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SHORT COMMUNICATIONS

THE EFFECT OF RENALASE-DERIVED PEPTIDES ON VIABILITY OF $HepG_2$ AND PC3 CELLS

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Renalase (RNLS) is a recently discovered protein, which plays different roles inside and outside cells. Intracellular RNLS is a FAD-dependent oxidoreductase (EC 1.6.3.5), while extracellular RNLS lacks its N-terminal peptide, FAD cofactor, and exhibits various protective effects in a non-catalytic manner. Certain evidence exists, that plasma/serum RNLS is not an intact protein secreted into the extracellular space, and exogenous recombinant RNLS is effectively degraded during short-term incubation with human plasma samples. Some synthetic analogues of the RNLS sequence (e.g. the Desir's peptide RP-220, a 20-mer peptide corresponding to the RNLS sequence 220–239) have effects on cell survival. This suggests that RNLS-derived peptides, formed during proteolytic processing, may have own biological activity. Based on results of a recent bioinformatics analysis of potential cleavage sites of RNLS (Fedchenko et al., Medical Hypotheses, 2022) we have investigated the effect of four RNLS-derived peptides as well as RP-220 and its fragment (RP-224) on the viability of two cancer cell lines: $HepG_2$ (human hepatoma) and PC3 (prostate cancer). Two RNLS-derived peptides (RP-207 and RP-220) decreased the viability of $HepG_2$ cells in a concentration dependent manner. The most pronounced and statistically significant effect (30–40% inhibition of cell growth) was observed at 50 μM concentration of each peptide. In the experiments with PC3 cells five of six RNLS-derived peptides had a significant impact on the cell viability. RP-220 and RP-224 decreased cell viability; however, no concentration dependence of this effect was observed in the range of concentrations studied (1–50 μM). Three other RNLS-derived peptides (RP-207, RP-233, and RP-265) increased viability of PC3 cells by 20–30%, but no concentration-dependence of this effect was found. Data obtained suggest that some RNLS-derived peptides may influence the viability of various cells and manifestation and direction of the effect (increase of decrease of the cell viability) is cell-type-specific.

Key words: renalase (RNLS); RNLS-derived peptides; cell viability; $HepG_2$ and PC3 cells

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INTRODUCTION

Renalase (RNLS) is a secretory protein discovered in 2005; it performs various functions inside and outside cells [1-5]. Intracellular RNLS exhibits the properties of a FAD-dependent oxidoreductase (EC 1.6.3.5) [5, 6], which oxidizes β-NAD(P)H isomeric forms reduced at position 2 or 6 of the nicotinamide ring instead of the metabolically active position 4 [7]. In the catalytically active form the FAD cofactor can be "accommodated" only in a full-length protein containing an N-terminal peptide [8, 9]. At the same time, this N-terminal peptide is considered as a signal for the extracellular localization of the protein, which is cleaved during the secretion of this protein into the extracellular space [10]. Extracellular RNLS lacking an N-terminal peptide is unable to bind FAD [9]. It exerts various protective effects on the cell through interactions with receptor proteins [11–14]. The detection of RNLS in the blood indicates that this protein can act remotely on resident cells of various organs [14]. However, according to our data, an intramolecular fragment corresponding to amino acid residues 100–116 of the RNLS sequence is not detected in the blood [15, 16]. In direct experiments, it was shown that short-term incubation of recombinant RNLS with blood plasma preparations caused a significant decrease in the level of the full-length protein [17]. This obviously suggests that RNLS entering the circulation undergoes proteolytic processing [17], and the resultant RNLS peptides have their own biological activity. Bioinformatic analysis performed using the Peptide Cutter and Procleave programs revealed potential cleavage sites, as well as proteolytic enzymes capable (or not capable) of RNLS processing [17]. In this regard, it was of interest to evaluate the effect of some of these peptides on cell viability. It has already been shown earlier that the Desir's RP-220 peptide (a 20-mer peptide corresponding to the amino acid sequence RNLS 220–239) had a significant impact on cell survival [14].

In this study we have investigated the effect of six peptides corresponding to several fragments of the RNLS amino acid sequence, including the Desir's peptide RP-220 and its fragment (RP-224), on the viability of two human cancer cell lines: $HepG_2$ (hepatoma) and PC3 (prostate cancer).

