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.

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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.

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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

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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

antineoplastic compounds MI-1 compared with existing anticancer drug 5-fluorouracil (5FU).

Materials and methods

All experimental procedures were conducted in the manner prescribed by the approval principles of bioethics, legislation and regulations of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and "General ethical principles of animal experiments" approved in First National Congress of Bioethics (Kyiv, 2001). Forty white nonlinear male rats weighing from 170 to 200 g were used. The animals were kept under standard vivarium conditions at natural light. Rats were divided into four groups: I – control, III-V – 20 weeks DMH (20 mg/kg) [5] and the next 6 weeks: III – received nothing, IV – MI-1 in dose 2.7 mg/kg (on average 10^{-4}mol/l in blood) in 0.1 ml of sunflower oil intragastrically daily, V – intraperitoneal 5-FU injections (45 mg/kg).

Animals were anesthetized and decapitated on the 27 week of the experiment. The middle segments of the rat small intestine were taken for histology investigation. The tissue samples were fixed and treated by conventional histological methods for making paraffin section [6]. The sections were stained with hematoxylin and eosin. The morphometric study of the intestinal mucosa was accomplished using a digital system connected to an optic microscope. Morphometrics included the total thickness of intestinal mucosa, villi height, crypt depth, diameter of villi and their stroma, absorption cell height (μm) and cross-sectional area of their nuclei (μm^2), number of goblet cells per villus and their cross-section area (μm^2). Statistical analysis of the experimental results was performed using Microsoft Excel for PC.

Results. The small intestinal mucosa of control rats has a typical structure. The thickness of the mucosa is 643.8 ± 43.8 μm. Villi have the right form. The villi height is 412.5 ± 55.3 μm. Villous stroma has blood and lymph capillaries. Diameter of the villi is 107.6 ± 1.6 μm and its stroma – 37.8 ± 2.4 μm. The largest populations in intestinal epithelial cells is absorptive cells that cover the villi. The absorptive cells height is 31.1 ± 1.9 μm, the cross-sectional area of their nuclei – 27.1 ± 2.0 μm^2. Between absorptive cells are goblet cells that produce mucus, number of this cells per villus is 22.8 ± 1.2 pcs and their cross-section area is 90.5 ± 0.8 μm^2. The intestinal epithelium forms tubular invagination around the ***villi*** – it is intestinal crypts. The crypts depth is 203.6 ± 9.7 μm in control.

There are significant morphological changes in the rats small intestine mucosa after 6 weeks after last DMH injection. Carcinogen causes swelling of the villi, epithelial desquamation, stagnation in the vessels of the villi. ***There is a tendency*** the thickness of the mucosa to increase (700.4 ± 42.4 μm). The villi height nonsignificant increase – 498.0 ± 39.7 μm (fig.1)

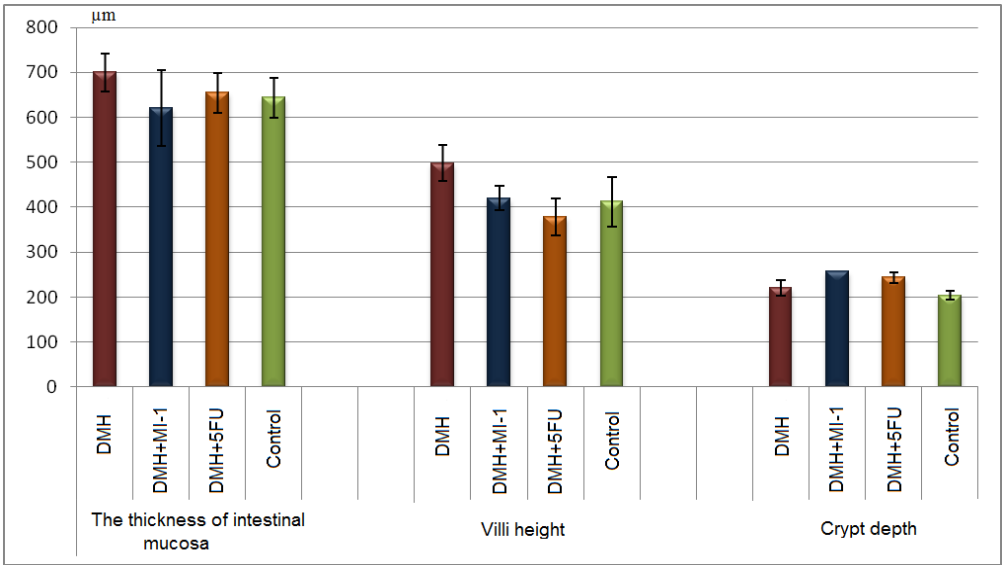

Fig. 1. The thickness of intestinal mucosa, villi height and crypt depth under the influence of the MI-1 and 5FU on the background of DMH -induced colon cancer

