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ORIGINAL CONTRIBUTIONS

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HUMAN BETA-DEFENSIN-2 CONTROLS CELL CYCLE IN MALIGNANT EPITHELIAL CELLS: *IN VITRO* STUDY

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***Aim*: In the present research we analyze the mechanism of human beta-defensin-2 (hBD-2) influence on cultured malignant epithelial cell growth. *Materials and Methods*: The analysis of a concentration-dependent effect of recombinant hBD-2 (rec-hBD-2) on cell growth patterns and cell cycle distribution has been performed *in vitro* with 2 cell lines (human lung adenocarcinoma A549 cells and human epidermoid carcinoma A431 cells) using MTT test, flow cytometry and direct cell counting. To study intracellular localization of hBD-2 immunocytofluorescent and immunocytochemical analyses were applied, and effect of hBD-2 on signal cascades involved in cell cycle regulation has been studied by Western blotting. *Results*: According to our data, rec-hBD-2 exerts a concentration-dependent effect on the viability of cultured A549 and A431 cells. It causes proproliferative effect at concentrations below 1 nM, significant suppression of cell proliferation at concentration range from 10 nM to 1 μM ($p<0.05$), and cell death at higher concentrations. Using flow cytometry we have demonstrated that hBD-2 dependent growth suppression is realized via cell cycle arrest at G1/S phase ($p<0.05$). Also, we have registered significant activation of pRB and decreased expression of Cyclin D1 in cells treated with the defensin compared to untreated control cells, while the expression of p53 remains unaffected. The study of intracellular localization of hBD-2 in these cells has revealed that exogeneously added defensin molecules enter the cells, are distributed throughout the cytoplasm and could be detected in cell nuclei. The model study using A549 cells treated with 1,25-$(OH)_2D_3$ has shown similar cell growth suppression effect of native endogenously produced hBD-2. *Conclusion*: The results of our study suggest that in malignant epithelial cells hBD-2 may control cell growth via arrest of G1/S transition and activation of pRB.**
***Key Words*: human beta-defensin-2, cell cycle, signaling pathway, pRB, intracellular localization.**

INTRODUCTION

Human beta-defensins (hBDs) belong to a family of antimicrobial peptides that constitute an important part of the innate immune defense system. hBDs are expressed mostly in epithelial tissues. Up to date, four hBDs, hBD-1–4 have been identified in human tissues. These molecules are small, with a molecular weight ranging from 3.5 to 4.5 kDa, possess high positive net charge and hydrophobic properties that allow their interaction with microbial membrane, its penetration or its destruction. They exert direct antimicrobial action and are active against bacteria, fungi, viruses; in parallel, according to the recent data, they possess multiple biologic activities, in particular, immunomodulatory ones, and are implicated in anti-tumor response [reviewed in 1, 2].

Biophysical properties of defensin molecules allow them to form dimers or octamers in water solutions and to create pores in target membrane or disrupt/penetrate the membranes causing lysis of bacterial and tumor cells [3, 4]. Interestingly, it has been documented that at lower concentrations defensins (at particular, alpha-defensins) may cause a concentration-dependent effect on cultured eucaryotic cells leading to stimulation of cell proliferation [5–7]. Moreover, *in vivo* effects of antimicrobial peptides could be also of opposite character dependent on their concentrations and patterns of tumor microenvironment [1].

In present research, we have studied the mechanism of growth suppressing action of human beta-defensin-2 (hBD-2) *in vitro*. hBD-2, a 41 residue peptide, was originally isolated from extracts of lesional scales from psoriatic skin in 1997 [8]; its expression is characteristic for skin, respiratory tract and gastrointestinal epithelium. hBD-2 expression is inducible upon treatment with bacterial products and some cytokines [9]. Similarly to neutrophylic defensins, HNPs, this antimicrobial peptide has been shown to affect cell proliferation and viability in a concentration-dependent manner [10, 11].

In this work, we have shown that recombinant hBD-2 (rec-hBD-2) exogenously added into cell cultivation medium at a wide concentration range causes stimulation of cell proliferation in picomolar concentrations and a killing effect at concentrations higher than 100 nM; rec-hBD-2 at concentrations of 10–100 nM is capable to enter cells, it accumulates in cell nuclei and causes cell growth suppression via cell cycle arrest in G1/S phase and activation of pRB.

