Exp Oncol 2006
28, 4, 288–292

Experimental ONCOLOGY

REGULATED EXPRESSION OF HUMAN BETA-DEFENSIN-2 LEADS TO ALTERED PHENOTYPE AND GROWTH PATTERNS OF CULTURED HUMAN EMBRYONAL KIDNEY CELLS

E. Zhuravel, D. Lytvyn, M. Soldatkina, S. Zeleniy, T. Shestakova, P. Pogrebnoy*
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine

Aim: **To create cell line with regulated expression of human beta-defensin-2 (hBD-2) and evaluate the influence of expressed peptide on its phenotypic and growth patterns.** ***Materials and Methods:*** **Using cloning techniques, on the base of human embryonic kidney cells of HEK293T line, stable T-rex HEK-hBD2-m cell subline expressing mature biologically active hBD-2 molecule upon the presence of tetracycline in culture medium was generated. The morphological patterns, growth characteristics and colony forming activity of these cells were studied using routine techniques.** ***Results:*** **T-rex HEK-HBD2-m cell subline was shown to express both mRNA and hBD-2m protein upon the presence of 1 μg/ml tetracycline in culture medium as it was demonstrated by RT-PCR and immunocytochemical approach. Upon prolonged expression of hBD-2, the cells acquired special features: they lost ability to grow in monolayer *in vitro* and to form colonies in soft agar, characteristic to parental HEK293T cells, but possess higher growth rate and longer survival in FBS-free medium than wild type cells.** ***Conclusion:*** **Expression of hBD-2 in T-rex HEK-HBD2-m cell subline results in specific biological consequences that favor cell survival.**
Key Words: **human beta-defensin-2, regulated expression, colony formation, HEK293 cells.**

It is accepted now that immune system plays an important role in the control of tumor growth. Numerous experimental studies carried out in recent years have revealed that the character of interaction between tumor and host organism is much more complex and multifactorial than it has been postulated by the theory of immune control. Among the factors of such interaction one may hypothetically mention defensins — small cationic antimicrobal peptides that are the components of innate immunity.

In the last decade it has been shown that a number of human tumors are characterized by hyperexpression or down-regulation of certain defensin genes and in some cases serum levels of defensins may serve as additional diagnostic markers of neoplastic process [1–7]. However, it remains unknown yet what is a functional role of defensin expression in tumor cells — whether these antimicrobials may protect the host from tumor development by killing tumor cells, or benefit malignization and metastasis. It has been revealed that defensins may exert oppositely directed influence on cultured cells dependent on their local concentration [8, 9]. Experiments carried *in vitro* demonstrated that defensins may cause a strong proliferative response in cultured cells in a concentration-dependent fashion, stimulating cell proliferation in micromolar concentration range [10] and promote motility and invasiveness of cancer cells *in vitro* [11]. However, in higher concentrations defensins are usually cytotoxic and cause lysis of tumor cells [8] or may induce cell death via mitochondrial injury and intracellular signalling pathways [12]. *In vivo* conditions are more complicated, because in tumor microenvironment defensins produced by malignant cells or by blood cells infiltrating tumor may interact with various cell receptors or be bound by HMW proteins of blood plasma. Up to date, just few reports are discussing possible local concentrations of defensins in oral squamous cell carcinoma [13].

To answer the question on the potential role of defensins in cancer it looks reasonable to create an appropriate model system to study various effects of defensins *in vitro* and *in vivo*. Such system should represent the regulated expression of antimicrobial peptide with possibility to evaluate the exact level of peptide's production per cell and the one where such production may be stopped in a given moment.

In present study we aimed to create cell line with regulated expression of human beta-defensin-2 (hBD-2) on the base of human embryonic kidney cells of HEK293T line and evaluate the influence of endogeneously produced hBD-2 on its phenotypic and growth patterns.

MATERIALS AND METHODS

Cell lines. HEK293T (human embryonic kidney) cells were purchased from the Europian Collection of Animal Cell Cultures. Cells were cultivated in DMEM with high glucose content supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G sodium salt, 100 μg/ml streptomycin sulfate and 0.25 μg/ml amphotericin B as fungizone.

