and adenocarcinoma of the breast duct cells. Cell proliferation was examined by a BrdU-based proliferation assay. Cell viability was assayed by the fluorescence method. It has been established that RHAMM-target peptides at a concentration of $2x10^{-7}$ M inhibited on ~ 55 % proliferation of MDA-MB-231 cells, on ~ 85 % proliferation of PC3m-LN4, and ~ 50 % proliferation of SKOV3 cells for 24 h. The results showed that the peptides inhibited the viability of ovarian cancer cells. In particular, peptide EEDFGEEAEEEA inhibited ovarian cancer cells' viability by 54%, peptide VEGEGEEGEEY by 63%, and peptide FTEAESNMNDLV by 57%. RHAMM-target peptides did not affect fibroblasts (non-tumor cells) and fibroblasts $RHAMM^{(-/-)}$. This work showed that RHAMM-target peptides at low concentrations of inhibited cancer cells' proliferation and viability. This effect was RHAMM mediated. RHAMM-target peptides are promising candidates for anti-cancer drugs.

Keywords: peptides; cancer; RHAMM/HMMR; proliferation; cell viability.

1. Introduction

Cancer is the second most fatal disease in the world after cardiovascular disease. Malignant neoplasms are still a severe problem to the scientific and medical community. Cancer is not a separate disease but a group of diseases characterized by abnormal cells' unregulated growth. The driving force behind this uncontrolled growth is a series of mutations that cause aberrant expression of gene products required to regulate cell proliferation, survival, and growth. Consequently, cancer arises from defects in the most basic biological functions of cells: the ability to respond to growth signals, activate cell death programs to eliminate unnecessary, excess, or damaged cells, and the formation of new blood vessels and the ability to penetrate tissues. Currently, prostate cancer is one of the most common malignant neoplasms in men. The incidence of prostate cancer ranks 7th-8th place (about 6%) [1]. A feature of prostate cancer in countries is the late diagnosis when the tumor is diagnosed at stage III-IV [2]. Breast cancer is the most common form of cancer among women globally, with an

incidence rate of 99.4 per 100,000 women aged 13 to 90 years [2]. Ovarian cancer is considered one of the most aggressive types of malignant tumors in women. According to statistics, more than 225 thousand new ovarian carcinoma cases are registered every year globally, of which about 140 thousand ends in death [3]. Despite the progress achieved in the diagnosis of ovarian carcinoma, about 75% of it is detected at the late stages. Therefore, the relevance of the development of targeted anti-cancer drugs is determined by the high incidence of oncological diseases worldwide, the low survival rate of cancer patients, and the expensive cancer treatment.

The challenge facing clinicians and researchers seeking effective therapeutic approaches for cancer treatment is to eliminate cancer cells while maintaining normal, healthy tissue. In a new era of personalized/precision medicine, the therapeutic treatment aims to use tumor- and patient-specific genetic and molecular characteristics to select specific targeted therapy for each patient [4–6]. The antitumor efficacy of a drug or compound is limited by its membrane permeability, targeted delivery, and tumor localization. The ability to target cancer tissues and concentrate on the desired therapeutic agent in these tissues can be achieved using tumor-specific markers, especially those found on the tumor or in the tumor vasculature. The identification and characterization of tumor markers is the foundation of the knowledge-based on which tumor-targeting peptides (TTP), also known as homing peptides, are developed. Tumor-targeted peptides can bind directly to a tumor marker (receptors) and, as a consequence, internalize the drug (cargo) into the cell. One of the first CPPs to be discovered was the TAT peptide, obtained from the human immunodeficiency virus [7, 8]. Tumor-targeted peptides hold great promise because their main mechanism of action targets a specific molecular target of tumor cells, which leads to their death. Therefore, the identification of such peptides, the study of the molecular mechanisms of their interaction with receptors in tumor cells is very urgent.

The RHAMM/HMMR oncoreceptor (hyaluronan-mediated motility receptor) is such a tumor marker for diagnosing and treating cancer [9]. RHAMM/HMMR is a multifunctional protein that can reside inside and on the cell surface [10, 11]. Intracellular RHAMM is a nuclear protein that binds to mitotic spindle tubulin as well as motor proteins such as dynein [12, 13]. RHAMM plays a key role in the formation of the mitotic spindle, as well as in the regulation of correct chromosomal segregation and genomic stability [14, 15]. It has been established that overexpression of RHAMM, as well as its absence, leads to deformation of the mitotic spindle [16]. These effects of RHAMM are a consequence of its docking with MEK1/ERK1,2 since damaged mitotic spindles resulting from loss of RHAMM expression can be restored by MEK1 activity [17].

Hyaluronic acid (HA) is a physiological ligand and signaling molecule of the RHAMM receptor [18, 19]. HA plays an important role in the progression and prognosis of cancer through RHAMM-mediated signaling pathways. Hyaluronic acid is an unsulfonated poly-(2-acetamido-2-deoxy-D-gluco)-D-glucuronoglycan composed of repeating residues of D-glucuronic acid and D-N-acetylglucosamine. Increased levels of HA and RHAMM are observed in various types of tumors, including carcinomas of breast, prostate, lung, bladder, and ovarian cancer [20-23]. It has been demonstrated that HA specifically binds with high affinity to the RHAMM of intact cells [24, 25]. Later, it was found that RHAMM and HA play an important role in the processes of proliferation, migration, invasion, angiogenesis of tumor cells, inflammation, wound healing, as well as in the development and progression of malignant neoplasms [26-31]. RHAMM is also involved in regulating the cell cycle since its expression

increases in the G2/M phase of the cell cycle [32, 33]. As predicted, due to the ability of RHAMM to control gene expression of the cell cycle, RHAMM also affects cell proliferation, and blocking surface RHAMM or RHAMM in the mitotic spindle leads to blocking the G2/M phase of the cell cycle [34, 35].

It was shown on model tumor systems that the RHAMM receptor contains a binding site for HA, tubulin, and special regions necessary to manifest its oncogenic properties [36, 37].

Thus, the development of novel potent inhibitors designed to compete with HA and block the RHAMM-receptor and its downstream signaling pathway is urgently needed. RHAMM-target peptides that specifically interact with the RHAMM receptor have been identified and characterized earlier [38, 39]. It was shown that RHAMM-target peptides compete for the binding site with HA, selectively bind with recombinant RHAMM, and easily penetrate cancer cells [39]. However, RHAMM-target peptides' effect on the proliferation of tumor cells in the breast, prostate, and ovarian cancers has not been investigated. This work is devoted to studying the effect of RHAMM target peptides on tumor cells' proliferation and cell viability.

2. Materials and Methods

2.1. Materials.

DMEM (Dulbecco's modified Eagle's medium) and FBS (fetal bovine serum) were purchased from Multicell (Canada), HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid) 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), the cell proliferation ELISA, BrdU (colorimetric) kit was purchased from Roche Diagnostic (Laval, Quebec, Canada), AlamarBlue reagent was purchased from Invitrogen (USA).

2.2. Cell lines and cell culture.

The following cancer cells were used in work: human breast carcinoma cell line (MDA-MB-231), prostate cancer cell line (PC3m-LN4), ovarian cancer cell line (SKVO3), epithelial-like cell line derived from invasive adenocarcinoma of the human breast duct (MCF-7). Also, MEF (mouse embryonal fibroblasts) were used in this work. All cell lines were obtained from the American Type Culture Collection (Manassas, Virginia, Merck, USA). All these tumor cells, except MCF-7, are characterized by increased expression of RHAMM (Oncomine Research, https://www.oncomine.org/resource/login.html). MEF-RHAMM$^{(-/-)}$ cells were obtained according to the protocol described in the article [40]. The origin of all the indicated cell lines: human, mouse. Cells cultured in DMEM growth media supplemented with 10% (v/v) FBS and 10 mM HEPES at pH 7.2. All cultures were incubated in a humidified atmosphere of 5% CO_2 at 37^0C.