MATERIALS AND METHODS

Cell lines and treatment with recombinant hBD-2 peptide. Human non-small cell lung adenocarcinoma A549 cells were kindly provided by Dr. V. Kashuba (Karolinska Institute, Sweden) and human epidermoid carcinoma A431 cells were obtained from the Bank of Cell Lines from Human and Animal Tissues, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine (Kyiv, Ukraine). The cells were cultured *in vitro* in DMEM culture medium with high glucose content supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin G sodium, 100 μg/ml streptomycin sulfate in 5% CO_2 athmosphere at 37 °C.

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Abbreviations used: hBD-2 – human beta-defensin-2; pRB – retinoblastoma protein; rec-hBD-2 – recombinant hBD-2; VD3 – vitamin D_3.

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 purified by standard two-step procedure as described earlier [12]. Protein concentration was determined by the method of Bradford [13] and by UV absorbance at 280 nm using spectrophotometer Nanodrop-1000 (USA).

Cells were cultured for 24 h in serum-free medium or (dependent on experimental conditions) in culture medium supplemented with 2.5% FBS, to nearly 50% confluence and then treated by addition of rec-hBD-2 at various concentrations (from 10 pM to 100 μM) for 48 h. After the treatment, cells were triply washed with PBS, detached with trypsin, and counted in hemocytometer or were subjected to flow cytometry analysis. The percent of dead cells was analized using tripan blue staining. Each experiment was repeated in triplicate.

For flow cytometry analysis, the attached cells were harvested by trypsinization, pelleted at 4 °C (500 g) for 5 min, washed twice in PBS, and resuspended in 1 ml of hypotonic lysis buffer (0.1% sodium citrate, 0.1% Triton X-100, 5 μg/ml PI (Sigma, USA). The cells were incubated at room temperaure for 30 min in the dark, and analyzed by Becton Dickinson FACS Calibur. The data were analyzed with the use of CellQuest software package and ModFit LT2.0 program (BDIS, USA) for Mac computers. All analyses were performed three times.

To evaluate the effect of rec-hBD-2 on cell viability, MTT-test has been applied [14]. Shortly, A549 and A431 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 and λ_{max}=630.

In some experiments to study the effect of native endogenously produced hBD-2, A549 cells were treated with 40 ng/ml of 1,25-$(OH)_2$D3 for 48 h. Then the attached cells were triply washed with PBS and counted in hemocytometer.

RT-PCR analysis of hBD-2 expression. Total RNA was isolated from tissue samples by the method of Chomzynski and Sacchi [15]. For detection of expression of hBD-2 mRNA in tissue samples, semiquantitative RT-PCR analysis was performed using specific primers (*hBD-2* — F: -5'-gaagctcccagccatcagcc; R: — 5'-gtcgcacgtctctgatgaggga; *beta-actin* — F: -5'-ctggaacggtgaaggtgaca; R: 5'-aagggacttcttgtaacaatgca). The expression level of beta-actin (the house-keeping gene) served as a loading control. The products of RT-PCR were routinely analyzed by electrophoresis in agarose gel. The relative mRNA expression levels were analyzed using TotalLab Program.

Western-blot analysis. To analyse the level of expression and/or phosphorylation of different proteins, the cultured cells were treated with rec-hBD-2 for 48 h as described above, and then washed with PBS and lyzed in PBS solution containing 50 mM Tris-HCl (pH 7.0), 50 mM EDTA, 2% Triton X-100. The concentration of total protein was determined by the method of Lowry.

The proteins were separated by electrophoresis in 9–22% gradient polyacrylamide gel and transferred to nitrocellulose membrane Hybond-ECL, RPN3032D (Amersham Biosciences, USA). Nonspecific binding sites were blocked with 1X PBS-T, 5% BSA solution for 1 h, and the blots were incubated with primary Abs, and then with secondary polyclonal HRP-conjugated anti-rabbit IgG or anti-mouse IgG Abs (DakoCytomation, Denmark). The ECL Western blotting detection system (Amersham Pharmacia Biotech) was used to reveal immunoreactivity. For Western blot analysis, the following antibodies were used: anti-pRB-Abs, anti-p53-Abs, anti-CyclinD1-Abs (Cell Signaling Tech, USA) and MoAbs against beta-actine (Sigma, USA). All antibodies were used at the working dilutions according to manufacturer instructions.