Gene cloning and plasmid constructions. The genes for mature β-defensin-2 were cloned from total RNA isolated from A431 cells treated with 1 μg/ml EGF [14] by RT-PCR using specific primers. To hBD-2m (mature molecule): forward — 5′-ACT-TAA-GCT-TGC-CAT-GGG-TAT-AGG-CGA-TCC-TGT-TAC-3′, and reverse — 5′-TCT-TGG-AAT-TCT-CAT-GGC-TTT-TTG-CAG-CATTTT-G-3′) (GenBank accession no. AF040153). PCR products were digested with HindIII and EcoRI and cloned into the same sites of inducible expression vector, pcDNA4/TO/neo modified by us as follow: neomycin phosphotransferase gene was cut from the pcDNA3 plasmid (Invitrogen Life Technologies, USA) and ligated in the pcDNA4/TO (Invitrogen Life Technologies, USA). The obtained construct

Received: November 17, 2006.
*Correspondence: E-mail: pogrebnoy@onconet.kiev.ua
Abbreviations used: hBD-2 – human beta-defensin-2; hBD-2m – mature molecule of human beta-defensin-2; PDK1 – phosphoinositide-dependent kinase 1; RT-PCR – reverse transcription polymerase chain reaction.

pcDNA4/TO/neo was verified by the restriction analysis using endonucleases NruI and XhoI.

The correctness of insert in abovementioned construct carrying hBD-2m gene was verified by the DNA sequencing and plasmids were purified using a standard protocol [15].

Transfection HEK293T cells. The resulting constructs were cotransfected with the regulatory plasmid, pcDNA6/TR (Invitrogen Life Technologies, USA), which contained a selective marker — the blasticidin S deaminase gene, into HEK293T cells using FuGENE 6 Reagent (Roche Molecular Biochemicals, USA) according to the instructions of manufacturer. After transfection cells were selected in medium with 5 μg/ml blasicidin (Invitrogen Life Technologies, USA) and 600 μg/ml G418 (Gibco, USA) for 2 weeks. Resistant cells were treated with tetracycline to derepress the hybrid CMV/TetO_2 promoter in pcDNA4/TO/neo and to induce expression of hBD-2 mature form.

Expression of mature hBD2 mRNA. After 24 h incubation of obtained cell line T-rex-HEK-hBD-2m with tetracycline, mRNA was isolated and the level of expression of hBD-2 was analysed by RT-PCR with the use of abovementioned primers.

Immunocytochemical analysis of hBD-2 expression. Cells were sedimented by centrifugation, and cell pellet was fixed with 4% formaline for 4 h, washed with PBS and stored in 70° ethanol. Later the paraffin blocks were prepared, 4 μm slides were cut and analysed immunocytochemically using anti-hBD-2-MoAbs [16] at the dilution of 1 : 10 and 1 : 25. For the part of the samples, renewal of antigenic properties using Target Retrieval Solution (DAKO, USA) according to protocol of the manufacturer was applied. To block nonspecific binding, biotin-blocking system (DAKO, USA) was used. Anti-mouse antibodies labeled with biotin (Sigma, USA) were used at the dilution of 1 : 400; Vectastain ABC Kit (Vecor Lab., USA) with peroxidase probe was used as the third coating. As negative control, 1% BSA (DiaM, Russia) prepared on 0.02 M phosphate buffer (pH 7.2) was used instead of specific antibodies. As positive control, gastric tumor tissue samples expressing hBD-2 [16] were used. Peroxidase conjugate was developed by 0.05% 3'3'-diaminobenzidinetetrahydrochloride (Sigma, USA) solution containing 0.01% hydrogen peroxide. Nuclei were counterstained with Mayer's hematoxyline.

The study was performed using microscope Axioplan (Zeiss, Germany) equipped with the videocamera Sony DXC-151AP, EZ Capture, computer Pentium IV, and program for analysis of images (Media Cybernetic, USA). Ten fields of view were studied per sample.

Colony formation analysis. Analysis of colony forming ability of the cells was carried out by routine technique as described elsewhere [17] in DMEM medium supplemented with 15% FBS and 0.4% methylcellulose without/with 1 μg/ml tetracycline. The cells were dispensed in 6-well plates (1×10^3 cells per 1 ml of medium) and incubated in CO_2-incubator for 14–16 days. The colonies were visualized using staining procedure with 0.2% p-iodonitrotetrazolium violet dye.

