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ИНСТИТУТ ЦИТОЛОГИИ РОССИЙСКОЙ АКАДЕМИИ НАУК

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ПОРТФОЛИО АСПИРАНТА

СВЕРЧИНСКОГО Дмитрия Вадимовича

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1. Общие сведения

Лаборатория: Лаборатория защитных механизмов клетки (ЛЗМК)
Тема диссертационной работы: Низкомолекулярные модуляторы шаперонной активности белка Hsp70 в применении к терапии социально значимых заболеваний
Научный руководитель: Маргулис Борис Александрович, д.б.н.
Год поступления в аспирантуру: 2016

2. Научные публикации

Статьи:

1. Lazarev, V. F., **Sverchinskyi, D**. V., Ippolitova, M. V., Stepanova, A. V., Guzhova, I. V., & Margulis, B. A. (2013). Factors Affecting Aggregate Formation in Cell Models of Huntington's Disease and Amyotrophic Lateral Sclerosis. *Acta naturae*, *5*(2), 81-9.

2. Gurskiy, Y. G., Garbuz, D. G., Soshnikova, N. V., Krasnov, A. N., Deikin, A., Lazarev, V. F., **Sverchinskyi, D.,** Margulis, B. A., Zatsepina, O. G., Karpov, V. L., Belzhelarskaya, S. N., Feoktistova, E., Georgieva, S. G., … Evgen'ev, M. B. (2016). The development of modified human Hsp70 (HSPA1A) and its production in the milk of transgenic mice. *Cell stress & chaperones*, *21*(6), 1055-1064.

3. **Sverchinsky, D. V**., Lazarev, V. F., Semenyuk, P. I., Mitkevich, V. A., Guzhova, I. V. and Margulis, B. A. (2017), Peptide fragments of Hsp70 modulate its chaperone activity and sensitize tumor cells to anticancer drugs. *FEBS Letters*, 591: 4074-4082. doi:10.1002/1873-3468.12913

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Тезисы:

1. Химический препарат МК30 подавляет активность белка Hsp70 в клеточных моделях онуологических заболеваний. С. А. Нисканен, В. Ф. Лазарев, **Д. В. Сверчинский**, Е. С. Чухно, И. В. Гужова, Б. А. Маргулис. Сборник тезисов V молодежной конференции по молекулярной и клеточной биологии Института цитологии РАН. СПб, 2016.- 76 с.

2. Пептидный фрагмент белка теплового шока Hsp70 ингибирует его шаперонную активность и увеличивает противоопухолевую активность доксорубицина. **Д.В. Сверчинский**, В.Ф. Лазарев, А.Д. Никотина, И.В. Гужова, Б.Н. Маргулис. Успехи молекулярной онкологии, 2016 том 4, с 80-81.

3. Малые молекулы, подавляющие функцию шаперона бтш70, как средства противоопухолевой терапии. В.Ф. Лазарев, **Д.В. Сверчинский**, С.А. Нисканен, Е.Р. Михайлова, И.В. Гужова, Б.А. Маргулис. Acta Naturae спецвыпуск 2016 том 2, стр. 74

4. Hsp70 chaperone inhibitors: tools for search and anti-cancer activity. V. Lazarev, **D. Sverchinsky**, S. Niskanen, R. Suezov. I. Guzhova, B. Margulis. Stress management mechanisms and pathways, 2018.

5. Peptide parts of Hsp70 inhibitits own chaperonic activity: possible application in anti-cancer therapy. **D. Sverchinsky**, V. Lazarev, I. Guzhova, B. Margulis. Stress management mechanisms and pathways, 2018.

6. Модуляторы активности шаперона hsp70 и их противоопухолевый потенциал. **Д.В. Сверчинский,** В.Ф. Лазарев, И.В. Гужова, Б.А. Маргулис. Acta Naturae спецвыпуск 2017, стр. 153.

7. Ингибиторы молекулярных шаперонов: инструменты для поиска и противоопухолевая активность. В.Ф. Лазарев, **Д.В. Сверчинский**, С.А. Нисканен, И.В. Гужова, Б.А. Маргулис. Acta Naturae спецвыпуск 2017, стр. 38.