2.3. Peptide synthesis.

RHAMM-target peptides (EEDFGEEAEEEA, VEGEGEEGEEY, FTEAESNMNDLV) were obtained as previously described [38, 39].

2.4. Cell proliferation ELISA BrdU (colorimetric) assay.

Prostate cancer cell line (PC3M-LN4), tumor cells of ovarian cancer (SKOV3), and human breast cancer cells (MDA-MB-231), adenocarcinoma of the human breast duct (MCF-7) were cultured in flat-bottomed 96-well plates (tissue culture grade) at density 1, 000 cells/well and allowed cells to adhere and grow for 24 hrs in an incubator at 37°C, 5 % CO_2 and 95 % humidity, before proceeding with the assay. Then RHAMM-target peptides 10 мкг/мл ($2x10^{-7}$M) were added to cells and incubated for 24 hrs at 37°C or left untreated as the control. The cell proliferation ELISA (Enzyme-linked immunosorbent assay), BrdU (colorimetric) kit (Roche Diagnostic, Laval, Canada) has been used for the quantification of cell proliferation. Subsequently, BrdU (5-bromo-2'-deoxyuridine, 100 µM) was added to the cells (10 µl/well), and the cells were re-incubated for 24 hrs. Then the culture medium was removed, and cells were fixed. DNA was denatured by adding FixDenat solution (90 % ethanol, incubated 5 min, following aspiration of ethanol and adding 2M HCl/0.5 % Triton X-100, 30 min incubation). The FixDenat solution was removed thoroughly by flicking off and tapping. Anti-BrdU antibody-working solution (100 µl/well) was added and incubated for 90 min at 25°C. Antibody conjugate was removed by flicking off, and wells were rinsed three times with 300 µl/well washing solution (1x PBS). The washing solution was then removed by tapping and adding 100 µl/well TMB (3, 3′, 5, 5′-tetramethylbenzidine substrate solution). The reaction was incubated at 25°C for 15 min. The reaction product was quantified by measuring absorbance in a microplate ELISA reader at 450 nm (reference wavelength: 690 nm).

2.5.Cell viability assay.

To analyze RHAMM-target peptides' effect on cells' viability, we used a fluorescent Alamar Blue®Cell Viability Assay (ThermoFisher Scientific, USA), the active ingredient of which is resazurin. This non-toxic, cell-permeable dye has a "blue" color and weak fluorescence. This method measures the activity of mitochondrial NADH dehydrogenases, which break down NADH into NAD and H^+, and the resulting proton reduces the "blue", weakly fluorescent resazurin to resofurin. Reduced resofurin is pink in color and highly fluorescent [41].

Ovarian cancer cell line (SKVO3), adenocarcinoma of the human breast duct (MCF-7), mouse embryonal fibroblasts (MEF), and MEF-RHAMM$^{(-/-)}$ cells were cultured in a 96-well plate (tissue culture grade) at a density of 1,000 cells/well and allowed cells to adhere and grow for 24 hrs in an incubator at 37°C, 5 % CO_2 and 95 % humidity, before proceeding with the assay. Then RHAMM-target peptides 10 мкг/мл ($2x10^{-7}$M) were added to cells and incubated for 48 hrs at 37℃ or left untreated as the control. Then 1/10th volume of Alamar Blue reagent (resazurin) was added directly to cells in culture medium and measured fluorescence in 48 hrs. Fluorescence intensity was measured at 570/590 nm, using a fluorescence microplate reader.

2.6. Statistical analysis.

Prism 4 software (GraphPad Software, Inc.) was used to perform tests for statistical significance. All experiments were performed in triplicate and repeated three times. For statistical comparison, one-way analysis of variance (ANOVA) was used with *P<0.05 considered significant.

3. Results and Discussion

3.1. RHAMM-target peptides inhibited cell proliferation of cancer cells.

We measured the effect of RHAMM-target peptides on the proliferation of various cancer cells in which RHAMM expression levels vary from high (PC3m-LN4, MDA-MB-231, SKVO3 cell lines) to low (MCF-7 cell line) [42, 43]. To clarify whether RHAMM-target peptides can inhibit the growth of breast cancer cells, we treated MDA-MB-231 cells with RHAMM-target peptides at a low concentration of 10 мкг/мл ($2x10^{-7}$M) for 24 hrs. Then cell proliferation was examined by an ELISA BrdU-based proliferation assay described in "Materials and methods". Results showed that RHAMM-target peptides inhibited the proliferation of MDA-MB-231 cells (Figure 1).

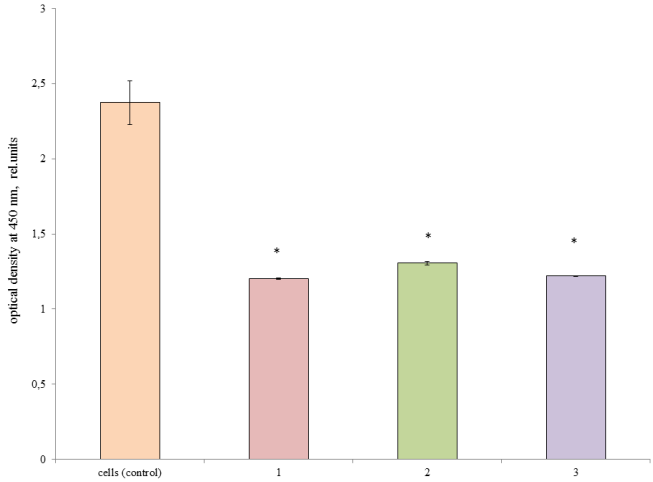

Figure 1. Effect of RHAMM-target peptides on the proliferation of MDA-MB-231 cells: cells (without peptides, control), 1-cells treated with peptide EEDFGEEAEEEA, 2- cells treated with peptide VEGEGEEGEEY, 3-cells treated with peptide FTEAESNMNDLV.

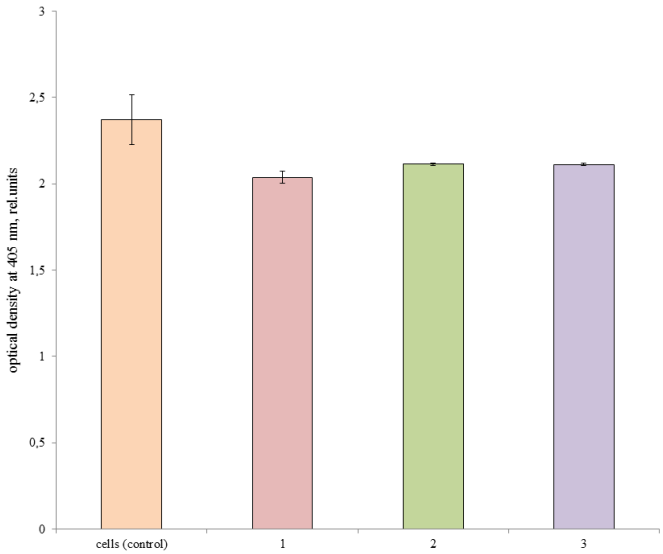

Figure 2. Effect of RHAMM-target peptides on the proliferation of MCF-7 cells: cells (without peptides, control), 1-cells treated with peptide EEDFGEEAEEEA, 2- cells treated with peptide VEGEGEEGEEY, 3-cells treated with peptide FTEAESNMNDLV.

All three peptides demonstrated the inhibition of proliferation of MDA-MB-231 cells up to 55 %. At the same time, treatment of MCF-7 cells, characterized by low expression of

RHAMM receptor, showed that the RHAMM-target peptides had practically no effect on the proliferation of these cells (Figure 2).

Thus, we have shown that RHAMM-target peptides act selectively on cancer cells, inhibit cell proliferation with a high receptor content, and do not affect cells with a low receptor content. This indicates that the action of the peptides is RHAMM mediated. In addition, functional analysis with RHAMM target peptides showed that these peptides restrict the proliferation of MDA-MB-231 cells at low peptide concentration ($2x10^{-7}$M).