RESULTS AND DISCUSSION

Generation of T-REX-HEK-hBD-2 cell sublines. For cloning and expression of hBD-2 gene in mammalian cells two-vector T-REx system was choosed. Such system contains expressing vector pcDNA4/TO designed for cloning of the gene of interest, and pcDNA6/TR vector coding Tet repressor. Co-transfection of these two vectors allows to perform tetracycline-regulated expression of the gene of interest in mammalian cells. Plasmid pcDNA4/TO contains hybrid promoter providing high level of tetracycline-regulated expression in different types of cells. Promoter is composed from two parts — early promoter of human cytomegalovirus (CMV) and region of two tetracycline operators (TetII O_2). For selection of stable cell lines, the gene of resistance to zeocin is introduced in this plasmid. Two TetII O_2 sequences present the region of binding of 4 Tet repressor molecules (two Tet homodimers). Tet repressor is expressed by pcDNA6/TR plasmid. In the absence of tetracycline expression of the cloned gene is suppressed through the binding of Tet repressor homodimers with TetII O_2 sequences. Addition of tetracycline to the cells derepresses hybrid CMV/ TetII O_2 promoter in pcDNA4/TO plasmid via its binding with Tet repressor and allows expression of the cloned gene.

T-REx system responded to all our requirements — cloning of hBD-2 gene, except the site of resistance to zeocin — this antibiotic is too expensive and rare; that's why we decided to replace it by another selective marker — Neomycin: the gene of zeocin resistance has been removed from pcDNA4/TO by restriction at NruI/XhoI sites, and neomycin gene cut from the plasmide pcDNA3.1+ (Invitrogen, USA) was inserted. The generated vector was named pcDNA4/TO/neo.

After this 151 bp fragment received by HindIII/EcoRI restriction was cloned into pcDNA4neo plasmid by HindIII/EcoRI sites. Selection of recombinant clones was performed by restriction analysis allowed to choose clone pcDNA4/TO/neo/hBD2m, containing the sequence coding mature hBD-2 molecule.

Next, HEK293T cells were cotransfected with pcDNA6/TR plasmid and pcDNA4/TO/neo/hBD2m by standard procedure. Selection of clones was based on blasticidin/G418 resistance. As the positive control, pcDNA4/TO/lacZ vector was used. Co-transfection of pcDNA4/TO/lacZ and pcDNA6/TR resulted in induction of β-galactosidase gene upon addition of tetracycline, and may be registered by staining using X-Gal.

Expression of hBD-2 in T-REX-HEK-hBD-2 cell sublines. As we have shown earlier, the highest level of defensin expression in engineered cell line could be achieved in 20 h after induction with 1 μg/ml tetracycline. RT-PCR analysis has demonstrated that the highest level of transcription of gene coding mature molecule of hBD-2 is registered in cell subline T-rex HEK-hBD2-m, induced with 1 μg/ml tetracycline, at lower level — in cell lines T-rex-HEK-hBD2-m grown in the absence of antibiotic, whilst in parental HEK293T cells expression of hBD-2 mRNA wasn't registered by the applied technique (Fig. 1). These data indicated that to some extent

the tet-regulated promoter is leaking (the most possible explanation is that there are trace amounts of tetracycline in the components of culture medium). Unfortunately, we were unable to analyze the expression of defensin molecules using Western blot analysis because anti-hBD-2 MoAbs generated in our lab earlier [16] fail to recognize native antigen in this assay. For this reason production of hBD-2 peptide in cell subline has been studied using immunocytochemical approach with the use of anti-hBD-2-mAbs (that method was appropriate for detection of hBD-2, as it was estimated using the samples of human gastric tumors characterized by hyperexpression of hBD-2 [16] or A431 cells induced by EGF as a positive control (data not presented)). As one may see from Fig. 2, the distribution of cells by hBD-2 content looks as follow: parental HEK293T cells < T-rex HEK-HBD2-m Tetr- < T-rex HEK-HBD2-m Tetr+.