8. Пептидные фрагменты Hsp70, как потенциальное средство в комбинированной противоопухолевой терапии. **Сверчинский Д.В.,** Лазарев В.Ф., Гужова И.В., Маргулис Б.А. Сборник тезисов международной конференция студентов, аспирантов и молодых ученых «Ломоносов-2018».

9. Peptide parts of Hsp70 chaperone as potential agents of anticancer combination therapy. **D Sverchinsky**, V Lazarev, I Guzhova, B Margulis - FEBS OPEN BIO, 2018.

3. Участие в научных конференциях, симпозиумах, семинарах, выставках

1. II Всероссийская конференция по молекулярной онкологии (Москва, 6-8 декабря 2016) – стендовый доклад.

2. 8th International Congress on Stress Responses in Biology and Medicine (13-17 August 2017, Turku, Finland) – стендовый доклад

3. VIII Российский симпозиум «Белки и пептиды». (22-24 сентября 2017, Москва, Россия) – устный доклад.

4. Международная конференция студентов, аспирантов и молодых ученых «Ломоносов-2018» (9-13 апреля 2018, Москва, Россия) – устный доклад.

10. 5. 43rd FEBS Congress, Biochemistry Forever (July 7-12, 2018 Prague, Czech Republic) – стендовый доклад.

4. Участие в грантах

- РНФ №14-50-00068, направление «Трансформированные и раковые стволовые клетки как мишени для противоопухолевых средств».

- РНФ №18-74-10087, «Поиск и верификация новых мишеней для комбинированной терапии вторичных повреждений после черепно-мозговой травмы»

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5. Научно-педагогическая деятельность

Научное руководство бакалаврами, магистрами, специалистами

Отсутствует.

Чтение лекций, проведение семинарских и практических занятий

Отсутствует.

6. Дополнительная информация

***7.* Сведения об освоении основной образовательной программы подготовки научно-педагогических кадров в аспирантуре** *(результаты сданных экзаменов, зачетов, кандидатских экзаменов, сведения о педагогической практике). Указать название дисциплины, время (месяц и год) сдачи, полученную оценку.*

Сведения о сдаче кандидатских экзаменов

№ п/п	***Дисциплина***	***Дата сдачи***	***Оценка***	***Место сдачи***
1.	История и философия науки	20.05.2017	хорошо	ИНЦ РАН
2.	Английский язык	20.04.2017	отлично	ИНЦ РАН
3.	Клеточная биология, цитология, гистология			ИНЦ РАН

Сведения о сдаче других дисциплин

№ п/п	***Дисциплина***	***Дата сдачи***	***Оценка***	***Место сдачи***
1.	Научно-исследовательская деятельность	29.11.2017	отлично	ИНЦ РАН
2.	Педагогика высшей школы	26.12.2017	отлично	ИНЦ РАН
3.	Клеточная биология, цитология, гистология	31.01.2018	Зачет (хорошо)	ИНЦ РАН

RESEARCH ARTICLES

Factors Affecting Aggregate Formation in Cell Models of Huntington's Disease and Amyotrophic Lateral Sclerosis

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ABSTRACT **Most neurodegenerative pathologies stem from the formation of aggregates of mutant proteins, causing dysfunction and ultimately neuronal death. This study was aimed at elucidating the role of the protein factors that promote aggregate formation or prevent the process, respectively, glyceraldehyde-3-dehydrogenase (GAPDH) and tissue transglutaminase (tTG) and Hsp70 molecular chaperone. The siRNA technology was used to show that the inhibition of GAPDH expression leads to a 45–50% reduction in the aggregation of mutant huntingtin, with a repeat of 103 glutamine residues in a model of Huntington's disease (HD). Similarly, the blockage of GAPDH synthesis was found for the first time to reduce the degree of aggregation of mutant superoxide dismutase 1 (G93A) in a model of amyotrophic lateral sclerosis (ALS). The treatment of cells that imitate HD and ALS with a pharmacological GAPDH inhibitor, hydroxynonenal, was also shown to reduce the amount of the aggregating material in both disease models. Tissue transglutaminase is another factor that promotes the aggregation of mutant proteins; the inhibition of its activity with cystamine was found to prevent aggregate formation of mutant huntingtin and SOD1. In order to explore the protective function of Hsp70 in the control of the aggregation of mutant huntingtin, a cell model with inducible expression of the chaperone was used. The amount and size of polyglutamine aggregates were reduced by increasing the intracellular content of Hsp70. Thus, pharmacological regulation of the function of three proteins, GAPDH, tTG, and Hsp70, can affect the pathogenesis of two significant neurodegenerative diseases.**