Then we measured RHAMM-target peptides' effect on the proliferation of the ovarian cancer cell line (SKVO3), characterized by overexpression of the RHAMM receptor [44]. The results showed that incubation of cells SKVO3 with peptides for 24 hours of inhibited cell proliferation by ~ 50% (Figure 3).

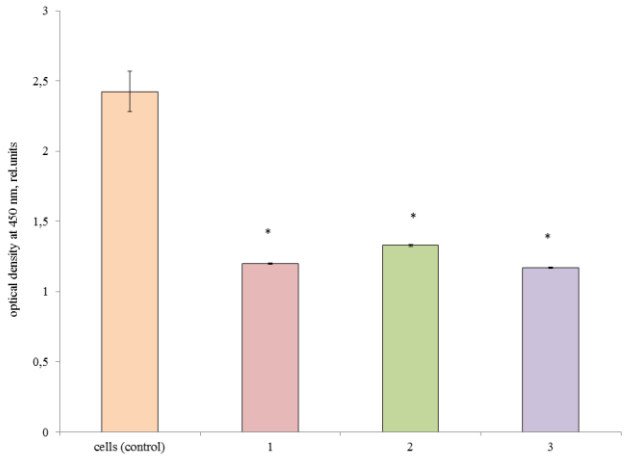

Figure 3. Effect of RHAMM-target peptides on the proliferation of SKVO3 cells: cells (without peptides, control), 1-cells treated with peptide EEDFGEEAEEEA, 2- cells treated with peptide VEGEGEEGEEY, 3-cells treated with peptide FTEAESNMNDLV.

We also investigated the effect of the RHAMM-target peptide on the proliferation of prostate cancer cells (PC3m-LN4), which are also characterized by increased expression of RHAMM receptor [45, 46]. The study results showed that the peptides (at concentration $2x10^{-7}$M) significantly inhibited prostate cell proliferation by up to 85% (Figure 4).

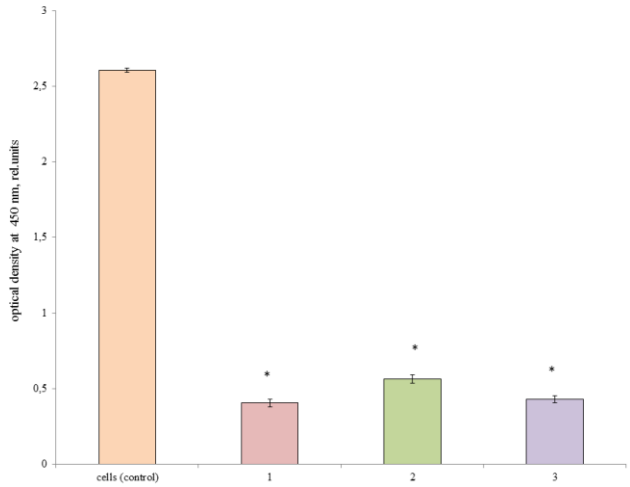

Figure 4. Effect of RHAMM-target peptides on the proliferation of PC3m-LN4 cells: cells (without peptides, control), 1-cells treated with peptide EEDFGEEAEEEA, 2- cells treated with peptide VEGEGEEGEEY, 3-cells treated with peptide FTEAESNMNDLV.

Thus, the peptides suppressed prostate cells' proliferation, most strongly in comparison with other cell lines. This is probably due to the physiological, metabolic characteristics of prostate cells and the highest expression of the RHAMM receptor in these cells.

It should be emphasized that the proliferation of cancer cells was significantly suppressed by low concentrations of RHAMM-target peptides, which indicates that RHAMM-target peptides are promising candidates for anti-cancer drugs.

3.2. RHAMM-target peptides inhibit the viability of cancer cells.

Because the RHAMM-target peptides inhibited cancer cells' proliferation, we would like to examine the effect of these peptides on the viability of cancer cells. For this, we measured the effect of RHAMM-target peptides on the viability of ovarian cancer (SKVO3) and adenocarcinoma of the human breast duct (MCF-7) cells. RHAMM-target peptides ($2x10^{-7}$M) were incubated with cells for 48 hours. Cell viability was assayed using resazurin (AlamarBlue reagent) as described in Research Methods. The results showed that the peptides inhibited the viability of ovarian cancer cells (Figure 5).

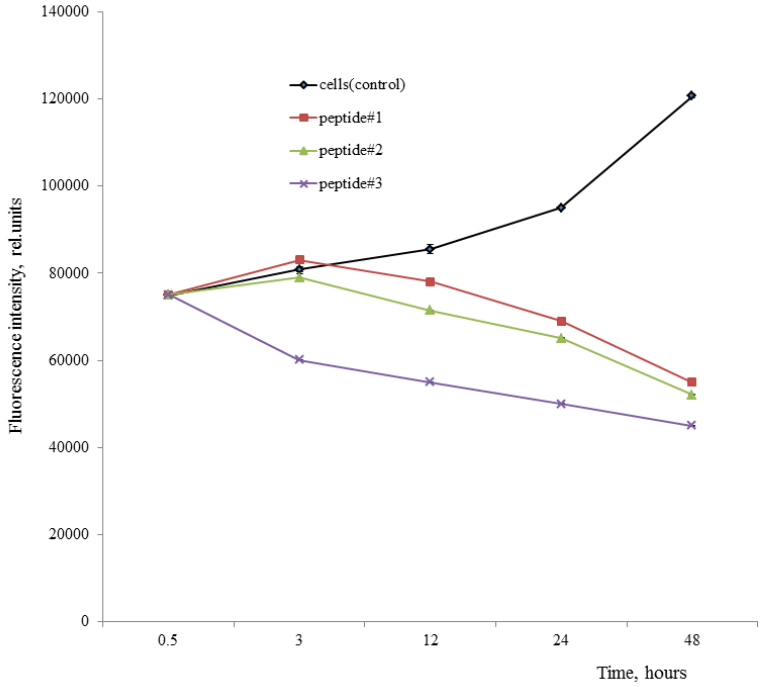

Figure 5. Effect of RHAMM-target peptides on cell viability ovarian cancer cells (SKVO3): cells (without peptides, control), 1-cells treated with peptide EEDFGEEAEEEA, 2- cells treated with peptide VEGEGEEGEEY, 3-cells treated with peptide FTEAESNMNDLV. The values obtained after peptide treatment are statistically significant relative to the control, *P <0.05.

At the same time, the treatment of MCF-7 cells with peptides showed that they insignificantly affected cell viability. Besides, we showed that peptides also did not affect the viability of fibroblasts and RHAMM-knockout cells (Table 1).

This indicates the selective action of the RHAMM-target peptides on certain tumor cell lines characterized by a high level of RHAMM expression. This is consistent with previously published data that RHAMM is important for peptide action [47].

Thus, our results showed that RHAMM-target peptides inhibit cancer cells' proliferation and viability, characterized by a high level of RHAMM expression. This indicates that the peptides' action is RHAMM-mediated and is probably due to blocking the receptor and

affecting its signaling pathways. These data suggest that RHAMM-target peptides can be used as drugs for targeted therapy of cancer.

Table 1. Effect of RHAMM-target peptides on cell viability MCF-7, MEF, and MEF- RHAMM$^{(-/-)}$ cells. The values obtained after peptide treatment are statistically significant relative to the control, *P <0.05.

#	Cell type	Cell viability (fluorescence intensity at 590 nm, rel. units)
1	MCF-7	16243
2	MCF-7+peptide EEDFGEEAEEEA	13332
3	MCF-7+peptide VEGEGEEGEEY	14536
4	MCF-7+peptide FTEAESNMNDLV	15128
5	MEF	14625
6	MEF +peptide EEDFGEEAEEEA	13981
7	MEF+peptide VEGEGEEGEEY	13256
8	MEF+peptide FTEAESNMNDLV	13016
9	MEF-RHAMM$^{(-/-)}$	14869
10	MEF-RHAMM$^{(-/-)}$ + peptide EEDFGEEAEEEA	13987
11	MEF- RHAMM$^{(-/-)}$ + peptide VEGEGEEGEEY	13568
12	MEF-RHAMM$^{(-/-)}$ + peptide FTEAESNMNDLV	13201

Currently, chemotherapy is used as the first step treatment for cancer therapy. However, platinum-based chemotherapy causes severe toxicity to cancer cells. It destroys healthy cells with a rapid replication rate due to its ability to disrupt DNA repair and produce side effects [48, 49]. The need for novel anti-cancer drugs with higher specificity and selective targeting of malignant tissues is increasingly urgent.