The diameter of villi increases by 9% compared with the control (117.5 ± 3.5 μm) (fig.2) . The diameter of villi stroma is 52.5 ± 6.8 μm (fig.2). The absorption cells height is 37.3 ± 1.2 μm which is 20% more than in the control (fig.2). The cross-sectional area of the absorption cells nuclei significantly increases compared with control (27.5%) and amounts to 34.6 ± 2.1 μm^2 (fig.3). Number of goblet cells is 27.3 ± 2.9 pcs on the villus, which is 20% higher than control values. At the same time the cross-sectional area of goblet cells increases – 105.5 ± 11.9 μm^2 (fig.3). The crypts depth is 220.1 ± 17.3 μm (fig.1).

The action of MI-1 for 6 weeks at a dose of 2.7 mg/kg after 20 weeks injection of DMH restores the rat small intestine mucosa. The mucous thickness is 620.4 ± 84.9 μm (fig.1). The form of villi is right. The villi height is 420.3 ± 27.4 μm and has no significantly different with control (fig.1). The villi diameter is also in the range of control values (114,3 ± 9,8 μm). The villi stroma does not change, its diameter is 45.9 ± 4.9 μm (fig.2). The height of the absorption cells is 29.9 ± 2.2 μm, the cross-sectional area of their nuclei is 34.4 ± 1.8 μm^2 (fig. 2,3). Number of goblet cells on the villous is near the control (21.3 ± 1.4 pcs), but the cross section area of the goblet cells significantly increases – 113.1 ± 4.2 μm^2 (25% compared with control) (fig.3). The crypts histoarchitectonics doesn't change, but there is a tendency their depth to increase (by 26% compared to control – 257.3 ± 0.3 μm) (fig.1).

The are some morphological changes in the rat small intestinal mucosa under the 5-fluorouracil action at a dose of 45 mg/kg for 6 weeks after a 20-week injection of DMH. The mucous thickness is in the range of control values – 654.7 ± 44.3 μm (fig.1). The villi height is 379.2 ± 41.1 μm (fig.1). There is a tendency villi diameter (101.8 ± 2.1 μm) to reduce relative to control (fig.2).