KEYWORDS **neurodegenerative pathologies; glyceralgehyde-3-phosphate dehydrogenase; chaperones; mutant proteins; aggregation.**

ABBREVIATIONS: EGFP – enhanced green fluorescence protein; ALS – amyotrophic lateral sclerosis; HSP – heat shock protein; HD – Huntington's disease; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; HNE – hydroxynonenal; SDS – sodium dodecyl sulfate; PAAG – polyacrylamide gel; SOD – superoxide dismutase; tTG – tissue transglutaminase; PBS – phosphate buffer saline.

INTRODUCTION

Progressive neuronal death in certain parts of the brain is the culprit in most neurodegenerative disorders. The development of these pathologies starts from intra- (Parkinson's and Huntington's diseases) or extracellular accumulation (Alzheimer's disease) of the aggregates of mutant proteins or their oligomers [1]. These structures are toxic for brain cells; they cause immediate neuronal death, although there is some evidence that they can exist in neurons for dozens of years and turn into an active toxic factor only at some moment in time [2].

There are two hypotheses for the aggregate formation of mutant proteins. According to one, the aggregates can form due to the formation of hydrogen bonds between the β-sheets of a damaged or a mutant protein molecule [3]. These structures are inaccessible to strong dissociating solvents, in particular to sodium dodecylsulfate (SDS). The high density of the aggregating material presumably prevents the cell from using proteolytic systems, proteasomes, and phagosomes to fight the aggregates [4]. According to the second hypothesis, amyloid aggregates can form due to covalent cross-links between mutant protein molecules and other cell proteins. The formation of such cross-links is typical of the so-called polyglutamine pathologies, which are based on mu-

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ORIGINAL PAPER

The development of modified human Hsp70 (HSPA1A) and its production in the milk of transgenic mice

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Abstract The production of major human heat shock protein Hsp70 (*HSPA1A*) in a eukaryotic expression system is needed for testing and possible medical applications. In this study, transgenic mice were produced containing wild-type human Hsp70 allele in the vector providing expression in the milk. The results indicated that human Hsp70 was readily expressed in the transgenic animals but did not apparently preserve its intact structure and, hence, it was not possible to purify the protein using conventional isolation techniques. It was suggested that the protein underwent glycosylation in the process of expression, and this quite common modification for proteins expressed in the milk complicated its isolation. To check this possibility, we mutated all presumptive sites of glycosylation and tested the properties of the resulting modified Hsp70 expressed in *E. coli*. The investigation demonstrated that the modified protein exhibited all beneficial properties of the wild-type Hsp70 and was even superior to the latter for a few parameters. Based on these results, a transgenic mouse strain was obtained which expressed the modified Hsp70 in milk and which was easy to isolate using ATP columns. Therefore, the developed construct can be explored in various bioreactors for reliable manufacture of high quality, uniform, and reproducible human Hsp70 for possible medical applications including neurodegenerative diseases and cancer.

Keywords Heat shock protein 70 · Transgenic mice · Glycosylation · Site-directed mutagenesis · *Escherichia coli*

David G. Garbuz and Nataliya V. Sochnikova contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12192-016-0729-x) contains supplementary material, which is available to authorized users.

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Introduction

Heat shock protein 70 (Hsp70) proteins and their co-chaperones have been studied in various prokaryotic and eukaryotic organisms chiefly because of their participation in protein folding under normal and stress conditions and because of their apparent role in aging and various pathologies, such as neurodegeneration and cancer (Fleshner and Johnson 2005; Calderwood et al. 2007; Kim et al. 2013; Evgen'ev et al. 2014).