Confocal microscopy and immunocytochemistry. A431 and A549 cells were plated into 6 cm Petri dishes on glass cover slides. Control cells were grown in fresh growth media supplemented with 2.5% FBS, while experimental cells were additionally treated for 48 h with 1 μM rec-hBD-2. After the treatment, cells were washed in PBS and fixed for 1 h at -20 °C in methanol-acetone (1:1) solution. Cells were then washed three times with PBS and blocked with 5% normal goat serum (VectorLaboratories, Inc., Burlingame, CA) in PBS for 1 h at room temperature. Next, cells were incubated for 1 h with rabbit anti-hBD-2 polyclonal antibody (Santa-Cruz, USA) diluted 1:50 in blocking solution. Following this, cells were washed and incubated for 1 h with goat anti-rabbit IgG-FITC antibody (Sigma F0382) at a dilution of 1:80. Finally, the cells were incubated with 0.5 μg/ml DAPI (4–6-diamidino-2-phenylindole). Confocal images were obtained using a Zeiss LSM 510 META confocal microscope equipped with lasers for 405 nm and 488 nm excitation for DAPI and FITC, correspondingly. Images were collected with Plan-Apochromate 63X/1,4 Oil DIC lens (Zeiss, Germany) in multi-track mode using line switching with averaging of 16 readings. Excitation of FITC and DAPI was collected with LP 505 and LP 420 filters.

Also, to detect intracellular localization of recombinant hBD-2, the cells were treated with 1 μM hBD-2 for 48 h, washed with PBS, fixed in cold methanol/acetone (1:1) mixture for 24 h at -20 °C, blocked with 5% normal goat serum for 1 h at RT. For immunocytochemical detection of hBD-2 staining we have used rabbit polyclonal anti-hBD-2 Abs (Santa-Cruz, USA) at the dilution 1:100. EnVision System and DAB (DAKO, Denmark) were used for visualization. The slides were analized using Axioplan microscope (Zeiss, Germany). On average, 10 fields were examined for each sample,

Statistical analysis. 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

Our research has been performed *in vitro* using rec-hBD-2 exogenously added to the cell culture medium. Control experiment was carried out on the model of vitamin-D_3-induced expression of native hBD-2. Recombinant hBD-2 was expressed in bacterial cells as GST-hBD-2 fusion protein and purified by standard procedure as described earlier [13]. The purified defensin preparation was of 98% purity as justified by the data of electrophoretic analysis, WB, HPLC, and active in antimicrobial test against *Pseudomonas aeruginosa* (data not shown).

The analysis of rec-hBD-2 effect on cell growth patterns in vitro. To study the effect of exogenous rec-hBD-2 on growth patterns of cultured A431 and A549 cells, the cells were treated with rec-hBD-2 that was added into serum-free culture medium at different concentrations (from 10 pM up to 100 μM) for 48 h. Using MTT-analysis, we have revealed that hBD-2 caused a concentration-dependent effect on cell growth rates, in particular, it stimulated cell proliferation at concentration range from 10 pM to 1 nM, and significantly suppressed cell growth at concentration from 10 nM to 1 μM (Fig. 1). Treatment of both cell lines with recombinant defensin at concentrations higher than 1000 nM resulted in cell death. The data of MTT-test were supported by the results of direct cell counting (Fig. 2). These effects were hBD-2-specific as far as pretreatment of rec-hBD-2 with anti-hBD-2 Abs completely abolished the above mentioned effects (Fig. 2).

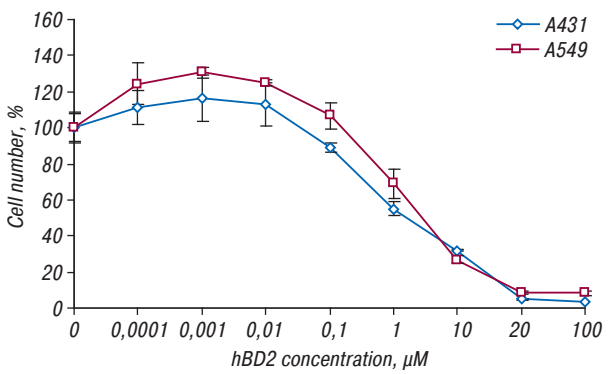

Fig. 1. Exogenous rec-hBD-2 affects the number of viable cultured cells of A431 and A549 lines. Cells were cultured in 96-well plates in serum free culture medium and treated with exogenously added recombinant hBD-2 at concentrations from 10 pM to 100 μM for 48 h. The number of viable cells was evaluated by MTT analysis. The data of three independent experiments are presented as the mean ± SD