RHAMM is the ideal molecular target for anti-cancer therapeutic agents because it is hyper expressed in tumor tissues, such as breast, prostate, ovarian cancer, leukemia, colon cancer, and endometrial carcinoma as multiple myeloma [16, 50-56]. RHAMM mRNA and protein expression are downregulated by p53, a central regulator of the cell cycle, tumor suppressor, and major mediator of apoptosis in response to stress signals [17, 32-34, 57, 58]. Intracellularly RHAMM interacts with the tumor suppressor BRCA1 at the mitotic spindle regulating spindle integrity and stability [16, 35, 36]. Based on this, blocking RHAMM with selective drugs (peptides) will inhibit its function and probably, mimic the action of p53, which inhibit tumor spread and growth. Additionally, probably, posttranscriptional regulation and protein stability are also important in the regulation of RHAMM function.

Previously, we demonstrated that HA-mimetic peptides interact with the HA binding domain of RHAMM with high selectivity and specificity [39]. These HA-mimetics peptides have an analogous distribution of negative charges, such as seen in HA. They interact with cell surface RHAMM and easily penetrate inside of cancer cells. We demonstrated that these RHAMM-target peptides were stable in serum. They blocked RHAMM/HA interactions in various *in vitro* assays. These RHAMM-target peptides probably bind to MAPs and motor proteins because their molecules were possessing a similar degree of negative charges, such as seen in HA. Our previous study showed that these RHAMM-target peptides induced apoptosis and inhibited cell viability of breast and prostate cancer cells [39, 59]. These results indicated that these RHAMM-target peptides might be used as therapeutic agents for treating cancers. RHAMM and HA hyper-expression or accumulation are linked to poor outcome [60-67]. However, the effect of RHAMM-target peptides on cell proliferation MDA-MB-231, PC3m-LN4, SKVO3, MCF-7, and on cell viability MCF-7, SKVO3, MEF, MEF-RHAMM$^{(-/-)}$ cells has not been studied. Therefore, this work was devoted to these studies. It is known that cancer cells have differences in transcriptional activity, signal transduction, and metabolism, so they respond differently to drugs.

In the present work, we examined RHAMM-target peptides' effect on cancer cells, using different cell lines: MDA-MB-231, PC3m-LN4, SKVO3, and MCF-7. Analysis of the data in Oncomine (www.oncomine.org) showed that all cells, except MCF-7, have an RHAMM overexpression profile [68, 69]. However, since these cancer cell lines have different metabolism and RHAMM expression levels, we expected to demonstrate the different responses to RHAMM-target peptides treatment. It is known that breast tumor MDA-MB-231 cells are epithelial, adenocarcinoma cells with highly metastatic characteristics, higher RHAMM, CXCR4 expression, constitutively active PI-3K/AKT pathway, higher SDF-1α expression, and higher cell motility and migration [70-73]. Breast cancer MDA-MB-231 cells preferentially metastasize to the lymph node, bone marrow, lung, and liver [74, 75]. In contrast, the MCF-7 cells are luminal cell lines retained several characteristics mammary epithelium, including the ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes. They show a lower RHAMM expression level [76-78]. It was demonstrated that tumor necrosis factor-alpha (TNF alpha) inhibits MCF-7 breast cancer cells' growth, and treatment with anti-estrogens can modulate the secretion of insulin-like growth factor-binding proteins [79-81].

It is known that an ovarian cancer cell line (SKOV3) was derived from the ascites of a female with an ovarian serous cystadenocarcinoma [82]. Ovarian cancer cells are hormone-sensitive. They express hormone receptors, the progesterone receptor, and the estrogen receptor, which correlate with cancer patients' survival [83]. It has been shown that overexpression RHAMM is a prognostic indicator for ovarian cancer [44, 83]. It was found that RHAMM expression is increased in ovarian cancer cells as compared to normal cells [44, 84]. It was shown that in 91% of patients with ovarian cancer, positive RHAMM staining was found, which was localized mainly in the cytoplasm, cell membrane, cystic fluid, and, sometimes, in the nucleus [44]. The increase in RHAMM levels observed in ovarian cancer has been demonstrated to correlate with invasiveness and disease progression [44].

Cells prostate carcinoma (PC3m-LN4) derived from metastatic site bone are hormone-sensitive and also have several features [85]. In particular, it was shown that these cells have novel molecular features and major genetic alterations, including hypermethylation of tumor-suppressor genes [85, 86]. It was found that in prostate tumors and metastases, there is also a significant overexpression of RHAMM compared to benign prostate tissue [87-91]. In addition, it showed that androgen stimulation leads to overexpression of RHAMM and hyaluronan-stimulated activation of the RHAMM-ROCK1 cascade in PC3m-LN4 cells, suggesting that RHAMM plays a role in androgen-dependent as well as in the castration-resistant stage of prostate cancer [88, 89].

Thus, scientific research results have shown that these cell lines have different metabolic and physiological characteristics. This study showed that RHAMM-target peptides significantly inhibited the proliferation of cancer cells (PC3m-LN4, SKVO3, and MDA-MB-231). However, their effect on MCF-7 cells was not significant. Different effects of RHAMM-target peptides on MDA-MB-231 and MCF-7 cells can be explained by a lower level RHAMM expression in MCF-7 cells compared with MDA-MB-231 cells. This effect of RHAMM-target peptides on cells is consistent with the interaction of RHAMM with the mitotic spindle, effect RHAMM on microtubule spicing, stability, and regulator role of RHAMM in the cell cycle [92-95]. We can suggest that RHAMM-target peptides bind with RHAMM, suppress its function, and inhibit proliferation of PC3m-LN4, SKVO3, and MDA-MB-231 cancer cells. In the case of a low expression of the RHAMM receptor in the cells, the peptides cannot

significantly affect the cell viability. In the complete absence of the receptor, peptides cannot bind to the receptor and affect its function. Their results indicate that RHAMM-target peptides' effect on the proliferation of cancer cells is selective and RHAMM mediated. We also demonstrated that RHAMM–target peptides inhibited cancer cells' proliferation at low concentration, which is a real benefit for using these peptides as anti-cancer agents.

4. Conclusions

Based on our results, we can conclude that RHAMM–target peptides selectively attack the cancer cells. They promise anti-cancer agents. The strategy of suppressing the viability and proliferation of cancer cells for established tumors can improve clinical outcomes. In general, our results indicate that RHAMM–target peptides are attractive anti-cancer agents because they inhibit the proliferation and cell viability of different cancer cells. These findings suggest that RHAMM–target peptides can be used as peptide drugs that are target-specific, cost-effective, and easily designed for cancer therapy. We can conclude that RHAMM-targeted peptides are potential therapeutic agents. In the future, targeted cancer peptides that can specifically kill tumor cells will avoid side effects, lower doses of the chemotherapeutic drugs, and decrease cancer cells' chemo-resistance, prevent metastasis.