Hsp70, in humans encoded by the HSPA1A gene, is a key component of the machinery protecting the cell from various stress conditions (Nollen et al. 2000; Calderwood et al. 2007; Hartl et al. 2011; Radons 2016). Briefly, Hsp70 binds partially unfolded or misfolded proteins and either assists in their refolding or directs them to a safe disposal (Mayer 2010; Duncan et al. 2015). Hsp70 may also have additional functions, including acting as cytokine-like molecules (Asea et al. 2000; Asea 2008a; Calderwood et al. 2007; Multhoff and Hightower 2011; Ghosh et al. 2015; Radons 2016). Thus, in

FEBS Letters

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Peptide fragments of Hsp70 modulate its chaperone activity and sensitize tumor cells to anti-cancer drugs

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Most Hsp70 chaperone inhibitors exert anti-cancer effects; however, their high cytotoxicity proposed the use of peptide fragments of the chaperone as safer modulators of its activity and as complements to customary drugs. One such peptide, ICit-2, was found to inhibit substrate-binding and refolding activities of the chaperone. Using various approaches, we established that ICit-2 binds Hsp70, which may explain its inhibitory action. ICit-2 penetrates A-431 cancer cells and, in combination with doxorubicin (Dox), enhances the cytotoxicity and growth inhibitory effect of the drug. Similarly, using the Bl6 mouse melanoma model, we found that ICit-2 inhibits the rate of tumor growth by 48% compared to Dox alone, confirming that the peptide can be employed to sensitize resistant tumors to cytostatic medicines.

Keywords: anti-cancer; chaperone; Hsp70; molecular docking; peptidomimetic; substrate-binding

The molecular chaperone Hsp70 plays a significant role in the protection of tumor cells from a great number of anti-cancer drugs, particularly those inducing apoptosis [1]. Cancer cells typically contain an increased level of Hsp70 and so its expression or its function should be targeted to overcome chaperone-based protection. To date, only a few Hsp70 chaperone inhibitors have been tested in various cell and animal tumor models [2].

One of the small molecule inhibitors of Hsp70, VER-155008, an adenosine derivative with a high affinity to the nucleotide-binding domain (NBD) of the protein, was found to inhibit growth and stimulate apoptosis of cells from several tumor lines [3]. Another Hsp70 inhibitor, 2-phenylethynesulfonamide (known as pifithrin-μ or PES), also recognizes NBD, causing conformational alterations and the disruption of its interaction with co-chaperones [4]. Similarly, JG-98, a novel compound that dissociates the link between Hsp70 and Bag-3, suppresses pro-survival signaling and reduces the proliferation rate in a variety of cancer cells [5]. The MKT-077 molecule was also shown to bind to Hsp70 and thus inhibit the chaperone activity that leads to tumor cell senescence [6,7].

Most of Hsp70 inhibitors display anti-cancer activity in cellular and animal tumor models; however, their clinical application is hampered by their high cytotoxicity [8]. Searching for safer Hsp70 inhibitors, we focused on peptides and identified a study in which A17 peptide aptamer over-expressed in tumor cells inhibited Hsp70 chaperone activity and, in combination with cisplatin or etoposide, demonstrated a pronounced anti-tumor effect [9]. Moreover, the expression of A17 in combination with Hsp90

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Cell Death & Disease

ARTICLE Open Access

Sensitizing tumor cells to conventional drugs: HSP70 chaperone inhibitors, their selection and application in cancer models

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Abstract

Hsp70 chaperone controls proteostasis and anti-stress responses in rapidly renewing cancer cells, making it an important target for therapeutic compounds. To date several Hsp70 inhibitors are presented with remarkable anticancer activity, however their clinical application is limited by the high toxicity towards normal cells. This study aimed to develop assays to search for the substances that reduce the chaperone activity of Hsp70 and diminish its protective function in cancer cells. On our mind the resulting compounds alone should be safe and function in combination with drugs widely employed in oncology. We constructed systems for the analysis of substrate-binding and refolding activity of Hsp70 and to validate the assays screened the substances representing most diverse groups of chemicals of InterBioScreen library. One of the inhibitors was AEAC, an N-amino-ethylamino derivative of colchicine, which toxicity was two-orders lower than that of parent compound. In contrast to colchicine, AEAC inhibited substrate-binding and refolding functions of Hsp70 chaperones. The results of a drug affinity responsive target stability assay, microscale thermophoresis and molecular docking show that AEAC binds Hsp70 with nanomolar affinity. AEAC was found to penetrate C6 rat glioblastoma and B16 mouse melanoma cells and reduce there the function of the Hsp70-mediated refolding system. Although the cytotoxic and growth inhibitory activities of AEAC were minimal, the compound was shown to increase the antitumor efficiency of doxorubicin in tumor cells of both types. When the tumors were grown in animals, AEAC administration in combination with doxorubicin exerted maximal therapeutic effect prolonging animal survival by 10–15 days and reducing tumor growth rate by 60%. To our knowledge, this is the first time that this approach to the high-throughput analysis of chaperone inhibitors has been applied, and it can be useful in the search for drug combinations that are effective in the treatment of highly resistant tumors.