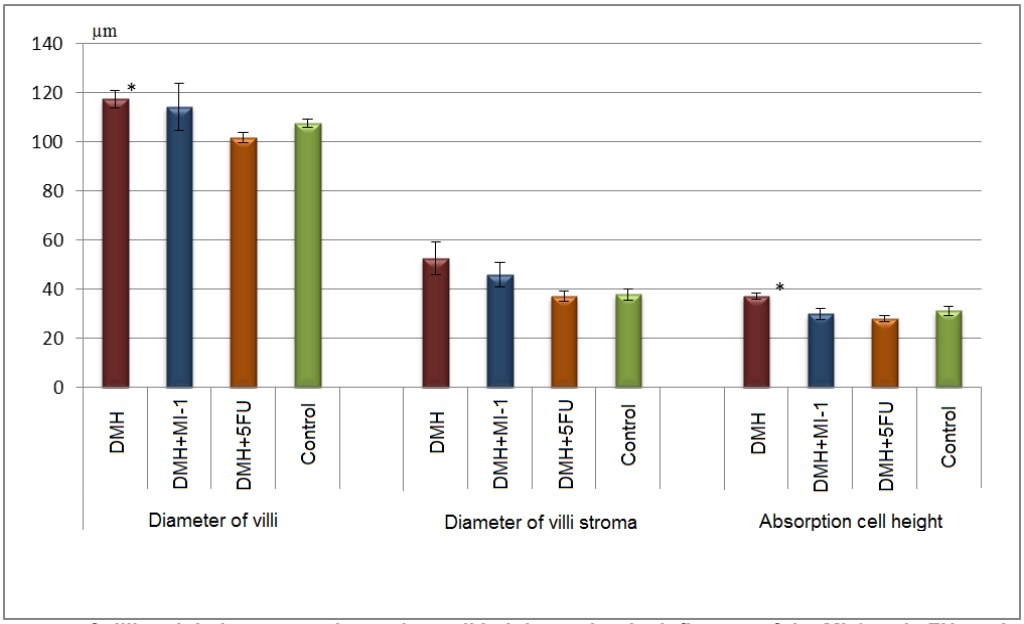

Fig. 2. The diameter of villi and their stroma, absorption cell height under the influence of the MI-1 and 5FU on the background of DMH-induced colon cancer. Significant differences compared to the corresponding control: * – p ≤ 0,05

There is swelling of the villi but its significant reduction compared with DMH. The villous stroma diameter is smaller compared to the DMH and it is equal to 37.0 ± 2.2 µm (fig.2). The height of rat small intestine mucosa absorption cells has no significantly different with control and is 28,1 ± 1,2 µm (fig.2). The cross-sectional area of absorption cells nuclei is 27.7 ± 2.2 µm^2 (fig.3).

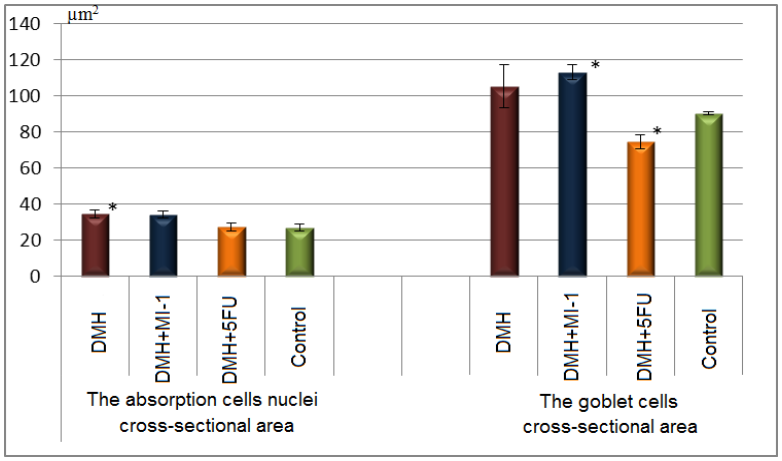

Fig. 3. The cross-sectional area of the absorption cell nuclei and goblet cells under the influence of the MI-1 and 5FU on the background of DMH -induced colon cancer.. Significant differences compared to the corresponding control: * – p ≤ 0,05

However, the number of goblet cells is a significant increased compared with control (19%) and is 27.2 ± 1.0 pcs. At the same time the cross-sectional area of goblet cells is a significant decreased (17%) and equals 77.7 ± 3.9 µm^2 (fig.3). Crypts has normal structure, the depth is not considerably different from control and is 243.9 ± 12.1 µm (fig.1).

Conclusion. Maleimide derivative MI-1 reduces swelling of the villi, epithelial desquamation, stagnation in the vessels of the villi that cause DMH. The changes in the crypts depth and goblet cells area indicating improvement of levels of the epithelium physiological regeneration and activation of adaptive mechanisms under the influence of MI-1. 5-Fluorouracil reduced swelling of the villi and villi diameter compared with the administration of the DMH. The increasing number of goblet cells and reducing their area, indicating the activation of protective processes in rat small intestine mucose under 5FU action. Thus, MI-1 and 5FU reduces the rat small intestine mucosa damage caused by the exposure of DMH. Positive effect of MI-1 is more pronounced than 5FU. Maleimide derivative MI-1 is a promising compound for further research.

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ВПЛИВ ПОХІДНОГО МАЛЕІМІДУ І 5-ФТОРУРАЦИЛУ НА МОРФОЛОГІЧНИЙ СТАН ТОНКОЇ КИШКИ ЩУРІВ З ХІМІЧНО-ІНДУКОВАНИМ РАКОМ ТОВСТОЇ КИШКИ

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.