Funding

This research was funded by the Ministry of Science and Higher Education of the Russian Federation and performed by the state task, state registration # AAAA-A19-119071890015-6.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Valerio, M.; Cerantola, Y.; Eggener, S.E.; Lepor, H.; Polascik, T.J.; Villers, A.; Emberton, M. New and established technology in focal ablation of the prostate: a systematic review. *Eur Urol.* **2017,** *71*, 17-34. https://doi: 10.1016/j.eururo.2016.08.044.
2. WHO. Breast cancer: prevention and control, **2019**. Available online: https://www.who.int/cancer/detection/breastcancer/en/.
3. Nikogosyan, S.O.; Kuznetsov, V.V. Sovremennaya diagnostika raka yaichnikov [Modern diagnosis of ovarian cancer]. *Rossiiskii onkologicheskii zhurnal [Russian Journal of Oncology]* **2013**, 52-56.
4. Dey, N.; Williams, C.; Leyland-Jones, B.; De, P. Mutation matters in precision medicine: A future to believe in. *Cancer Treat. Rev.* **2017**, *55*, 136-149, https://doi.org/10.1016/j.ctrv.2017.03.002.
5. Phillips, K.A.; Deverka, P.A.; Sox, H.C.; Khoury, M.J.; Sandy, L.G.; Ginsburg, G.S.; Tunis, S.R.; Orlando, L.A.; Douglas, M.P. Making genomic medicine evidence-based and patient-centered: a structured review and landscape analysis of comparative effectiveness research. *Genet. Med.* **2017**, *19*, 1081-1091, https://doi,org/10.1038/gim.2017.21.
6. Roukos, D.H. Spatiotemporal diversification of intrapatient genomic clones and early drug development concepts realize the roadmap of precision cancer medicine. *Drug Discov. Today* **2017**, *22*, 1148-1164, https://doi.org/10.1016/j.drudis.2017.03.014.

7. Bermejo-Jambrina, M.; Eder, J.; Helgers, L.C.; Hertoghs, N.; Nijmeijer, B.M.; Stunnenberg, M.; Geijtenbeek, T.B.H. C-type lectin receptors in antiviral immunity and viral escape. *Front. Immunol.* **2018**, *9*, 590, https://doi.org/10.3389/fimmu.2018.00590.
8. Ates, I.; Rathbone, T.; Stuart, C.; Bridges, P.H.; Cottle, R.N. Delivery Approaches for Therapeutic Genome Editing and Challenges. *Genes* **2020**, *11*, 1113, https://doi.org/10.3390/genes11101113.
9. Abatangelo, G.; Vindigni, V.; Avruscio, G.; Pandis, L.; Brun, P. Hyaluronic acid: redefining its role. *Cells* **2020**, *9*, 1743, https://doi.org/10.3390/cells9071743.
10. Underhill, C.B.; Toole, B.P. Binding of hyaluronate to the surface of cultured cells. *J. Cell Biol.* **1979**, *82*, 475-484, https://doi.org/10.1083/jcb.82.2.475.
11. Peters, A.; Sherman, L.S. Diverse Roles for Hyaluronan and Hyaluronan Receptors in the Developing and Adult Nervous System. *Int. J. Mol. Sci.* **2020**, *21*, 5988, https://doi.org/10.3390/ijms21175988.
12. Leng, Y.; Abdullah, A.; Wendt, M.K.; Calve, S. Hyaluronic acid, CD44 and RHAMM regulate myoblast behavior during embryogenesis. *Matrix Biol.* **2019**, *78-79*, 236-254, https://doi.org/10.1016/j.matbio.2018.08.008.
13. Assmann, V.; Gillett, C.E.; Poulsom, R.; Ryder, K.; Hart, I.R.; Hanby, A.M. The pattern of expression of the microtubule-binding protein RHAMM/IHABP in mammary carcinoma suggests a role in the invasive behaviour of tumour cells. *The Journal of Pathology* **2001**, *195*, 191-196, https://doi.org/10.1002/path.941.
14. Garantziotis, S.; Savani, R.C. Hyaluronan biology: A complex balancing act of structure, function, location and context. *Matrix Biol.* **2019**, *78-79*, 1-10, https://doi.org/10.1016/j.matbio.2019.02.002.
15. Jiang, J.; Mohan, P.; Maxwell, C.A. The Cytoskeletal Protein RHAMM and ERK1/2 Activity Maintain the Pluripotency of Murine Embryonic Stem Cells. *PLoS One* **2013**, *8*, e73548, https://doi.org/10.1371/journal.pone.0073548.
16. Maxwell, C.A.; Keats, J.J.; Belch, A.R.; Pilarski, L.M.; Reiman, T. Receptor for hyaluronan-mediated motility correlates with centrosome abnormalities in multiple myeloma and maintains mitotic integrity. *Cancer Res.* **2005**, *65*, 850-860.
17. Tolg, C.; Hamilton, S.R.; Morningstar, L.; Zhang, J.; Zhang, S.; Esguerra, K.V.; Telmer, P.G.; Luyt, L.G.; Harrison, R.; McCarthy, J.B. RHAMM promotes interphase microtubule instability and mitotic spindle integrity through MEK1/ERK1/2 activity. *J. Biol. Chem.* **2010**, *285*, 26461-26474, https://doi.org/10.1074/jbc.M110.121491.
18. Lennon, F.E.; Singleton, P.A. Role of hyaluronan and hyaluronan-binding proteins in lung pathobiology. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **2011**, *301*, L137-L147, https://doi.org/10.1152/ajplung.00071.2010.
19. Suzuki, M.; Takahashi, N.; Sobue, Y.; Ohashi, Y.; Kishimoto, K.; Hattori, K.; Ishiguro, N.; Kojima, T. Hyaluronan suppresses enhanced cathepsin K expression via activation of NF-κB with mechanical stress loading in a human chondrocytic HCS-2/8 cells. *Sci. Rep.* **2020**, *10*, 216, https://doi.org/10.1038/s41598-019-57073-8.
20. Simpson, M.A.; Lokeshwar, V.B. Hyaluronan and hyaluronidase in genitourinary tumors. *Frontiers in bioscience: a journal and virtual library* **2008**, *13*, 5664, https://doi.org/10.2741/3108.
21. Tolg, C.; Liu, M.; Cousteils, K.; Telmer, P.; Alam, K.; Ma, J.; Mendina, L.; McCarthy, J.B.; Morris, V.L.; Turley, E.A. Cell-specific expression of the transcriptional regulator RHAMM provides a timing mechanism that controls appropriate wound re-epithelialization. *J. Biol. Chem.* **2020**, *295*, 5427-5448, https://doi.org/10.1074/jbc.RA119.010002.
22. Reckamp, K.L. Molecular Targets Beyond the Big 3. *Thorac. Surg. Clin.* **2020**, *30*, 157-164, https://doi.org/10.1016/j.thorsurg.2020.01.004.
23. Morera, D.S.; Hennig, M.S.; Talukder, A.; Lokeshwar, S.D.; Wang, J.; Garcia-Roig, M.; Ortiz, N.; Yates, T.J.; Lopez, L.E.; Kallifatidis, G.; Kramer, M.W.; Jordan, A.R.; Merseburger, A.S.; Manoharan, M.; Soloway, M.S.; Terris, M.K.; Lokeshwar, V.B. Hyaluronic acid family in bladder cancer: potential prognostic biomarkers and therapeutic targets. *Br. J. Cancer* **2017**, *117*, 1507-1517, https://doi.org/10.1038/bjc.2017.318.
24. Evanko, S.P.; Potter-Perigo, S.; Petty, L.J.; Workman, G.A.; Wight, T.N. Hyaluronan Controls the Deposition of Fibronectin and Collagen and Modulates TGF-β1 Induction of Lung Myofibroblasts. *Matrix Biol.* **2015**, *42*, 74-92, https://doi.org/10.1016/j.matbio.2014.12.001.
25. Máiz Carro, L.; Martínez-García, M.A. Use of Hyaluronic Acid (HA) in Chronic Airway Diseases. *Cells* **2020**, *9*, 2210, https://doi.org/10.3390/cells9102210.