Introduction

Most of human tumors are known to contain high quantities of Hsp70 chaperone, suggesting that the protein is vital for the proper function of cancer cells[1]. Because of the cytoprotective power Hsp70 reduces the sensitivity of tumors to anti-cancer drugs (such as doxorubicin, etoposide, cisplatin, and others collectively known to induce apoptosis)[2], an effective therapy should be at least partially based on targeting chaperone activity in cancer cells. Inhibiting such activity would result in an improved response to chemotherapy with less severe side effects.

Some of anti-chaperone substances can inhibit the efficacy of the heat-shock response by reducing the heat shock factor 1-mediated transcription of heat shock protein genes, similar to the mechanisms of compounds such

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Article

Etoposide-Induced Apoptosis in Cancer Cells Can Be Reinforced by an Uncoupled Link between Hsp70 and Caspase-3

Dmitry V. Sverchinsky [1], Alina D. Nikotina [1], Elena Y. Komarova [1], Elena R. Mikhaylova [1], Nikolay D. Aksenov [1], Vladimir F. Lazarev [1], Vladimir A. Mitkevich [2], Roman Suezov [1], Dmitry S. Druzhilovskiy [3], Vladimir V. Poroikov [3], Boris A. Margulis [1] and Irina V. Guzhova [1,*]

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Abstract: The Hsp70 chaperone binds and inhibits proteins implicated in apoptotic signaling including Caspase-3. Induction of apoptosis is an important mechanism of anti-cancer drugs, therefore Hsp70 can act as a protective system in tumor cells against therapeutic agents. In this study we present an assessment of candidate compounds that are able to dissociate the complex of Hsp70 with Caspase-3, and thus sensitize cells to drug-induced apoptosis. Using the PASS program for prediction of biological activity we selected a derivative of benzodioxol (BT44) that is known to affect molecular chaperones and caspases. Drug affinity responsive target stability and microscale thermophoresis assays indicated that BT44 bound to Hsp70 and reduced the chaperone activity. When etoposide was administered, heat shock accompanied with an accumulation of Hsp70 led to an inhibition of etoposide-induced apoptosis. The number of apoptotic cells increased following BT44 administration, and forced Caspase-3 processing. Competitive protein–protein interaction and immunoprecipitation assays showed that BT44 caused dissociation of the Hsp70–Caspase-3 complex, thus augmenting the anti-tumor activity of etoposide and highlighting the potential role of molecular separators in cancer therapy.

Keywords: Hsp70; Caspase-3; apoptosis; anti-cancer drugs; biological activity prediction; PASS

Приложение 2

Участие в научных конференциях, семинарах и т.п.

Размещаются соответствующие копии документов

прессии C23 и B23 на мембране, в цитоплазме и в ядре клеток целого ряда опухолей позволяет рассматривать эти белки в качестве возможных мишеней для ингибиторов клеточной пролиферации. Для индукции избирательной гибели опухолевых клеток перспективны некоторые катионные пептиды (КП) – возможные лиганды мембранного C23, которые имеют дендритную структуру, устойчивы к внутриклеточной деградации и в рабочих концентрациях нетоксичны для нормальных клеток.

Задачи исследования. Анализ цитотоксической активности КП в отношении клеток меланомы кожи, рака яичников и глиобластомы человека.