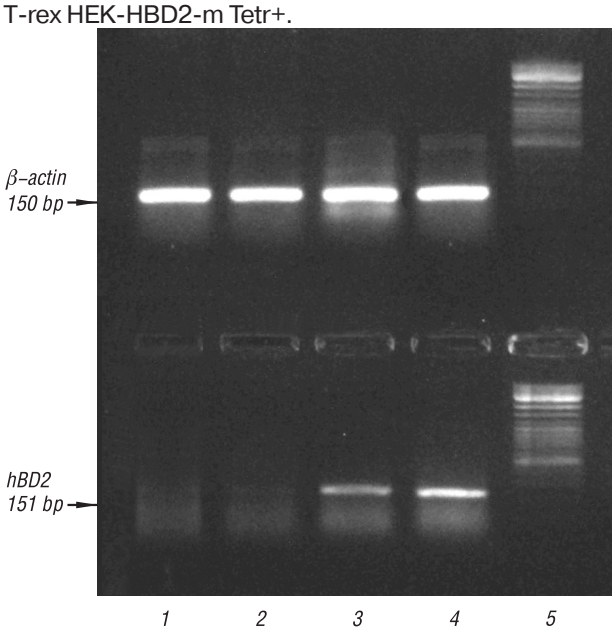

Fig. 1. RT-PCR analysis of hBD-2 expression. M — DNA ladder; 1, 2 — HEK293T cells without/with tetracycline incubation; 3, 4 — T-rex HEK-hBD2m cells without induction with tetracycline; and after induction with tetracycline respectively

Expression of hBD-2 leads to altered cell growth patterns and ability to form colonies in methylcellulose clonal culture. The study of growth patterns of cell subline T-rex HEK-hBD2-m compared to these of parental HEK293T cells was performed by methods of light microscopy and colony formation assay. We have registered the phenomenon that T-rex HEK-hBD2-m cells (contrary to parental strain) acquire ability for prolonged growth in culture (up to 148 h) even in the absence of FBS, i. e. on "starving" medium (upon such conditions, HEK293T cells die in 48–62 h). Using direct cell counting, it was found out that the number of T-rex HEK-hBD2-m cells cultivated in the presence of tetracycline in the "starving" medium, increased nearly 1.7 fold during the 1st day, and nearly 3-fold — till the end of the 2nd day *versus* 1.5- and 2-fold increase in T-rex HEK-hBD2-m cultivated without tetracycline (the number of parental cells did not alter significantly) (Table).

Table. The number of cells evaluated by direct cell counting

Cell line	Cell number (10^6 cells) (mean square deviation)			
	Initial	18 h	26 h	52 h
HEK293T Tet–	0.50	1.0 ± 0.25	0.40 ± 0.1*	0.50 ± 0.13*
HEK293T Tet+	0.50	0.52 ± 0.16*	0.34 ± 0.01*	0.40 ± 0.05*
T-rex HEK-hBD2-m Tet–	0.30	0.52 ± 0.09*	0.50 ± 0.12*	0.65 ± 0.05*
T-rex HEK-hBD2-m Tet+	0.30	0.56 ± 0.06*	0.60 ± 0.22	0.95 ± 0.05*

*Statistically significant, $p < 0.05$.

The growth-stimulatory patterns of hBD-2 expression in the cells are in agreement with our previous results obtained with the use of recombinant hBD-2 expressed in bacterial cells [10] that showed the promoting effect of rec-hBD-2 at the concentrations of 0.1–2 μg/ml on proliferation and viability of eukaryotic cells *in vitro*. Yet we can't evaluate the exact level of hBD-2 production per cell in generated cell sublines, but one may speculate that according to the observed biologic effect, its production may be close to the mentioned above concentration range.

Another specific pattern that has been noticed is the phenomenon of deprivation of ability to form confluent monolayer by HEK/hBD-2m cells cultured in the presence of 1 μg/ml tetracycline (Fig. 3, *a–d*). Whilst parental HEK293 cells are growing in typical monolayer, where the cells possess flattened or spread morphology, tetracycline-induced HEK/hBD-2m cells seem to loose this property forming multilayer clusters and possessing mostly rounded morphology. It looks like that normal cell-to-cell contacts are disturbed, as

