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BACTENECINS AS CELL-PENETRATING PEPTIDES

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БАКТЕНЕЦИНЫ КАК ПРОНИКАЮЩИЕ В КЛЕТКИ ПЕПТИДЫ

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Cell-Penetrating Peptides (CPPs) are molecules that can easily internalize into eukaryotic cells, as well as deliver across their membranes a variety of compounds (proteins, nucleic acids, liposomes, nanoparticles, etc.). CPPs are considered as promising components of anticancer drugs, serving for delivery of active ingredients into malignant cells, therefore, a detailed study of a mechanism of action of CPPs and search for novel, more effective peptides are vital tasks of current biological and medical research. An ability of proline-rich peptides bactenecins (ChBac5, ChBac3.4, mini-ChBac7.5Na) and their truncated variants to penetrate into eukaryotic cells has been explored. By means of flow cytometry and confocal microscopy, we found that these peptides, tagged with a fluorescent dye BODIPY FL, rapidly penetrated into tumor cells and, to a lesser extent, into normal mammalian cells *in vitro*. The dependence of the internalization process on the medium temperature and energy metabolism of target cells was studied. The obtained data on the cell-penetratin activity of caprine bactenecins confirm the prospect of further investigations of these peptides as prototypes of new compounds — carriers of drugs into malignant or infected cells.

Keywords: caprine bactenecins; proline-rich peptides; Cell-Penetrating Peptides.

Проникающие в клетки пептиды (Cell-Penetrating Peptides — CPP) — небольшие молекулы, обладающие способностью к интернализации в эукариотические клетки, а также переносящие через клеточные мембраны разнообразные соединения (белки, нуклеиновые кислоты, липосомы, наночастицы и т.п.). Благодаря таким свойствам CPP рассматриваются, как перспективные компоненты противоопухолевых препаратов, осуществляющие доставку действующих веществ в малигнизированные клетки. Поэтому детальное изучение механизмов действия CPP, а также поиск новых, более эффективных пептидов-переносчиков, являются актуальными задачами биологии и медицины. Изучена способность пролин-богатых пептидов бактенецинов ChBac5, ChBac3.4, mini-ChBac7.5Na и их укороченных аналогов проникать в эукариотические клетки. С помощью проточной цитофлуориметрии и конфокальной микроскопии установлено, что эти пептиды, меченые флуоресцентным красителем BODIPY FL, быстро проникают в опухолевые клетки и, в несколько меньшей степени, в нормальные клетки млекопитающих *in vitro*. Изучена зависимость процесса интернализации от температуры среды и энергетического метаболизма клеток-мишеней. Полученные данные о свойстве исследуемых бактенецинов проникать в эукариотические клетки подтверждают перспективность дальнейшего изучения этих пептидов как прототипов новых соединений-переносчиков лекарственных препаратов в малигнизированные или инфицированные клетки.

Ключевые слова: бактенецины Capra hircus; пролин-богатые пептиды; проникающие в клетки пептиды.

Introduction. A problem of drug delivery to the specific targets is of utmost importance in practical medicine. Many of antitumor preparations cannot effectively penetrate across membranes of malignant cells and need carriers to get to their intracellular targets. Cell-penetrating peptides (CPPs) are known as such carriers that are able to deliver varied cargos (proteins, nucleic acids, nanoparticles, liposomes, etc.) into eukaryotic cells. Despite many CPPs have been described: penetratin, fragments of viral proteins — TATp, peptide V22, etc., there is a need of a search of novel CPPs since the efficacy of existing peptides is not high for all types of tumor cells, the cell and tissue selectivity is not sufficient, and the stability of the peptides in biological liquids is low [3]. It was reported that some peptides of the innate immune system also can serve as CPPs [2]. The aim of the present study was an investigation of an ability of several cationic peptides — caprine bactenecins (ChBac5, ChBac3.4, mini-ChBac7.5Na and their analogous) to penetrate into varied types of mammalian cells *in vitro*. The listed peptides have been previously isolated by us from leukocytes of the domestic goat Capra hircus and their antibiotic

Material and methods. The studied peptides were obtained by solid-phase synthesis on the automatic peptide synthesizer Symphony X (Protein

Technologies, USA). The peptides were conjugated with a fluorescent dye BODIPY FL (Invitrogen, USA, Ex/Em 503/512) according to the dye manufacturer instruction. Labeled peptides were purified by high-performance liquid chromatography and their molecular masses were determined by MALDI TOF MS. Cellular uptake of the tagged peptides into cultured tumor and normal cells *in vitro* was quantitatively assessed using flow-cytometry on the device Navios (Beckman Coulter, USA) and observed by means of confocal microscopy (Leica TCS SL microscope and Zeiss LSM 510 Meta microscope, Germany).

Results and discussion. According to flow cytometry data, all studied peptides penetrate into target cells in non-toxic concentrations (0.2–5 μM) and within 5 minutes after adding to a cell culture can be detected in cells. The cellular uptake of the BODIPY-tagged bactenecins is similar or higher than that of a peptide TAT (47–57 fragment) labeled by the same protocol and used as a standard cell-penetrating peptide, described in the literature. The obtained values depend on the cell type and differ for each of the studied peptides. Bactenecins more effectively penetrate into tumor cells (K-562 — human erythromyeloid leukemia, THP-1 — human monocytic leukemia, U-937 — human hystiocytic limphoma) comparing to normal human cells (peripheral blood mononuclear cells (PMBC), erythrocytes of healthy donors) *in vitro*. The highest rates of the internalization into tumor cells have been demonstrated for a C-terminal fragment of ChBac5: this peptide 10 times more effectively penetrates into malignant cells THP-1 than into human PMBC and 2 times more effectively into cells K562 than into the normal mononuclear cells. All studied peptides are practically not able to internalize into erythrocytes in non-hemolytic concentrations. Study of an influence of the target cells energy metabolism and the temperature of the cultural medium on the peptides cell-penetrating efficacy revealed that lowering the temperature to 4 °C is the most critical parameter that decreases the peptides cellular uptake.

Confocal microscopy data confirm the established property of the investigated peptides to penetrate into cells.

Conclusion. The obtained data confirm the prospect of further investigations of bactenecins as prototypes of new effective CPPs for drug delivery into malignant or infected cells.

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