Flow cytometry analysis. To explore the mechanism implicated in the growth-inhibition effect of hBD-2 we have selected the hBD-2 concentration range of 10–1000 nM, and using flow cytometry analysis we have analyzed the influence of rec-hBD-2 exogenously added into the cell culture medium on cell cycle distribution of cultured A549 and A431 cells after 48 h incubation. Flow cytometry analysis of A431 cells treated with rec-hBD-2 has revealed (Fig. 3) a concentration-dependent cell growth arrest at G1/S phase ($p<0.05$). Similar results were registered for A549 cells treated with rec-hBD-2 (data not shown). At the same time treatment of cells with 10–1000 nM of hBD-2 had

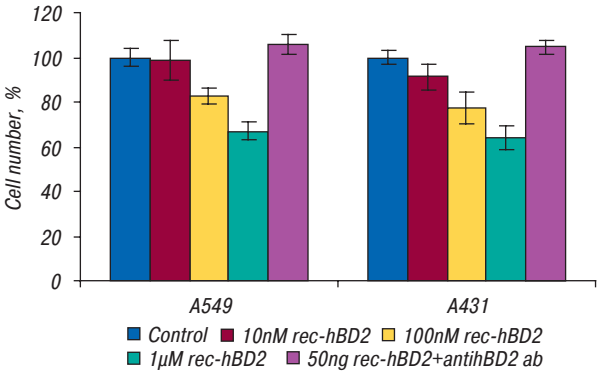

Fig. 2. A concentration-dependent effect of exogenous recombinant hBD-2 on the number of viable cultured A549 and A431cells. The number of attached cells was evaluated by direct cell counting. Cells were cultured in 96-well plates in serum free medium and treated with exogenously added rec-hBD-2 concentrations of 10, 100 nM and 1 μM for 48 h. To analyze the specificity of growth-suppressing activity of rec-hBD-2, 50 ng of hBD-2 were pre-incubated for 1 h with 0.8 μg of anti-hBD-2 Abs (Santa-Cruz, USA), and then added to the cell incubation medium for 48 h. The data of four independent experiments are presented as the mean ± SD. *The difference is significant as compared to control ($p < 0.05$)

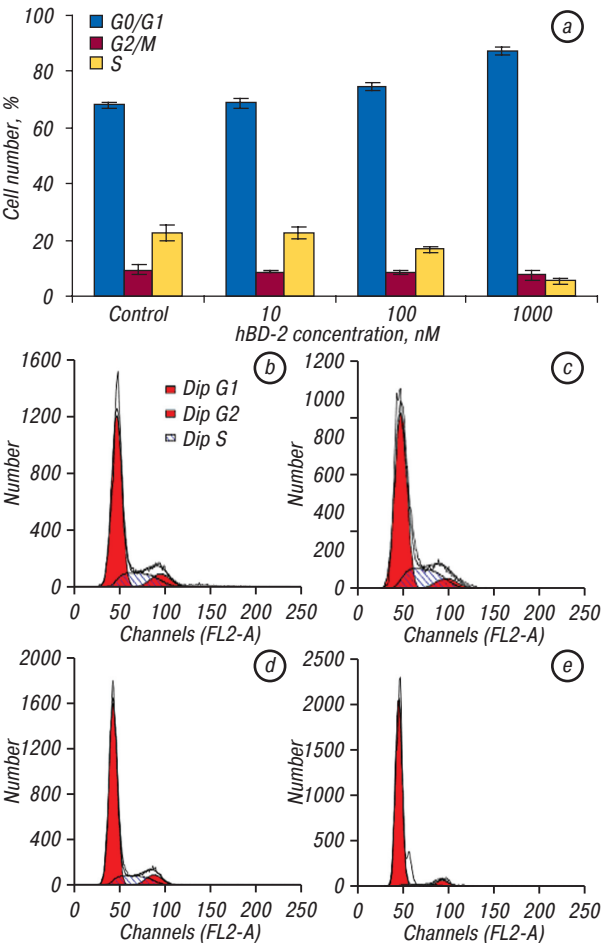

Fig. 3. Flow cytometry analysis of cell cycle distribution of A431 cells treated or not treated with rec-hBD-2 for 48 h (*a*-*e*). Cells were cultured in 10 cm Petri dishes in culture medium supplemented with 10% FBS to 50% confluency, then the medium was replaced by the one supplemented with 2.5% FBS, and the cells were treated by addition of rec-hBD-2 for 48 h. After the treatment, the cells were triply washed with PBS, detached with trypsin and subjected to flow cytometry analysis as described in Materials and Methods section: *a* — the data of three independent experiments are presented as the mean ± SD; *b* — control A431 cells; *c*, *d*, *e* — the cells treated with 10, 100, and 1000 nM rec-hBD-2 respectively