Материалы и методы. Для изучения цитотоксической активности на культурах клеток меланомы кожи линий melIS, melH, рака яичников линии RYa и 2 штаммов глиобластомы линий Glb-Sh и Glb-17 использовали 3 КП с дендритной структурой и 1 КП, меченый нетоксичным флуоресцентным красителем C-5, полимеразную цепную реакцию (ПЦР) с праймерами к ранее картированным «горячим» точкам этих генов с последующим конформационно-чувствительным электрофорезом в полиакриламидном геле и прямым секвенированием ампликонов с измененной структурой ДНК (полиморфные варианты или мутации). Уровень экспрессии генов *NPM/NCL/Tp53* определяли с помощью ПЦР с обратной транскрипцией, белков – с помощью вестерн-блоттинга.

Результаты. В клетках изучаемых линий уровень экспрессии B23/C23 по сравнению с клетками в группе контроля был выше в 3,5/4,6–6,2/8,2 раза в зависимости от линии. Через 3 суток инкубации опухолевых клеток с немечеными пептидами уровень экспрессии матричной РНК (мРНК) NPM/NCL снизился в 1,5/1,1–2,1/3,6 раза. В то же время уровень экспрессии мРНК Tp53 повысился в 1,8–3,5 раза. С помощью МТТ-теста и посредством окрашивания клеток после инкубации с КП красителем Хекст 33342, трипановым синим и йодистым пропидием выявлены апоптозные ядра. Установлена токсичность КП для всех исследованных линий опухолевых клеток. С учетом результатов вестерн-блоттинга избирательная токсичность КП может быть связана с взаимодействием C23/B23/p53, инактивацией p53 и конкурентным связыванием КП с C23 и последующей активацией p53.

Выводы. Исследована цитотоксичность одного из КП с дендримерной структурой молекулы. Выявлена избирательная гибель клеток в культурах меланомы кожи, глиобластомы, рака яичника, но не в культуре подкожных фибробластов человека. Обсуждаются возможные механизмы избирательной цитотоксичности КП с участием шаперонов B23/C23 и p53.

Пептидный фрагмент белка теплового шока Hsp70 ингибирует его шаперонную активность и увеличивает противоопухолевую активность доксорубицина

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Введение. Важную роль в защите опухолевой клетки играет молекулярный шаперон Hsp70 (heat shock proteins 70), поддерживающий белки в нативной конформации. В нормальных клетках уровень Hsp70 небольшой, однако в малигнизированных клетках его экспрессия значительно повышается, что является фактором снижения эффективности противоопухолевой терапии. Для того, чтобы усилить действие противоопухолевых агентов, необходимо подавить защитную систему трансформированной клетки, основанную на Hsp70.

Задачи исследования. Многие вещества, ингибирующие Hsp70 и исследуемые в качестве противоопухолевых средств, обладают высокой токсичностью и низкой стабильностью. В то же время пептиды зачастую обладают хорошей пенетрирующей способностью и стабильностью, а также менее токсичны. Поэтому целью нашей работы стал поиск пептидов, способных влиять на его шаперонную активность, и изучение цитотоксического эффекта таких пептидов в комплексе с противоопухолевыми препаратами.

Материалы и методы. Сначала мы провели скрининг пептидов – фрагментов Hsp70 – в целях поиска ингибиторов функции Hsp70 с помощью тестов на узнавание и рефолдинг субстратного белка. Выявленные ингибиторы мы проверяли на способность уменьшать протеолиз Hsp70, указывающую на возможность таких соединений связываться с Hsp70.

Далее методами иммунофлуоресцентной микроскопии и проточной цитометрии мы изучили способность пептидов–ингибиторов Hsp70 проникать во внутриклеточное пространство.

Наконец, мы оценили цитотоксический эффект пептидов на опухолевые клетки в сочетании с доксорубицином в опытах по измерению динамического сопротивления и тестах по оценке активности внеклеточной лактатдегидрогеназы.

Результаты. В ходе нашей работы мы выявили пептиды, подавляющие шаперонную функцию Hsp70. Один из этих пептидов, ICit-2, уменьшает эффективность протеолиза Hsp70, что подтверждает его взаимодействие с данным шапероном.

Потенциальный терапевтический пептид должен обладать способностью проникать во внутриклеточное пространство. Пенетрирующие свойства пептида ICit-2

Приложение 3.

Дополнительная информация

Размещаются соответствующие копии документов