26. Ma, X.; Pearce, J.D.; Wilson, D.B.; English, W.P.; Edwards, M.S.; Geary, R.L. Loss of the hyaluronan receptor RHAMM prevents constrictive artery wall remodeling. *J. Vasc. Surg.* **2014**, *59*, 804-813, https://doi.org/10.1016/j.jvs.2013.03.047.
27. Toole, B.P. Hyaluronan: from extracellular glue to pericellular cue. *Nature Reviews Cancer* **2004**, *4*, 528-539, https://doi.org/10.1038/nrc1391.
28. Bourguignon, L.Y.W.; Wong, G.; Earle, C.; Krueger, K.; Spevak, C.C. Hyaluronan-CD44 interaction promotes c-Src-mediated twist signaling, microRNA-10b expression, and RhoA/RhoC up-regulation, leading to Rho-kinase-associated cytoskeleton activation and breast tumor cell invasion. *J. Biol. Chem.* **2010**, *285*, 36721-36735, https://doi.org/10.1074/jbc.M110.162305.
29. Bohaumilitzky, L.; Huber, A.-K.; Stork, E.M.; Wengert, S.; Woelfl, F.; Boehm, H. A trickster in disguise: Hyaluronan's ambivalent roles in the matrix. *Front. Oncol.* **2017**, *7*, 242, https://doi.org/10.3389/fonc.2017.00242.
30. Shigeeda, W.; Shibazaki, M.; Yasuhira, S.; Masuda, T.; Tanita, T.; Kaneko, Y.; Sato, T.; Sekido, Y.; Maesawa, C. Hyaluronic acid enhances cell migration and invasion via the YAP1/TAZ-RHAMM axis in malignant pleural mesothelioma. *Oncotarget* **2017**, *8*, 93729, https://doi.org/10.18632/oncotarget.20750.
31. Wu, R.-L.; Huang, L.; Zhao, H.-C.; Geng, X.-P. Hyaluronic acid in digestive cancers. *J. Cancer Res. Clin. Oncol.* **2017**, *143*, 1-16, https://doi.org/10.1007/s00432-016-2213-5.
32. Chen, Y.-T.; Chen, Z.; Nancy Du, Y.-C. Immunohistochemical analysis of RHAMM expression in normal and neoplastic human tissues: a cell cycle protein with distinctive expression in mitotic cells and testicular germ cells. *Oncotarget* **2018***; 9*, 20941-20952, https://doi.org/10.18632/oncotarget.24939.
33. Matou-Nasri, S.; Gaffney, J.; Kumar, S.; Slevin, M. Oligosaccharides of hyaluronan induce angiogenesis through distinct CD44 and RHAMM-mediated signalling pathways involving Cdc2 and γ-adducin. *Int. J. Oncol.* **2009**, *35*, 761-773, https://doi.org/10.3892/ijo_00000389.
34. Jiang, B.; Zhou, L.; Lu, J.; Wang, Y.; Liu, C.; You, L.; Guo, J. Stroma-Targeting Therapy in Pancreatic Cancer: One Coin With Two Sides? *Front. Oncol.* **2020**, *10*, 2272, https://doi.org/10.3389/fonc.2020.576399.
35. Akiyama, Y.; Jung, S.; Salhia, B.; Lee, S.; Hubbard, S.; Taylor, M.; Mainprize, T.; Akaishi, K.; van Furth, W.; Rutka, J.T. Hyaluronate Receptors Mediating Glioma Cell Migration and Proliferation. *J. Neurooncol.* **2001**, *53*, 115-127, https://doi.org/10.1023/a:1012297132047.
36. Riehl, T.E.; Alvarado, D.; Ee, X.; Ciorba, M.A.; Stenson, W.F. Hyaluronic acid promotes Lgr5+ stem cell proliferation and crypt fission through TLR4 and PGE2 transactivation of EGFR. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **2020**, *319*, G63-G73, https://doi.org/10.1152/ajpgi.00242.2019.
37. Skandalis, S.S.; Karalis, T.; Heldin, P. Intracellular hyaluronan: Importance for cellular functions. *Semin. Cancer Biol.* **2020**, *62*, 20-30, https://doi.org/10.1016/j.semcancer.2019.07.002.
38. Luyt, L.G.; Turley, E.A.; Esguerra, K.V. Rhamm binding peptides. Google Patents: 2011.
39. Esguerra, K.V.N.; 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). *Integrative Biology* **2015**, *7*, 1547-1560, https://doi.org/10.1039/c5ib00222b.
40. Tolg, C.; Hamilton, S.R.; Nakrieko, K.-A.; Kooshesh, F.; Walton, P.; McCarthy, J.B.; Bissell, M.J.; Turley, E.A. Rhamm−/− fibroblasts are defective in CD44-mediated ERK1,2 motogenic signaling, leading to defective skin wound repair. *J. Cell Biol.* **2006**, *175*, 1017-1028, https://doi.org/10.1083/jcb.200511027.
41. Schreer, A.; Tinson, C.; Sherry, J.P.; Schirmer, K. Application of Alamar blue/5-carboxyfluorescein diacetate acetoxymethyl ester as a noninvasive cell viability assay in primary hepatocytes from rainbow trout. *Anal. Biochem.* **2005**, *344*, 76-85, https://doi.org/10.1016/j.ab.2005.06.009.
42. Purnell, M.C. Bio-electric field enhancement: the influence on hyaluronan mediated motility receptors in human breast carcinoma. *Discov. Med.* **2017**, *23*, 259-267.
43. He, Z.; Mei, L.; Connell, M.; Maxwell, C.A. Hyaluronan Mediated Motility Receptor (HMMR) Encodes an Evolutionarily Conserved Homeostasis, Mitosis, and Meiosis Regulator Rather than a Hyaluronan Receptor. *Cells* **2020**, *9*, 819, https://doi.org/10.3390/cells9040819.
44. Buttermore, S.T.; Hoffman, M.S.; Kumar, A.; Champeaux, A.; Nicosia, S.V.; Kruk, P.A. Increased RHAMM expression relates to ovarian cancer progression. *Journal of Ovarian Research* **2017**, *10*, 66, https://doi.org/10.1186/s13048-017-0360-1.