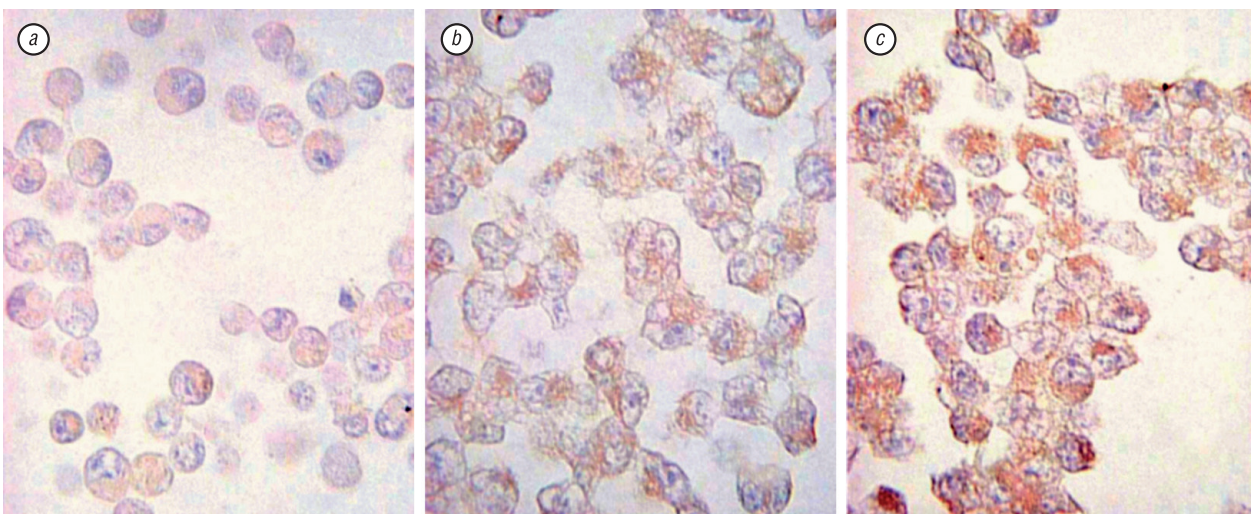

Fig. 2. Immunocytochemical analysis of hBD-2 expression in wild type HEK cells (*a*), T-rex HEK-hBD2m Tetr- (*b*), T-rex HEK-hBD2m Tetr+ (*c*). x 100

well as adhesion properties of the cells. We speculate that such effect may be explained in part by possible alteration of cell adherence in hBD-2-producing cells. The morphological features of tetracycline-induced T-rexHEK-hBD-2m cells closely resembled these of HEK293T cells expressing chymeric constructs of ADAM9 (a desintegrin and metalloprotease 9), molecule interacting with β-integrins in renal cells and affecting their adherence properties [19]. There are some reports evidencing the possible link between alpha-defensins and adhesion molecules. It has been shown that alpha-defensins may specifically inhibit alpha5beta1-integrin-dependent migration of bovine retinal endothelial cells to FN *in vitro* [20]. Another research demonstrated that human neutrophil peptides (HNPs) promote the binding of fibronectin to alpha5beta1 integrin and may affect endothelial cell adhesion and migration in FN-dependent manner [21]. So, there are some grounds to speculate that specific morphological and growth patterns of T-rexHEK-hBD-2m cells could be related to hBD-2-dependent alteration of adhesive properties of the cells.

Finally we have performed colony-formation assay using 0.4% methylcellulose without/with 1 μg/ml tetracycline, and found out that after 2 weeks of growth HEK293T cells formed multiple colonies, but T-rexHEK-hBD-2m cells seeded at the same density of 1 x 10^3 cells/ml did not and form only single colonies in tetracycline free medium. So, one may conclude that expression of hBD-2 in T-rexHEK-hBD-2m cells may result in the loss of the pattern of malignization (namely, ability to form colonies in semiliquid medium) characteristic for parental HEK293T cells.