no effect on cell mortality: the percent of apoptotic cells in control and hBD-2 treated A431 cells didn't differ significantly and did not exceed 4%. There data were supported by the results of tripan blue staining.

The analysis of rec-hBD-2 effect on signal pathways involved in cell cycle regulation in vitro. To explore how rec-hBD-2 affects specific signal cascades involved in cell cycle regulation in cultured cells, we have studied the state of some proteins which activity is required for G1/S cell cycle transition. We have analyzed phosphorylation of pRB, expression levels of cyclin D1 and p53 in A549 and A431 cells treated with 1 μM rec-hBD-2 in comparison to control (untreated) cells. We have shown, rec-hBD-2 treatment resulted in the dramatic reduction of pRB phosphorylation, and significant decrease of cyclin D1 expression, while p53 expression remained unaffected (Fig. 4). So, one may conclude that G1/S cell cycle arrest in hBD-2 treated cells could be possibly realized via activation of pRB.

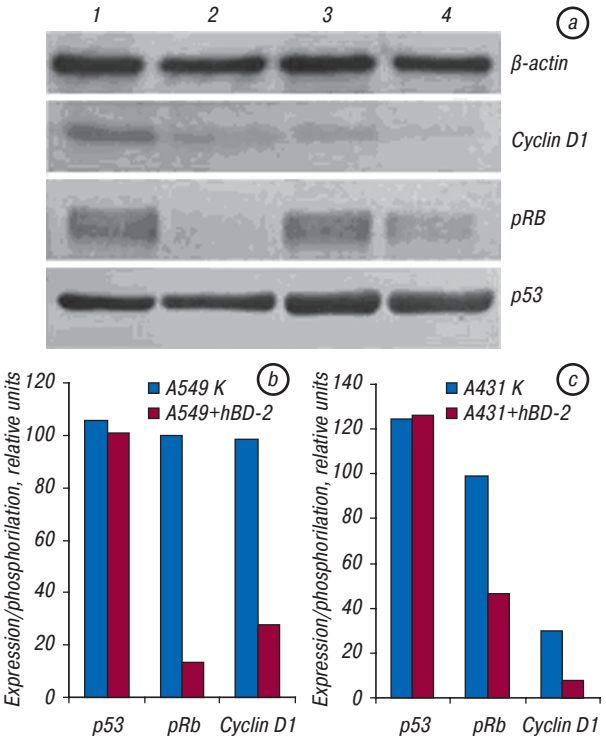

Fig. 4. Western blot analysis of pRB phosphorylation and expression levels of cyclin D1 and p53 in A549 and A431 cells treated with 1 μM rec-hBD-2 for 48 h. ***a***: line 1 — intact A549 cells; line 2 — A549 cells treated with 1 μM rec-hBD-2; line 3 — intact A431 cells; line 4 — A431 cells treated with 1 μM rec-hBD-2; ***b, c*** — graphic representations of Western blot analysis data counted with the use of GelPro 4.0 program

The study of intracellular localization of hBD-2. To analyze whether exogenously added rec-hBD-2 enters the cell, two methods were used — immunocytochemical analysis and immunocytofluorimetry. In both cases we have compared hBD-2-specific immunostaining in defensin-treated A431 and A549 cells *versus* intact control cells, and have detected hBD-2 specific immunostaining in the fist case. According to our results (Fig. 5), rec-hBD-2 enters the cells, is distributed in cytoplasm and is accumulated in cell nuclei of A431 cells (Fig. 5) and A549 cells (Fig. 7).