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ВЛИЯНИЕ ПРОИЗВОДНОГО МАЛЕИМИДА И 5-ФТОРУРАЦИЛА НА МОРФОЛОГИЧЕСКОЕ СОСТОЯНИЕ ТОНКОЙ КИШКИ КРЫС С ХИМИЧЕСКИ-ИНДУЦИРОВАННЫМ РАКОМ ТОЛСТОЙ КИШКИ

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.

UDK: 577.3.616-006.04:618

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IN VITRO TESTING FOR PREDICTION OF TOXITY AND FOR SCREENING OF POTENTIAL CARCINOGENS AND ANTI–CANCER DRUGS

Cell-based technologies can be used for screening of potential carcinogens and evaluation of stem cell quality for clinical applications. 3D spheroids can serve as intermediate in vitro/ in vivo model for evaluation of anti-carcinogenic effects of therapeutic substances.

Key words: mesenchymal stem cells, MCF7 cell line, cell transformation, cell differentiation, spheroids, transformed foci.

Introduction. Cell-based technologies have broad experimental applications including pre-clinical studies in drug discovery and in regenerative medicine. Multi-potent stem cells have many clinical applications due to their capacity to be expanded *in vitro,* differentiate into several lineages, and participate in tissue regeneration. Clinical application of stem cells requires quality control to avoid potential carcinogenic effects of non-differentiated cells. Cell transformation (foci formation) and cell differentiation assays can provide information about stem cell quality and also can be used to evaluate drug effects.

Standard two-dimensional cell cultures for testing effects of anticancer agents are simple and convenient. However, they have significant limitations in reproducing the complexity and pathophysiology of *in vivo* tumor tissue [31; 33; 34; 37; 38; 39; 46]. Three-dimensional culture systems are of increasing interest in cancer research since tissue architecture and the extracellular matrix (ECM) significantly influence tumor cell responses to micro–environmental signals [43].

Vinci et al. [45] discovered differential sensitivity to targeted agents between two-dimensional and three-dimensional cultures, and also demonstrated enhanced potency of some agents against cell migration and invasion compared to cell proliferation. 2D and 3D *in vitro* functional assays can enhance the biological relevance of early preclinical cancer studies. These assays will increase the translational predictive value of *in vitro* drug evaluation studies and reduce the need for *in vivo* studies by more effective triaging of compounds.

Methods. Cells: Mouse mesenchymal stem cell line was obtained from the Tulane University Center for Gene Therapy under Material Transfer Agreement. These cells were originally derived from femurs and tibiae of C57BL mice. Cells were grown in IMDM (Invitrogen/GIBCO, CA) that was supplemented with 10% Premium Select FBS (Atlanta Biologicals, GA), 10% HS (Hyclone, UT), 100 U/mL penicillin, 100 µg/mL streptomycin (Invitrogen, CA). Colony formation and differentiation assays were performed periodically with iron oxide loaded and unloaded cells according to the instructions provided by the cell supplier. Briefly, cells were grown to 70-90% confluency in 6-well tissue culture plates, then incubated for 3 weeks in osteogenic or adipogenic differentiation medium, with medium change twice a week, and then stained for 10 min with 2 ml of 40 mM Alizarin red (pH 4.1; Sigma) for mineral deposits [36] or Oil Red-O (Sigma) for fat globules [18, 32], respectively. 3D transformed foci were comprised of cells that lost contact inhibition. We determined transformation frequency as ratio of foci number and the number of plated cells.

For tumor spheroid assay, we used MCF7 breast cancer cell line, kindly provided by Dr. I.Gut. We used modified standard method for multi-cellular spheroid generation [11]. Confluent cells were trypsinized, and single-cell suspension was seeded on low-adhesive substrate with density 1.0x105 cells/ml in growth medium with 0.24% carboxymethyl-cellulose (CMC). We generated spheroids in 6-wells test-plates (Nunclon, Belgium) and Petry dishes. Dishes with cells were placed on shaker with low rotation (150 rpm) for one hour. The number of live cells was determined in wells using MTT-colorymetric test. Cell count was performed after 48 hours incubation using a Trypan Blue dye [25]. Distribution of cells between different phases of the cell cycle and apoptotic index were assessed by flow cytometry [26]. Glucose absorption and LDG-