UDK 616-006.04:577.122.612.014

O. Gerastchenko, PhD student, E. Zhuravel, PhD.,
M. Soldatkina, PhD., P. Pogrebnoy, Doctor Biol Sc.
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Kyiv

EXPRESSION OF HUMAN BETA-DEFENSINS-1-4 MRNA IN NAMALWA AND JURKAT CELLS

The expression profile of human beta-defensins-1-4 in human Burkitt lymphoma Namalwa cell line and Jurkat leukemic T-cell line has been analyzed. It has been shown that in Namalwa and Jurkat cells, hBD-1, hBD-2 and hBD-3 genes are expressed, while hBD-4 expression is absent. Treatment of Namalwa and Jurkat cells with 0.1-1000 nM of recombinant hBD-2 results in significant concentration-dependent suppression of viability of these cell lines.

Keywords: human beta-defensins, expression, lymphoma cell line, leukemic cell line.

Introduction. Human beta-defensins (hBDs), cationic peptide antibiotics and important components of innate immunity system in humans, are mainly expressed in epithelial cells of different origin and provide first-line defense of epithelial surfaces from microbial challenge. The family of hBDs includes more than 30 members, however, up to date, the most studied members of beta-defensin family are hBD-1 and hBD-2, and to the lesser extent – hBD-3 and hBD-4. hBD-1 is supposed to be constitutively expressed in epithelial cells, while three other hBDs – hBD-2,-3, and -4 are peptides with inducible character of expression. All these beta-defensins demonstrate potent antimicrobial activity against a variety of bacterial pathogens, that's why direct microbial killing is supposed to be their main function. However, experimental data suggest that defensins are multifunctional molecules with a wide spectrum of important biological activities and could be involved not only in local immediate antimicrobial response but also in chemotaxis, modulation of inflammatory response, angiogenesis, and wound healing [1, 2]. In a number of studies there have been documented abilities of hBDs to influence many vital cell processes – cell proliferation, viability, differentiation, and apoptosis, and it has been shown that such effects of hBDs are concentration-dependent and could be exerted against many cell types [3-5]. In a similar manner hBDs may affect growth patterns of tumor cells and may play a role in promotion or suppression of human cancer cell growth. According to our data [6], recombinant hBD-2 (rec-hBD-2) causes significant suppression of lung cancer cell growth *in vitro* via cell cycle regulation. It is of interest to analyze whether hBD-2 could exert its growth suppressing effects on growth patterns of human malignant white blood cells. In this work we have analyzed for the first time the expression patterns of hBD-1-4 mRNA in human Burkitt lymphoma Namalwa cell line and Jurkat leukemic T-cell line and influence of rec-hBD-2 on viability of mentioned malignant cells.

Materials and methods. Human Burkitt lymphoma Namalwa cell line and Jurkat leukemic T-cell line were obtained from the Bank of Cell Lines from Human and Animal Tissues, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine (Kyiv, Ukraine). The cells were cultured *in vitro* in DMEM culture medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G sodium, 100 μg/mL streptomycin sulphate in 5% CO_2 atmosphere at 37^oC.

To study the effect of exogenous defensin on cell growth, we have used the preparation of rec-hBD-2 expressed in bacterial cells as GST-hBD-2 fusion protein and purified by standard two-step procedure as described earlier [7]. Protein concentration was determined by UV absorbance at 280 nm using spectrophotometer Nanodrop-1000 (USA).

To evaluate the effect of rec-hBD-2 on cell viability, MTT-test has been applied [8]. Shortly, Namalwa and Jurkat cells were seeded into 96-well plates and incubated with rec-hBD-2 for 48 h in serum-free medium. Then the cells were treated with MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide), and colorimetric reaction was evaluated with the use of ELISA reader (Awareness Technology Inc, USA) at the wave lengths λ_{min} =545.

To evaluate the patterns of hBD-1-4 mRNA expression, semiquantative RT-PCR was used. Total RNA was isolated from Namalwa and Jurkat cells by the method of Chomzynski and Sacchi [9]. For detection of hBDs expression, semiquantitative RT-PCR analysis was performed using specific primers (see Table 1).