In conclusion, we may assume that stable cell subline T-rexHEK-hBD-2m expressing mature hBD-2 molecule in tetracycline-dependent manner has been created; whilst wild type cells do not express and produce hBD-2, T-rexHEK-hBD-2 cells express hBD-2 upon incubation with 1 μM tetracycline in incubation medium, as it has been shown by RT-PCR and immunocytochemical analysis. Our data have demonstrated that prolonged expression (> 48 h) of endogeneous hBD-2 resulted in a number of biological effects: hBD-2-producing cells lost normal adhesive properties as well as the ability to form colonies in agar, characteristic to parental HEK cells, but acquire some features favoring the accelerated growth and survival. Our preliminary data allow to suppose that some signal cascades in these cells (in particular, PDK1-Akt, and p70S6 kinase) are involved in mediating abovementioned events; the research of these enzymes and expression of adhesion molecules is currently under study.

ACKNOWLEDGEMENT

The work was supported by grant № 2.2.5.260/2004, the Program "Newest Medico-Biological Problems and

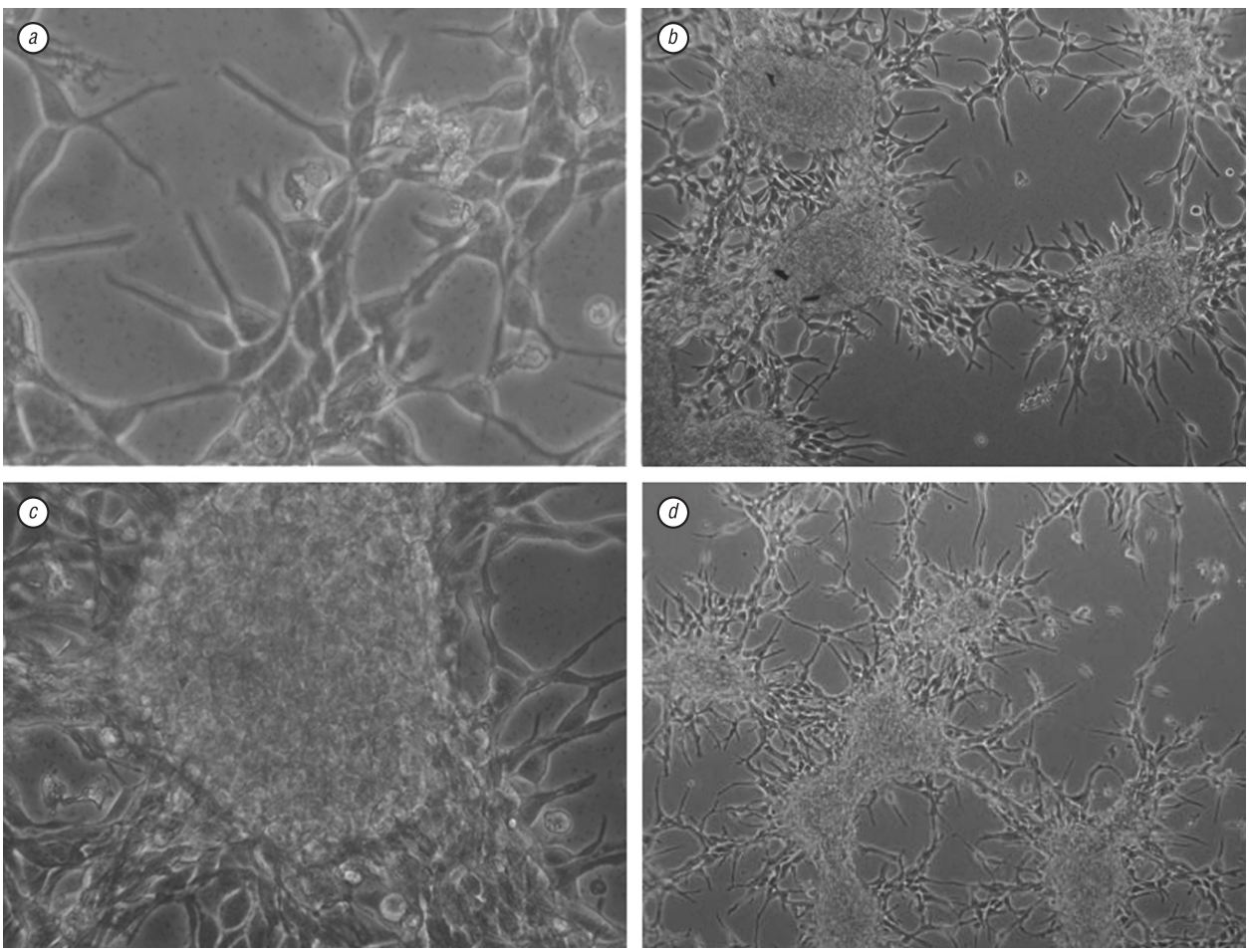

Fig. 3. The morphological features of T-rex-HEK-hBD-2m cells Tet+ (*b–d*) and of parental HEK293T cells (*a*) cultured on "starving" medium for 48 h