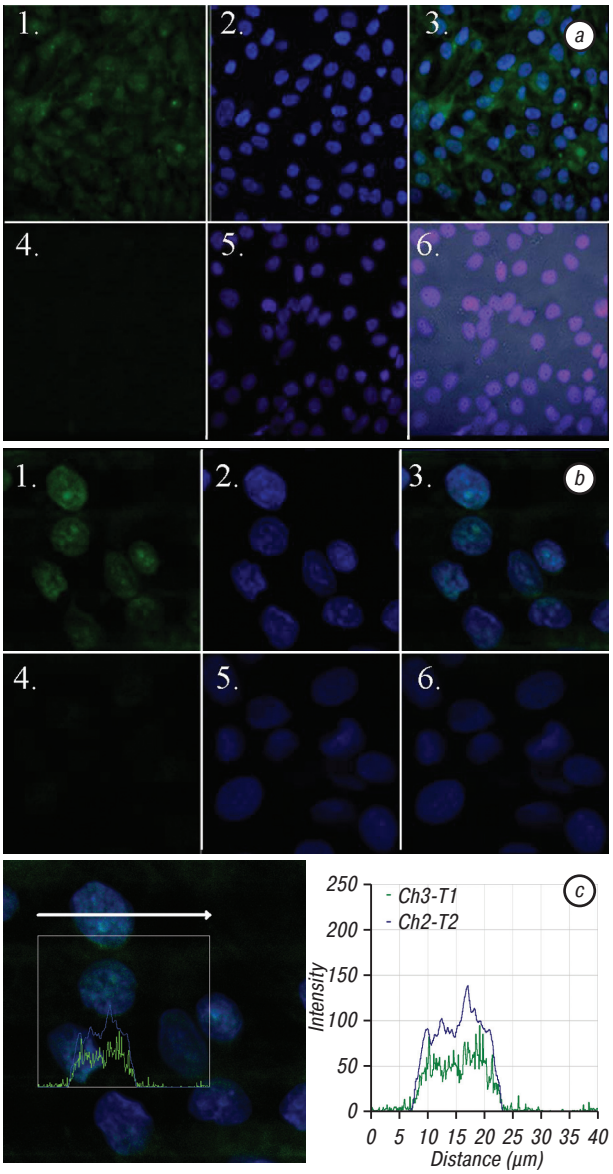

Fig. 5. Rec-hBD-2 is detected in cytoplasm (*a*) and nuclei (*b*, *c*) of hBD-2 treated A431 cells but not in control cells. Confocal microscopy was applied for analysis of immunofluorescent staining of hBD-2 in A431 cells treated (1–3) or not treated (4–6) with the recombinant defensin at the concentration of 1 μM for 48 h. 1, 4- staining with FITC-anti-hBD-2 Abs; 2, 5 — DAPI staining, 3, 6 — overlay. *c* — evaluation of hBD-2 specific nuclear staining of A431 cells treated with 1 μM rec-hBD-2

Modeling of action of native hBD-2 in vitro. To find out whether the data obtained with the use of recombinant defensin may reflect similar physiologic events related to the action of native hBD-2, we have designed an experiment on a model of A549 cells in which the hBD-2 mRNA expression is induced by treatment with metabolites of vitamin D_3 (VD3). First of all, using RT-PCR analysis we have determined that intact A549 cells do not express hBD-2 mRNA, while its expression in these cells could be induced if the cells are treated with $4 \cdot 10^{-7}$ M 1,25-$(0H)_2D_3$ or 25$(0H)D_3$ for 12 h (Fig. 6).

Next, with the use of direct cell counting we have revealed that A549 cells incubated with metabolites of VD3 for 48 or 72 h demonstrated significantly suppressed cell proliferation rate compared with the control cells (by 1.3 and 1.6 fold respectively, $p<0.05$),

and that such growth suppression could be eliminated by the addition of anti-hBD-2 Abs into the cell culture medium. Next, using immunocytochemical and immunofluorescent staining we have detected an appearance of hBD-2-specific immunostaining in cytoplasm and nuclei of A549 cells treated with VD3 metabolites for 48–72 h (see Fig. 7).

DISCUSSION

In our research we have received new data that allow to understand the possible mechanism of cell growth-suppressing activity of hBD-2 toward human cancer cells *in vitro*. As we have shown, rec-hBD-2 exerts a concentration-dependent effect toward cultured cells of two different lines — lung adenocarcinoma A549 cells and vulval epidermoid carcinoma A431 cells. This defensin stimulates cell proliferation at the concentrations below 1 nM, and suppress cell growth at the concentrations higher than 10 nM. Such dual effect has been reported earlier for some defensins [16–18], and beta-defensin-dependent stimulation of cell proliferation has been studied in detail by few research groups [19–22]. In particular, it has been shown that hBD-2,-3,-4 stimulate keratinocyte proliferation, and this process requires hBD-dependent phosphorylation of EGFR, STAT1 and STAT3 [21]. Another study [23] has shown that hBD-2-dependent proliferation of human conjunctival fibroblasts is mediated by activation of p42/44 MAP kinase and Akt. Killing effects of hBDs toward cultured human tumor cells have been studied in many works, and they are thought to be realized via membrane lysis [reviewed in 24].