***Table* 1. Primers for the genes of interest**

Gene	Primers
DEFB1 (hBD-1)	F:-5′-TGTTGCCTGCCAGTCGCCATGAG R: 5′-TCACTTGCAGCACTTGGCCTTCCC
DEFB4 (hBD-2)	F: – 5′-GAAGCTCCCAGCCATCAGCC R: – 5′-GTCGCACGTCTCTGATGAGGGA
DEFB103 (hBD3)	F: CCTGTTTTTGGTGCCTGTTCC R: CTTTCTTCGGCAGCATTTTCG
DEFB104 (hBD-4)	F: 5′-GAAGCTCCCAGCCATCAGCC R: 5′-GTCGCACGTCTCTGATGAGGGA
Beta-actin	F: 5′-CTGGAACGGTGAAGGTGACA R: 5′-AAGGGACTTCTTGTAACAATGCA

The expression level of beta-actin (the housekeeping gene) served as a loading control. The products of RT-PCR were routinely analyzed by electrophoresis in agarose gel.

The data are reported as the mean ± SD. The statistical significance of differences between mean values was assessed by the Student's *t*-test. Values $p < 0.05$ were considered as statistically significant.

Results and discussion. The results of semiquantative RT-PCR analysis have demonstrated that human Burkitt lymphoma Namalwa cells and Jurkat leukemic T-cells express hBD-1, -2 and -3 mRNA, while expression of hBD-4 mRNA in these cells is not detected (Fig. 1).

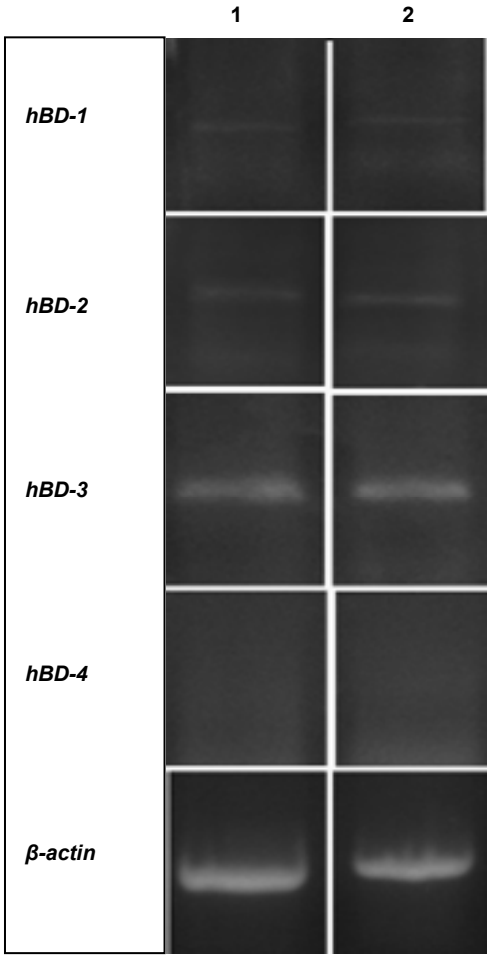

Fig. 1. Semiquantative RT-PCR analysis of hBD-1-4 mRNA expresssion in Jurkat (3, 5, 7, 9) and Namalwa (4, 6, 8, 10) cells. β-actin is used as a house-keeping gene

Up-to-date this is the first observation of beta-defensin mRNA expression in mentioned human lymphoma and leukemic cells, and so far the functional role of beta-defensin expression in malignant white blood cells remains unknown.

Next, we have analyzed an influence of rec-hBD-2 on viability of cultured Jurkat and Namalwa cells and have determined that the defensin causes a concentration-dependent effect on the numbers of viable cells (Fig. 2).