Environment", Part 2. "Biologically Active Compounds for Human Health" (Ukraine). Authors wish to express their gratitude to Dr. Liudmila Drobot (Ukraine) and Dr. Tatyana Klymenko (Germany) for their help and advices in solving gene-engineering tasks.

REFERENCES

1. **Sun CQ, Arnold R, Fernandez-Golarz C, Parrish AB, Almekinder T, He J, Ho SM, Svoboda P, Pohl J, Marshall FF, Petros JA.** Human {beta}-defensin-1, a potential chromosome 8p tumor suppressor: control of transcription and induction of apoptosis in renal cell carcinoma. Cancer Res 2006; **66**: 8542–9.

2. **Shnitsar VM, Lisovskiy IL, Soldatkina MA, Nespryadko SV, Turchak OV, Vinnitskaya AB, Pogrebniy PV.** Human beta-defensin 3 (hBD-3) expression in A431 cell line and human vulval tumors. Exp Oncol 2004; **26**: 328–30.

3. **Arimura Y, Ashitani J, Yanagi S, Tokojima M, Abe K, Mukae H, Nakazato M.** Elevated serum beta-defensins concentrations in patients with lung cancer. Anticancer Res 2004; **24**: 4051–**7.**

4. **Gambichler T, Skrygan M, Huyn J, Bechara FG, Sand M, Altmeyer P, Kreuter A.** Pattern of mRNA expression of beta-defensins in basal cell carcinoma. BMC Cancer 2006; **6**: 163.

5. **Muller CA, Markovic-Lipkovski J, Klatt T, Gamper J, Schwarz G, Beck H, Deeg M, Kalbacher H, Widmann S, Wessels JT, Becker V, Muller GA, Flad T.** Human alpha-defensins HNPs-1, -2, and -3 in renal cell carcinoma: influences on tumor cell proliferation. Am J Pathol 2002; **160**: 1311–**24.**

6. **Melle C, Ernst G, Schimmel B, Bleul A, Thieme H, Kaufmann R, Mothes H, Settmacher U, Claussen U, Halbhuber KJ, Von Eggeling F.** Discovery and identification of alpha-defensins as low abundant, tumor-derived serum markers in colorectal cancer. Gastroenterology 2005; **129**: 66–**73.**

7. **Albrethsen J, Bogebo R, Gammeltoft S, Olsen J, Winther B, Raskov H.** Upregulated expression of human neutrophil peptides 1, 2 and 3 (HNP 1-3) in colon cancer serum and tumours: a biomarker study. BMC Cancer 2005; **5**: 8.

8. **Kamysz W, Okroj M, Lukasiak J.** Novel properties of antimicrobial peptides. Acta Biochim Pol 2003; **50**: 461–9.

9. **Murphy CJ, Foster BA, Mannis MJ, Selsted ME, Reid TW.** Defensins are mitogenic for epithelial cells and fibroblasts. J Cell Physiol 1993; **155**: 408–13.

10. **Markeeva N, Lisovskiy I, Zhuravel E, Soldatkina M, Lyzogubov V, Usenko V, Potapov V, Pogrebnoy P.** Involvement of human beta-defensin-2 in proliferation of transformed cells of human cervix. Exp Oncol 2005; **27**: 308–14.

11. **Holterman DA, Diaz JI, Blackmore PF, Davis JW, Schellhammer PF, Cirica A, Semmes OJ, Vlahou A.** Overexpression of alpha-defensin is associated with bladder cancer invasiveness. Urol Oncol 2006; **24**: 97–108.

12. **Aarboiu J, Tjabringa GS, Verhoosel RM, Ninaber DK, White SR, Peltenburg LT, Rabe KF, Hiemstra PS.** Mechanisms of cell death induced by the neutrophil antimicrobial peptides alpha-defensins and LL-37. Inflamm Res 2006; **55**: 119–27.