Table 1. RNLS-derived peptides used in this study

No.	RNLS-derived peptide	Peptide position in the RNLS sequence	Length (residues)
1	RP-104 – NFVAPQGISSIIKHYLK	104–120	17
2	RP-207 – DVPWAGQYITSNPCIR	207–222	16
3	RP-220 – CIRFVSIDNKKRNIESSEIG	220–239	20
4	RP-224 – VSIDNKKRN	224–232	9
5	RP-233 – IESSEIGPSLVIHTTVPFGV	233–252	20
6	RP-265 – LVFQQLENILPGLPQP	265–280	16

MATERIALS AND METHODS

The PC3 (androgen-independent adenocarcinoma of the prostate) and $HepG_2$ (liver carcinoma) cell lines maintained in the collection of cell cultures of the Institute of Biomedical Chemistry were used in this study. They were cultivated in the RPMI-1640 (in the case of PC3) or DMEM (in the case of $HepG_2$) medium (PanEco, Russia) supplemented with 10% fetal calf serum (HyClone®, ThermoScientific, USA), 2 mM L-glutamine (PanEco), and 1% gentamicin (Biochemist, Russia). The RNLS-derived peptides, were synthesized by BelkiAntitela (Russia). Their amino acid sequences are shown in Table 1. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from Sigma-Aldrich (USA). All substances were dissolved in deionized water.

Cultivation of Cells and Determination of their Viability using the MTT Test

Cells were seeded in a 96-well plate (Costar, USA) (2×10^4 cells/100 μl per well) and cultivated for 24 h at 37°C in a humified atmosphere containing 5% CO_2. After that, the medium was removed and the test compounds were added in fresh growth medium (100 μl) at final concentrations of 1 μM; 5 μM; 10 μM; 25 μM; and 50 μM. Cells were incubated with RNLS peptides for 72 h under the same conditions.

The effect of the studied RNLS peptides on the cell viability was assessed using the MTT assay [18]. Briefly, after incubation with peptides, the medium was removed and 100 μl of MTT (1 mg/ml) in culture medium was added to each well. The plates were additionally incubated for 3 h at 37°C in a humified atmosphere containing 5% CO_2. Next, the medium was removed from the plates, and 100 μl of dimethyl sulfoxide was added to each well to dissolve the formazan crystals formed in living cells. Optical density was determined at 570 nm using a GENiosPlus plate analyzer (TECAN, Switzerland). All data presented here represent the results of three or four parallel experiments.

RESULTS AND DISCUSSION

Two of the six RNLS peptides tested (RP-207 and RP-220) reduced the viability of $HepG_2$ cells in a concentration-dependent manner (Fig. 1a). The most pronounced and statistically significant effect (30–40% inhibition of cell growth) was noted at the maximum concentration of each peptide (50 μM). Interestingly, the RP-224 peptide, containing the nine-residue sequence of the Desir's peptide (RP-220), basically had no effect on the viability of $HepG_2$ cells.

In experiments with PC3 cells, five of the six RNLS peptides influenced the cell viability (Fig. 1b). RP-220 and RP-224 reduced the cell viability, but no concentration dependence of this effect was observed in the range of studied concentrations (1–50 μM). Three other RNLS peptides (RP-207, RP-233, and RP-265) increased the viability of PC3 cells by 20-30%, but again no dependence of this effect on the concentration of the studied peptides was found. In contrast to experiments on $HepG_2$ cells, the effect of RP-220 and RP-224 on PC3 cells was comparable. The absence of a concentration dependence of the action of RNLS peptides on PC3 cells requires a separate study. It is quite possible that in order to reveal a dose-dependent effect, it will be necessary to change the time of culturing cells with the studied peptides.

Thus, the data obtained indicate that some peptides formed during the proteolytic cleavage of RNLS can affect the viability of various cells, but the manifestation and direction of the effect (increase or decrease in cell viability) depend on the type of cells they act on.