At the same time the mechanism of action of defensins at concentration range between pro-proliferative and cytolytic doses was not analyzed yet, and our research is the first one in this field. We have analyzed the growth suppressing arm of hBD-2-concentration curve, namely its concentrations between 10 and 1000 nM. These medium concentrations could be considered close enough to physiologic margins reported in some related publications [25, 26].

First of all we have studied effect of nanomolar concentrations of rec-hBD-2 on cell cycle distribution and signal pathways that regulate G1/S cell cycle transition. The obtained results have shown that exogenously added rec-hBD-2 caused G1/S cell cycle arrest in both cell lines via significant dephosphorylation of pRB and inhibition of cyclin D1 expression, while p53 expression remained unaffected. So, the growth inhibitory action of rec-hBD-2 could be in part realized through pRB activation, and this fact is of significant importance. It is well recognized that the pRB plays a tumor suppressive function and is able to promote cell cycle exit via the control of activity of E2F transcription factors regulating a number of genes, involved into the G1 to S transition [27]. As far as we know, it is a first report on a new biologic activity of hBD-2 as a native activator of pRB.

According to the data of immunocytochemical and immunocytofluorescent analysis, rec-hBD-2 enters the cells, may be found in cytoplasm and also — in cell nuclei; the latest fact points on a possibility that this defensin may play a role in gene transcription. Accord-

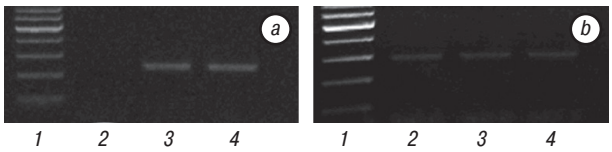

Fig. 6. RT-PCR analysis of hBD-2 mRNA expresssion in A-549 cells (*a*). The cells A549 ($2,5x10^5$) were grown in 3 cm Petri dishes, cultured for 24 h in serum free medium, and then treated with 1,25- $(0H)_2D_3$ or $(25)(0H)D_3$ at the concentrations of 40 ng/ml for 12 h. *a* — 1 — DNA ladder (Fermental, Lithuania); 2 — control A549 cells; 3- the cells treated with 40 ng/ml 1,25-$(0H)_2D_3$; 4 — the cells treated with 40 ng/ml $25(0H)D_3$. β-actin is used as a house-keeping gene (*b*)