45. Menon, S.; Gujral, S.; Bakshi, G.; Tongaonkar, H.B. Bilateral testicular metastasis from prostatic adenocarcinoma mimicking an intertubular pattern of seminoma and expressing Rhamm. *J. Cancer Res. Ther.* **2010**, *6*, 97, https://doi.org/10.4103/0973-1482.63560.
46. Gust, K.M.; Hofer, M.D.; Perner, S.R.; Kim, R.; Chinnaiyan, A.M.; Varambally, S.; Moller, P.; Rinnab, L.; Rubin, M.A.; Greiner, J.; Schmitt, M.; Kuefer, R.; Ringhoffer, M. RHAMM (CD168) Is Overexpressed at the Protein Level and May Constitute an Immunogenic Antigen in Advanced Prostate Cancer Disease. *Neoplasia* **2009**, *11*, 956-963, https://doi.org/10.1593/neo.09694.
47. Akentieva, N.P.; Shushanov, S.S. RHAMM (receptor hyaluronan-mediated motility)-target peptides induce apoptosis in prostate cancer cells. *Vopr. Onkol.* **2016**, *62*, 514-518.
48. Hauner, K.; Maisch, P.; Retz, M. Nebenwirkungen der Chemotherapie. *Der Urologe* **2017**, *56*, 472-479, https://doi.org/10.1007/s00120-017-0338-z.
49. Duan, Z.-Y.; Liu, J.-Q.; Yin, P.; Li, J.-J.; Cai, G.-Y.; Chen, X.-M. Impact of aging on the risk of platinum-related renal toxicity: A systematic review and meta-analysis. *Cancer Treat. Rev.* **2018**, *69*, 243-253, https://doi.org/10.1016/j.ctrv.2018.07.002.
50. Schatz-Siemers, N.; Chen, Y.T.; Chen, Z.; Wang, D.; Ellenson, L.H.; Du, Y.N. Expression of the receptor for hyaluronic acid-mediated motility (RHAMM) in endometrial cancer is associated with adverse histologic parameters and tumor progression. *Appl. Immunohistochem. Mol. Morphol.* **2020,** *28*, 453-459, https: // doi: 10.1097/PAI.0000000000000763.
51. 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. Elevated hyaluronan and hyaluronan-mediated motility receptor are associated with biochemical failure in patients with intermediate-grade prostate tumors. *Cancer* **2014**, *120*, 1800-1809, https://doi.org/10.1002/cncr.28646.
52. Lugli, A.; Zlobec, I.; Günthert, U.; Minoo, P.; Baker, K.; Tornillo, L.; Terracciano, L.; Jass, J.R. Overexpression of the receptor for hyaluronic acid mediated motility is an independent adverse prognostic factor in colorectal cancer. *Mod. Pathol.* **2006**, *19*, 1302-1309, https://doi.org/10.1038/modpathol.3800648.
53. Tzankov, A.; Strasser, U.; Dirnhofer, S.; Menter, T.; Arber, C.; Jotterand, M.; Rovo, A.; Tichelli, A.; Stauder, R.; Günthert, U. In situ RHAMM protein expression in acute myeloid leukemia blasts suggests poor overall survival. *Ann. Hematol.* **2011**, *90*, 901-909, https://doi.org/10.1007/s00277-011-1159-6.
54. Schwertfeger, K.L.; Cowman, M.K.; Telmer, P.G.; Turley, E.A.; McCarthy, J.B. Hyaluronan, Inflammation, and Breast Cancer Progression. *Front. Immunol.* **2015**, *6*, 236, https://doi.org/10.3389/fimmu.2015.00236.
55. Klaric, M.; Haller, H.; Brncic Fischer, A.; Babarovic, E.; Prijic, A.; Eminovic, S. The Role of CD44 and RHAMM in Endometrial (Endometrioid Type) Cancer: An Immunohistochemical Study. *Appl. Immunohistochem. Mol. Morphol.* **2019**, *27*, https://doi.org/10.1097/PAI.0000000000000673.
56. Wei, J.; Hu, M.; Huang, K.; Lin, S.; Du, H. Roles of Proteoglycans and Glycosaminoglycans in Cancer Development and Progression. *Int. J. Mol. Sci.* **2020**, *21*, 5983, https://doi.org/10.3390/ijms21175983.
57. Sohr, S.; Engeland, K. RHAMM is differentially expressed in the cell cycle and downregulated by the tumor suppressor p53. *Cell Cycle* **2008**, *7*, 3448-3460, https://doi.org/10.4161/cc.7.21.7014.
58. Engeland, K. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death Differ.* **2018**, *25*, 114-132, https://doi.org/10.1038/cdd.2017.172.
59. Akent'eva, N.P.; Shushanov, S.S.; Kotel'nikov, A.I. Effects of RHAMM/HMMR-Selective Peptides on Survival of Breast Cancer Cells. *Bull. Exp. Biol. Med.* **2015**, *159*, 658-661, https://doi.org/10.1007/s10517-015-3041-3.
60. van Luijn, M.M.; van den Ancker, W.; Chamuleau, M.E.D.; Ossenkoppele, G.J.; van Ham, S.M.; van de Loosdrecht, A.A. Impaired antigen presentation in neoplasia: basic mechanisms and implications for acute myeloid leukemia. *Immunotherapy* **2009**, *2*, 85-97, https://doi.org/10.2217/imt.09.84.
61. Schmitt, M.; Casalegno-Garduño, R.; Xu, X.; Schmitt, A. Peptide vaccines for patients with acute myeloid leukemia. *Expert Review of Vaccines* **2009**, *8*, 1415-1425, https://doi.org/10.1586/erv.09.90.
62. Foster, A.E.; Okur, F.V.; Biagi, E.; Lu, A.; Dotti, G.; Yvon, E.; Savoldo, B.; Carrum, G.; Goodell, M.A.; Heslop, H.E.; Brenner, M.K. Selective elimination of a chemoresistant side population of B-CLL cells by cytotoxic T lymphocytes in subjects receiving an autologous hCD40L/IL-2 tumor vaccine. *Leukemia* **2010**, *24*, 563-572, https://doi.org/10.1038/leu.2009.281.
63. Greiner, J.; Schmitt, A.; Giannopoulos, K.; Rojewski, M.T.; Götz, M.; Funk, I.; Ringhoffer, M.; Bunjes, D.; Hofmann, S.; Ritter, G. High-dose RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia, myelodysplastic syndrome and multiple myeloma. *Haematologica* **2010**, *95*, 1191-1197, https://doi.org/10.3324/haematol.2009.014704.

64. Schmitt, M.; Schmitt, A.; Rojewski, M.T.; Chen, J.; Giannopoulos, K.; Fei, F.; Yu, Y.; Götz, M.; Heyduk, M.; Ritter, G.; Speiser, D.E.; Gnjatic, S.; Guillaume, P.; Ringhoffer, M.; Schlenk, R.F.; Liebisch, P.; Bunjes, D.; Shiku, H.; Dohner, H.; Greiner, J. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. *Blood* **2008**, *111*, 1357-1365, https://doi.org/10.1182/blood-2007-07-099366.
65. Giannopoulos, K.; Dmoszynska, A.; Kowal, M.; Rolinski, J.; Gostick, E.; Price, D.A.; Greiner, J.; Rojewski, M.; Stilgenbauer, S.; Döhner, H.; Schmitt, M. Peptide vaccination elicits leukemia-associated antigen-specific cytotoxic CD8+ T-cell responses in patients with chronic lymphocytic leukemia. *Leukemia* **2010**, *24*, 798-805, https://doi.org/10.1038/leu.2010.29.
66. Yang, H.S.; Zhang, D.M.; Deng, H.X.; Peng, F.; Wei, Y.Q. Antitumor and anti-angiogenesis immunity induced by CR-SEREX-identified Xenopus RHAMM. *Cancer Sci.* **2010**, *101*, 862-868, https://doi.org/10.1111/j.1349-7006.2009.01473.x.
67. Takayuki, A.; Koji, K.; Koichi, Y.; Jun, M.; Sadahiro, N.; Hirosuke, F.; Shoichi, K.; Masami, F.; Mikiko, F.; Yuji, H.; Michiyasu, S. Antitumor effects of vaccination with dendritic cells transfected with modified receptor for hyaluronan-mediated motility mRNA in a mouse glioma model. *Journal of Neurosurgery JNS* **2007**, *106*, 638-645, https://doi.org/10.3171/jns.2007.106.4.638.
68. Rhodes, D.R.; Kalyana-Sundaram, S.; Mahavisno, V.; Varambally, R.; Yu, J.; Briggs, B.B.; Barrette, T.R.; Anstet, M.J.; Kincead-Beal, C.; Kulkarni, P.; Varambally, S.; Ghosh, D.; Chinnaiyan, A.M. Oncomine 3.0: Genes, Pathways, and Networks in a Collection of 18,000 Cancer Gene Expression Profiles. *Neoplasia* **2007**, *9*, 166-180, https://doi.org/10.1593/neo.07112.
69. Rhodes, D.R.; Yu, J.; Shanker, K.; Deshpande, N.; Varambally, R.; Ghosh, D.; Barrette, T.; Pander, A.; Chinnaiyan, A.M. ONCOMINE: A Cancer Microarray Database and Integrated Data-Mining Platform. *Neoplasia* **2004**, *6*, 1-6, https://doi.org/10.1016/s1476-5586(04)80047-2.
70. 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*, 3150-3157, https://doi.org/10.1074/jbc.M109579200.
71. Daniel, S. Signaling Pathways Responsible for Cancer Cell Invasion as Targets for Cancer Therapy. *Curr. Cancer Drug Targets* **2004**, *4*, 327-336, https://doi.org/10.2174/1568009043332961.
72. Lee, B.-C.; Lee, T.-H.; Avraham, S.; Avraham, H.K. Involvement of the Chemokine Receptor CXCR4 and Its Ligand Stromal Cell-Derived Factor 1α in Breast Cancer Cell Migration Through Human Brain Microvascular Endothelial Cells11NIH grant NS39558 (S. Avraham), the Susan G. Komen Fellowship (S. Avraham), the Milheim Foundation (S. Avraham), CA97153 (H. Avraham), and K18 PAR-02-069 (H. Avraham). *Mol. Cancer Res.* **2004**, *2*, 327-338.
73. Pellerino, A.; Internò, V.; Mo, F.; Franchino, F.; Soffietti, R.; Rudà, R. Management of Brain and Leptomeningeal Metastases from Breast Cancer. *Int. J. Mol. Sci.* **2020**, *21*, 8534, https://doi.org/10.3390/ijms21228534.
74. Müller, A.; Homey, B.; Soto, H.; Ge, N.; Catron, D.; Buchanan, M.E.; McClanahan, T.; Murphy, E.; Yuan, W.; Wagner, S.N.; Barrera, J.L.; Mohar, A.; Verástegui, E.; Zlotnik, A. Involvement of chemokine receptors in breast cancer metastasis. *Nature* **2001**, *410*, 50-56, https://doi.org/10.1038/35065016.
75. Fu, J.-X.; Zou, Y.-N.; Long, L.; Wang, X.-J. Widespread Metastasis to the Stomach 10 Years After Primary Breast Cancer: A case report and review of the literature. *Medicine* **2020**, *99*, https://doi.org/10.1097/MD.0000000000022527.
76. Comșa, Ș.; Cimpean, A.M.; Raica, M. The story of MCF-7 breast cancer cell line: 40 years of experience in research. *Anti-cancer Res.* **2015**, *35*, 3147-3154.
77. Jain, N.; Nitisa, D.; Pirsko, V.; Cakstina, I. Selecting suitable reference genes for qPCR normalization: a comprehensive analysis in MCF-7 breast cancer cell line. *BMC Molecular and Cell Biology* **2020**, *21*, 68, https://doi.org/10.1186/s12860-020-00313-x.
78. Li, H.; Chen, C.; Wang, D. Low-frequency ultrasound and microbubbles combined with simvastatin promote the apoptosis of MCF-7 cells by affecting the LATS1/YAP/RHAMM pathway. *Mol Med Rep* **2018**, *18*, 2724-2732, https://doi.org/10.3892/mmr.2018.9273.
79. Moses, H.; Barcellos-Hoff, M.H. TGF-β biology in mammary development and breast cancer. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a003277, https://doi.org/10.1101/cshperspect.a003277.
80. Foulstone, E.J.; Zeng, L.; Perks, C.M.; Holly, J.M.P. Insulin-Like Growth Factor Binding Protein 2 (IGFBP-2) Promotes Growth and Survival of Breast Epithelial Cells: Novel Regulation of the Estrogen Receptor. *Endocrinology* **2013**, *154*, 1780-1793, https://doi.org/10.1210/en.2012-1970.