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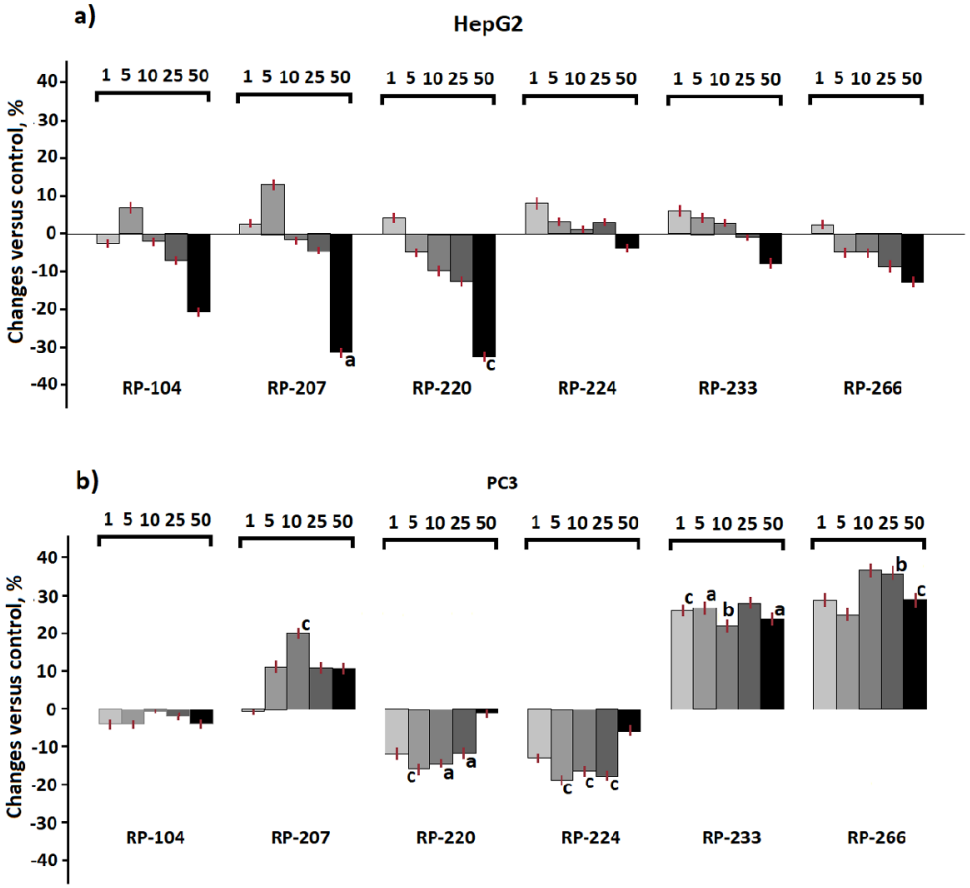

Figure 1. The effect of RNLS peptides on the viability of HepG$_2$ (**a**) and PC3 (**b**) cells. Letters indicate the significance of differences versus control: a – $p<0.05$; b – $p<0.02$; c – $p<0.01$.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or using animals as subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ВЛИЯНИЕ РЕНАЛАЗНЫХ ПЕПТИДОВ НА ЖИЗНЕСПОСОБНОСТЬ КЛЕТОК $HepG_2$ И PC3

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Реналаза (RNLS) — недавно открытый белок, который играет разные роли внутри и снаружи клеток. Внутриклеточная RNLS представляет собой FAD-зависимую оксидоредуктазу (КФ 1.6.3.5), в то время как внеклеточная RNLS, лишённая своего N-концевого пептида и кофактора FAD, проявляет различные защитные эффекты при помощи некаталитических механизмов. Накапливается всё больше данных, что RNLS плазмы/сыворотки не является интактным белком, секретируемым во внеклеточное пространство, а экзогенная рекомбинантная RNLS эффективно разрушается при кратковременной инкубации с образцами плазмы человека. Некоторые синтетические аналоги последовательности RNLS (например, пептид Дезира RP-220 — 20-членный пептид, соответствующий аминокислотной последовательности RNLS 220–239) влияют на выживаемость клеток. Это свидетельствует о том, что пептиды RNLS, образующиеся в ходе протеолитического процессинга, могут обладать собственной биологической активностью. Основываясь на результатах недавнего биоинформатического анализа потенциальных сайтов расщепления RNLS (Fedchenko et al., Medical Hypotheses, 2022) мы исследовали действие шести пептидов, соответствующих нескольким фрагментам аминокислотной последовательности RNLS, включая RP-220 и его фрагмент (RP-224), на жизнеспособность двух линий раковых клеток человека: $HepG_2$ (гепатома) и PC3 (рак предстательной железы). Два пептида RNLS (RP-207 и RP-220) концентрационно-зависимым образом снижали жизнеспособность клеток $HepG_2$. Наиболее выраженный и статистически значимый эффект (30–40% торможение роста клеток) отмечен при концентрации каждого пептида 50 мкМ. В экспериментах с клетками PC3 пять из шести пептидов RNLS оказывали влияние на жизнеспособность клеток. При этом RP-220 и RP-224 снижали жизнеспособность клеток, однако концентрационной зависимости этого эффекта в диапазоне исследованных концентраций (1–50 мкМ) не наблюдалось. Три других пептида RNLS (RP-207, RP-233, RP-265) повышали жизнеспособность клеток PC3 на 20–30%, но зависимости этого эффекта от концентрации исследуемых пептидов обнаружено не было. Полученные данные свидетельствуют о том, что некоторые пептиды, образующиеся в ходе протеолитического расщепления RNLS, могут влиять на жизнеспособность различных клеток, однако проявление и направленность эффекта (увеличение или снижение жизнеспособности клеток) зависят от типа клеток, на которые они действуют.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: реналаза (RNLS); RNLS пептиды; жизнеспособность клеток; клетки $HepG_2$ и PC3

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