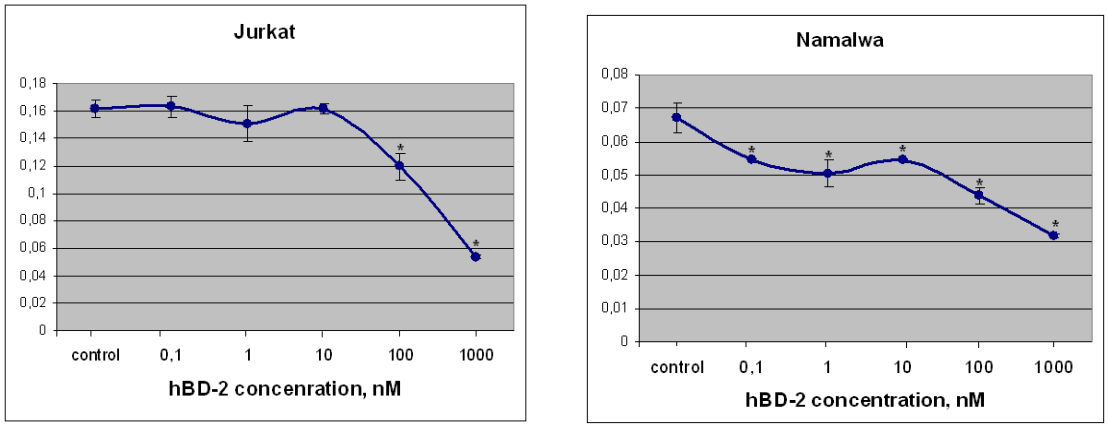

Fig. 2. Effect of exogenous rec-hBD-2 on the viability of cultured Jurkat and Namalwa cells (MTT analysis). The data of three independent experiments are presented as the mean ± SD. *The difference is significant as compared to control ($p < 0.05$)

In concentration range of 0.1-10 nM rec-hBD-2 has no significant effect on viability of Jurkat cells, but its action in higher concentrations (10-1000 nM) results in drastic suppression of Jurkat cell viability. Viabity of Namalwa cells treated with 0.1-1000 nM rec-hBD-2 was significantly lower ($p<0.05$) than that of control cells, so one may conclude that these lymphoma cells are more sensitive to the growth suppressive activity of hBD-2 than leukemic cells.

These data are in agreement with our previously published data on involvement of hBD-2 into the growth regula-

tion of human cancer A431 and A549 cell lines and a concentration-dependent effect of this defensin on proliferation and viability of human carcinoma cells. The most important observation is the fact that beta-defensins 1-3 are expressed by malignant white blood cells and that rec-hBD-2 could suppress Jurkat and Namalwa cell viability in nanomolar concentration range. Such concentrations are close to reported physiologic serum concentrations of hBD-2 registered in healthy individuals and in the cases of some human pathologies (psoriasis, atopic dermatitis, rheumatoid arthritis) [10]. So, it's of interest to further explore *in vivo* patterns of hBD-2 expression in leukemia and lymphoma cells; the knowledge on mechanisms of beta-defensin gene induction in human lymphoma and leukemia cells and biologic effects of human peptide antibiotics toward cancer cells will help to understand the involvement of defensins in tumorigenesis and their potential use in treatment of human pathologies.

Conclusions. Human Namalwa and Jurkat cells express hBD-1, hBD-2 and hBD-3 genes, and respond on growth suppressive activity of rec-hBD-2 in a concentration-dependent fashion.

Acknowledgement. *This study has been supported in part by grant 0112U002194 "The study of transcription programs of regulation of gene expression in normal and malignant cells" in the frame of NASU program "Functional Genomics and Metabolomics in System Biology".*

References

1. Auvynet C., Rosenstein Y. Multifunctional host defense peptides: antimicrobial peptides, the small yet big players in innate and adaptive immunity // FEBS. J. – 2009. – V. 276. – P. 6497-508.

2. Kohlgraf K.G., Pingel L.C., Dietrich D.E., Brogden K.A. Defensins as anti-inflammatory compounds and mucosal adjuvants// Future Microbiol. – 2010. – V.5. – P. 99-113.

3. Baroni A., Donnarumma G., Paoletti I., et al. Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells // Peptides. – 2009. – V.30. – P. 267-72.

4. Winter J., Pantelis A., Reich R., et al. Human beta-defensin-1, -2, and -3 exhibit opposite effects on oral squamous cell carcinoma cell proliferation // Cancer Invest. – 2011. – V.29. – P. 196-201.

5. Bullard R.S., Gibson W., Bose S.K., et al. Functional analysis of the host defense peptide human beta defensin-1: new insight into its potential role in cancer // Mol. Immunol. – 2008. – V. 45. – P. 839-48.

6. Zhuravel E., Shestakova T., Efanova O., et al. Human beta-defensin-2 controls cell cycle in malignant epithelial cells: in vitro study // Exp. Oncol. – 2011. – V. 33. – P. 114-20.

7. Lisovskiy I.L., Markeeva N.V., Shnitsar V.M., et al. Production of recombinant of hBD-2 – human antimicrobial peptide expressed in cervical and vulval cancer // Exp. Oncol. – 2003. – V.25. – P. 36-9.

8. Mosman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays // Immunol. Methods. – 1983. – V. 65. – P. 55-63.

9. Chomczynski P., Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction // Anal. Biochem. – 1987. – V. 162. – P. 156-9.