81. Vaziri-Gohar, A.; Zheng, Y.; Houston, K.D. IGF-1 Receptor Modulates FoxO1-Mediated Tamoxifen Response in Breast Cancer Cells. *Mol. Cancer Res.* **2017**, *15*, 489, https://doi.org/10.1158/1541-7786.MCR-16-0176.
82. Tian, H.; Lyu, Y.; Yang, Y.-G.; Hu, Z. Humanized Rodent Models for Cancer Research. *Front. Oncol.* **2020**, *10*, 1696, https://doi.org/10.3389/fonc.2020.01696.
83. Jiang, X.f.; Tang, Q.l.; Li, H.g.; Shen, X.m.; Luo, X.; Wang, X.y.; Lin, Z.q. Tumor-associated macrophages correlate with progesterone receptor loss in endometrial endometrioid adenocarcinoma. *J. Obstet. Gynaecol. Res.* **2013**, *39*, 855-863, https://doi.org/10.1111/j.1447-0756.2012.02036.x.
84. Rein, D.T.; Roehrig, K.; Schöndorf, T.; Lazar, A.; Fleisch, M.; Niederacher, D.; Bender, H.G.; Dall, P. Expression of the hyaluronan receptor RHAMM in endometrial carcinomas suggests a role in tumour progression and metastasis. *J. Cancer Res. Clin. Oncol.* **2003**, *129*, 161-164, https://doi.org/10.1007/s00432-003-0415-0.
85. Mimeault, M.; Johansson, S.L.; Batra, S.K. Marked improvement of cytotoxic effects induced by docetaxel on highly metastatic and androgen-independent prostate cancer cells by downregulating macrophage inhibitory cytokine-1. *Br. J. Cancer* **2013**, *108*, 1079-1091, https://doi.org/10.1038/bjc.2012.484.
86. Chu, L.W.; Pettaway, C.A.; Liang, J.C. Genetic abnormalities specifically associated with varying metastatic potential of prostate cancer cell lines as detected by comparative genomic hybridization. *Cancer Genet. Cytogenet.* **2001**, *127*, 161-167, https://doi.org/10.1016/s0165-4608(01)00389-2.
87. Thangavel, C.; Boopathi, E.; Liu, Y.; Haber, A.; Ertel, A.; Bhardwaj, A.; Addya, S.; Williams, N.; Ciment, S.J.; Cotzia, P.; Dean, J.L.; Snook, A., McNair, C.; Price, M.; Hernandez, J.R.; Zhao, S.G.; Birbe, R.; McCarthy, J.B.; Turley, E.A.; Pienta, K.J.; Feng, F.Y.; Dicker, A.P.; Knudsen, K.E.; Den, R.B. RB loss promotes prostate cancer metastasis. *Cancer Res.* **2017**, *77*, 982-995, https://doi.org/10.1158/0008-5472.CAN-16-1589.
88. Lin, S.-L.; Chang, D.; Chiang, A.; Ying, S.-Y. Androgen receptor regulates CD168 expression and signaling in prostate cancer. *Carcinogenesis* **2008**, *29*, 282-290, https://doi.org/10.1093/carcin/bgm259.
89. Lin, S.-L.; Chang, D.; Ying, S.-Y. Hyaluronan stimulates transformation of androgen-independent prostate cancer. *Carcinogenesis* **2007**, *28*, 310-320, https://doi.org/10.1093/carcin/bgl134.
90. Yu, Y.P.; Landsittel, D.; Jing, L.; Nelson, J.; Ren, B.; Liu, L.; McDonald, C.; Thomas, R.; Dhir, R.; Finkelstein, S.; Michalopoulos, G.; Becich, M.; Luo, J.-H. Gene Expression Alterations in Prostate Cancer Predicting Tumor Aggression and Preceding Development of Malignancy. *J. Clin. Oncol.* **2004**, *22*, 2790-2799, https://doi.org/10.1200/JCO.2004.05.158.
91. LaTulippe, E.; Satagopan, J.; Smith, A.; Scher, H.; Scardino, P.; Reuter, V.; Gerald, W.L. Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res.* **2002**, *62*, 4499-4506.
92. Yang, C.-W.; Su, J.-Y.; Tsou, A.-P.; Chau, G.-Y.; Liu, H.-L.; Chen, C.-H.; Chien, C.-Y.; Chou, C.-K. Integrative genomics based identification of potential human hepatocarcinogenesis-associated cell cycle regulators: RHAMM as an example. *Biochem. Biophys. Res. Commun.* **2005**, *330*, 489-497, https://doi.org/10.1016/j.bbrc.2005.03.005.
93. Godar, S.; Weinberg, R.A. Filling the mosaic of p53 actions: p53 represses RHAMM expression. *Cell Cycle* **2008**, *7*, 3479-3480, https://doi.org/10.4161/cc.7.22.7320.
94. Cho, R.J.; Huang, M.; Campbell, M.J.; Dong, H.; Steinmetz, L.; Sapinoso, L.; Hampton, G.; Elledge, S.J.; Davis, R.W.; Lockhart, D.J. Transcriptional regulation and function during the human cell cycle. *Nat. Genet.* **2001**, *27*, 48-54, https://doi.org/10.1038/83751.
95. Whitfield, M.L.; Sherlock, G.; Saldanha, A.J.; Murray, J.I.; Ball, C.A.; Alexander, K.E.; Matese, J.C.; Perou, C.M.; Hurt, M.M.; Brown, P.O.; Botstein, D. Identification of Genes Periodically Expressed in the Human Cell Cycle and Their Expression in Tumors. *Mol. Biol. Cell* **2002**, *13*, 1977-2000, https://doi.org/10.1091/mbc.02-02-0030.