13. **Sawaki K, Mizukawa N, Yamaai T, Yoshimoto T, Nakano M, Sugahara T.** High concentration of beta-defensin-2 in oral squamous cell carcinoma. Anticancer Res 2002; **22**: 2103–**7.**

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

15. **Maniatis T, Frich E, Sambrook J.** Molecular cloning. Moskow: Mir, 1984; p. 423 (In Russian).

16. **Markeeva N, Lisovskiy I, Lyzogubov VV, Usenko V, Soldatkina M, Merentsev S, Zaitsev S, Kondratskii Yu, Tofan A, Osinskiy S, Pogrebnoy P.** Expression of beta-defensin-2 in human gastric tumors: a pilot study. Exp Oncol 2005; **27**: 130–5.

17. **Xu MJ, Tsuji K, Ueda T, Mukouyama YS, Hara T, Yang FC, Ebihara Y, Matsuoka S, Manabe A, Kikuchi A, Ito M, Miyajima A, Nakahata T.** Stimulation of mouse and human primitive hematopoiesis by murine embryonic aorta-gonad-mesonephros-derived stromal cell lines. Blood 1998; **92**: 2032–40.

18. **Liu L, Wang L, Jia HP, Zhao C, Heng HHQ, Schutte BC, McCray PB Jr, Ganz T.** Structure and mapping of the human beta-defensin HBD-2 gene and its expression at sites of inflammation Gene 1998; **222** (2), 237–244.

19. **Mahimkar RM, Visaya O, Pollock AS, Lovett DH.** The disintegrin domain of ADAM9: a ligand for multiple b1 renal integrins. Biochem J 2005; **385:** 461–8.

20. **Economopoulou M, Bdeir K, Cines DB, Fogt F, Bdeir Y, Lubkowski J, Lu W, Preissner KT, Hammes HP, Chavakis T.** Inhibition of pathologic retinal neovascularization by alpha-defensins. Blood 2005; **106**: 3831–8.

21. **Chavakis T, Cines DB, Rhee JS, Liang OD, Schubert U, Hammes HP, Higazi AA, Nawroth PP, Preissner KT, Bdeir K.** Regulation of neovascularization by human neutrophil peptides (alpha-defensins): a link between inflammation and angiogenesis. FASEB J 2004; **18**: 1306–8.

ИЗМЕНЕНИЕ ФЕНОТИПА И ОСОБЕННОСТЕЙ РОСТА В КУЛЬТУРЕ ЛИНИИ ЭМБРИОНАЛЬНЫХ КЛЕТОК ПОЧКИ ПРИ РЕГУЛИРУЕМОЙ ЭКСПРЕССИИ БЕТА-ДЕФЕНСИНА-2

Цель: **создать линию клеток с регулируемой экспрессией бета-дефенсина-2 человека (hBD-2) и проанализировать влияние экспрессии этого пептида на особенности фенотипа и рост клеток.** ***Материалы и методы:*** **клеточная сублиния T-rex HEK-hBD2-m, экспрессирующая биологически активную зрелую форму hBD-2 при индукции клеток тетрациклином, получена путем клонирования на основе эмбриональных клеток почки человека линии HEK293T. Морфологические особенности, характеристики роста и показатели колониеобразования в полужидкой среде исследовали стандартными методами.** ***Результаты:*** **с помощью методов РТ-ПЦР и иммуноцитохимии показано, что сублиния клеток T-rex HEK-HBD2-m экспрессирует hBD2 в присутствии 1 μг/мл тетрациклина в среде инкубации. Продолжительная экспрессия hBD-2 приводила к тому, что клетки утрачивали способность образовывать монослой при культивировании *in vitro* и образовывать колонии в полужидкой среде, но характеризовались более высокой скоростью роста и способностью к более продолжительному выживанию в бессывороточной среде, чем исходная линия клеток HEK293T.** ***Выводы:*** **экспрессия hBD-2 в сублинии клеток T-rex HEK-HBD2-m cell обусловливает специфические биологические эфеекты, способствующие выживанию клеток.**

Ключевые слова: **бета-дефенсин-2 человека, регулируемая экспрессия, колониеобразование, клетки линии HEK293.**