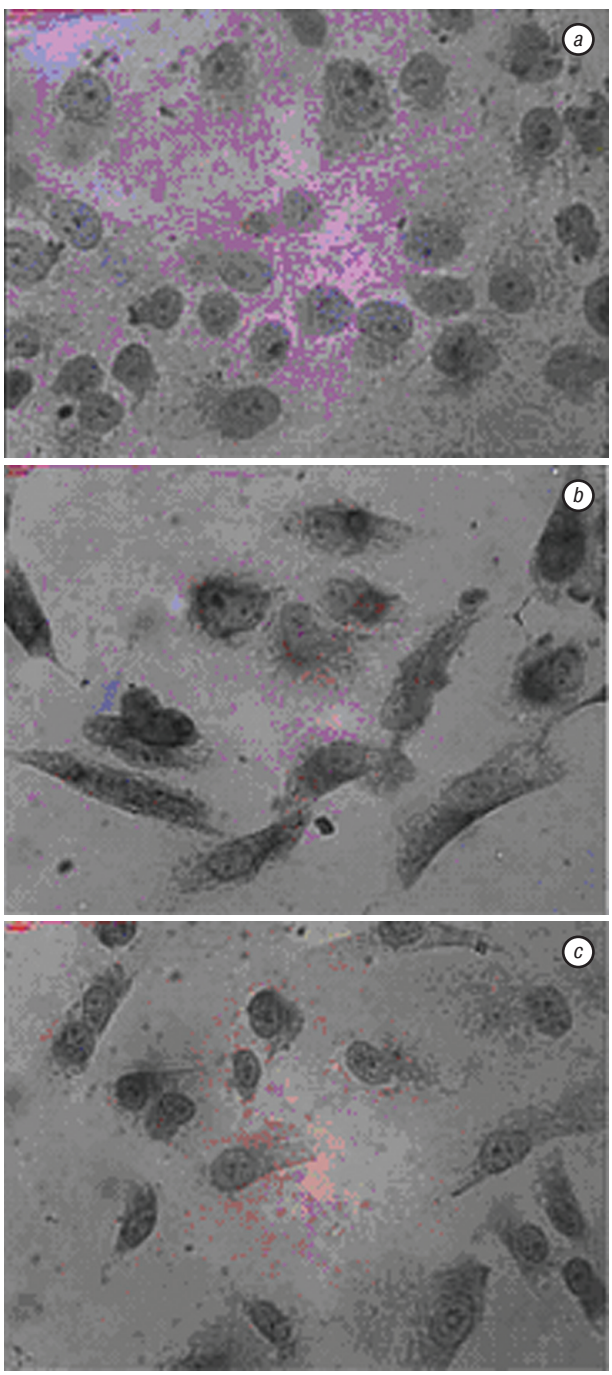

Fig. 7. Immunocytochemical staining of hBD-2 in intact A459 cells (*a*) and the cells treated with the 1000 nM rec-hBD-2 for 48 h (*b*) or 40 ng/ml 1,25-$(0H)_2D_3$ for 48 h (*c*). Magnification x400

ing to recent data, some antimicrobial peptides were found in cancer cell nuclei, in particular, hBD-1 [28] and cathelicidine LL-37 [29]. However, biologic role of nuclear translocation of these peptides have got no explanations so far.

To check if the observed effects of rec-hBD-2 may reflect similar physiologic events related to the action of native hBD-2 and are not related to artifacts, we have analyzed cell growth patterns of A549 cells in which hBD-2 mRNA expression is induced by treatment with VD3 metabolites. We have demonstrated that VD3-treated A549 cells express hBD-2 mRNA and possess lower growth rates than control cells that do not express the defensin. It is known that VD3 metabolites regulate more than two hundred genes, not only this one; so, we have analyzed if a depletion of newly secreted defensin molecules from culture medium of 1,25-$(0H)_2D_3$-treated cells will restore growth rates of these cells. So, in a model experiment with A549 cells we have induce hBD-2 expression (treatment with 40 ng/ml 1,25-$(0H)_2D_3$; 24 h after such induction we have added anti-hBD-2 Abs into cell culture medium to bind defensin molecules that are secreted from the cells; and 48 h after the beginning of VD3 treatment we have revealed that anti-hBD-2-Abs treated hBD-2 expressing A549 cells do not differ by cell counts from control A549 cells. So, we conclude that growth suppression of A549 cells in which hBD-2 expression was induced by VD3 metabolites, is related to action of native defensin. Also, we have observed specific hBD-2 immunostaining in (1,25)$(0H)_2$D3 treated cells (Fig. 7) and its nuclear localization (data not shown). So, one may conclude that autocrine effect of native hBD-2 toward A549 cells is similar to that of recombinant defensin.

All these data allowed us to propose a hypothesis on a possible mechanism of hBD-2 dual concentration-dependent action on eucaryotic cells *in vitro*. Let's remember that this peptide is shown to be involved in wound healing *in vitro* and *in vivo* stimulating keratinocyte proliferation and migration [10, 21]; at the same time this process couldn't be endless. From other side, biophysical studies evidence on an ability of the defensin to form oligomers in water solutions [3] — hBD-2 may exist in monomeric, dimeric or octameric forms, and the latest ones, according to the authors conclusion, are responsible for the disruption of bacterial membranes. It's tempting to speculate that the concentration-dependent mode of influence of hBD-2 could be related to different mechanisms of action of monomeric and oligomeric forms of these molecules — possibly, receptor-mediated for monomeric forms (with pro-proliferative mode of action), membrane-penetrating ones for dimeric forms (with growth suppressing activity) and cytolytic activity characteristic for octamers. However, such hypothesis is a speculative one and requires further experimental research.

In conclusion, the data of the present research allow to suggest that human beta-defensin-2 may act as a native regulator of cell growth — either positive at low concentrations or negative at higher ones, and further research in this field may help to reveal new activities of this peptide antibiotic in epithelial cells.

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