10. Jansen P.A., Rodijk-Olthuis D., Hollox E.J., et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin // PLoS One. – 2009. – V.4. – e4725.

Received to editorial board 09.07.13

О. Геращенко, асп., О. Журавель, канд. біол. наук, М. Солдаткіна, канд. біол. наук, П. Погрібний, д-р біол. наук
Інститут експериментальної патології, онкології і радіобіології імені Р.Є. Кавецького НАНУ, Київ

ЕКСПРЕСІЯ МРНК БЕТА-ДЕФЕНСИНІВ ЛЮДИНИ 1-4 В КЛІТИНАХ ЛІНІЙ *NAMALWA* ТА *JURKAT*

Досліджено профіль експресії генів бета-дефенсинів людини 1-4 в клітинах Namalwa (лімфома Беркітта) та Jurkat (Т-клітинна лейкемія). Встановлено, що в клітинах Namalwa та Jurkat експресовано гени hBD-1, hBD-2 та hBD-3, але ген hBD-4 не експресовано. Інкубація клітин Namalwa та Jurkat з рекомбінантним hBD-2 в концентрації 0.1-1000 нМ мала наслідком значуще концентраційно-залежне пригнічення життєздатності вказаних клітин.

Ключові слова: бета-дефенсини людини, експресія, лінія клітин лімфоми, лінія клітин лейкемії.

О. Геращенко, асп., О. Журавель, канд. биол. наук, М. Солдаткина, канд. биол. наук, П. Погрибной, д-р биол. наук
Инстиут экспериментальной патологии, онкологии и радиобиологии имени Р.Е. Кавецкого, Киев

ЭКСПРЕССИЯ МРНК БЕТА-ДЕФЕНСИНОВЧЕЛОВЕКА 1-4 В КЛЕТКАХ ЛИНИЙ *NAMALWA* И *JURKAT*

Проанализирован профиль экспрессии генов бета-дефенсинов человека 1-4 в клетках Namalwa (лимфома Беркитта) и Jurkat (Т-клеточная лейкемия). Установлено, что в клетках Namalwa и Jurkat экспрессированы гены hBD-1, hBD-2 и hBD-3, но не экспрессирован ген hBD-4. Инкубация клеток Namalwa и Jurkat с рекомбинантным hBD-2 в концентрации 0.1-1000 нМ приводила к значительному концентрационно-зависимому угнетению жизнеспособности указанных клеточных линий.

Ключевые слова: бета-дефенсины человека, экспрессия, линия клеток лимфомы, линия клеток лейкемии.

UDC 57.044:616.018:612.33+616.34-006

O. Babuta, PhD student, N. Kutuzova, student, O. Lynchak, PhD
Taras Shevchenko National University of Kyiv, Kyiv

THE INFLUENCE OF MALEIMIDE DERIVATIVE AND 5-FLUORACIL ON MORPHOLOGICAL STATE OF THE SMALL INTESTINE OF RATS WITH CHEMICAL-INDUCED COLON CANCER

The influence of maleimide derivative (1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrole-2,5-dione, MI-1) and 5-fluorouracil (5FU) for 6 weeks on the small intestine mucosa of rats with 1,2-dimethylhydrazine-induced colon cancer. Found that MI-1 and 5FU reduces the rat small intestine mucosa damage caused by the exposure of DMH. The positive effect of MI-1 is more pronounced than 5FU.

Keywords: maleimide derivative, 5-fluorouracil, 1,2-dimethylhydrazine, colon cancer, small intestine.

Introduction. Despite the significant development of modern science the problem of oncologic diseases remains unresolved for medicine and biology. Researchers at many laboratories work to develop new anticancer medications. The promising substance is maleimide derivative 1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrole-2,5-dione (MI-1). This substance was created by *insilico* design as an inhibitor of ATP-binding site of proteinkinases and was synthesized in Chemical Faculty of Taras Shevchenko National University of Kyiv [1-3]. Most anticancer drugs except antineoplastic effects have also high cytotoxicity. Therefore it is important to study the influence of potential antitumor substances for the state not only affected organs, but also healthy, especially those through which exogenous compounds come and metabolize [3, 4]. Thus the aim of our research is to study the morphological state of the small intestine of rats with 1,2-dimethylhydrazine (DMH)-induced colon cancer under the influence of a potential