Exp Oncol 2008
30, 2, 153–156

SHORT COMMUNICATIONS

Experimental ONCOLOGY

EXPRESSION OF HUMAN BETA-DEFENSINS-1, 2 AND 4 mRNA IN HUMAN LUNG TUMOR TISSUE: A PILOT STUDY

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Aim: **To analyze the patterns of human beta-defensin-1, 2, 4 (hBDs) expression in human lung tumors.** ***Materials and Methods:*** **Tissue samples of surgically resected human lung tumors (squamous cell carcinoma (SCC), n = 10; adenocarcinoma (AC), n = 10) paired with conditionally normal tissue samples were analized for expression of hBD-1, 2, 4 mRNA by semiquantitive RT-PCR.** ***Results:*** **In a number of studied lung cancer tissue samples, overexpression of defensin mRNA was registered: hBD-1 mRNA (50% of SCC and 60% AC), hBD-2 mRNA (60% of SCC and 50% of AC) or hBD-4 (40% of SCC and 20% AC). No correlation was detected between the levels of hBD-1, hBD-2 and hBD-4 mRNA and histological type, differentiation grade of the tumor, and the stage of the disease, as well as the content of hBD-2 peptide in blood serum of lung cancer patients.** ***Conclusion:*** **Human beta-defensins-1 and -2 are often up-regulated in human lung tumors.**

Key Words: **human beta-defensin, human lung tumors, expression.**

Defensins — small cationic antimicrobial peptides — are recognized presently as an important component of innate immunity that protects the host from invading microorganisms [1]. In the last years it has been established that apart from direct antimicrobial action defensins possess multiple biological activities, in particular, chemokine activity, and a number of immunomodulatory activities as well [2, 3].

Beta-defensins (hBDs) are peptides composed of 40–45 amino acid residues with characteristic cysteine pairing bonds 1–5, 2–4, 3–6 [4]. Expression of beta-defensins is found predominantly in epidermis and epithelium lining different organs, in particular, in respiratory and gastrointestinal tract — the largest surfaces of body contacting with antimicrobial agents. In respiratory tract the distribution of hBDs is shown to be complementary — hBD-2 is expressed in type-2 alveolar epithelial cells, while expression of hBD-3 is observed in bronchus and bronchioli [5]. It is known also that hBD-1 expression is constitutive, while expression of hBD-2 and hBD-3 genes is induced upon the influence of LPS and some cytokines [6, 7].

There is a number of reports that have demonstrated an importance of beta-defensin expression for antibacterial protection of respiratory tract of mammals and man as well as the role of defensin malfunction in different respiratory pathologies including cystic fibrosis, asthma, pneumonia [7–9].

Moreover, there are some data evidencing on possible implication of defensins, in particular, hBD-1, in lung cancer [10]. The authors have evaluated the serum hBD-1 and hBD-2 levels in two groups of patients with lung cancer and pneumonia, and in healthy individuals, and found significant elevation of hBD-1 in blood serum of lung cancer patients compared to control [10]. The serum hBD-2 level was also increased in lung cancer patients compared to healthy donors, but the difference was found to be insignificant. However, the data on the patterns of expression of defensin genes in human lung cancer cells are scarce, and the functional role of hBDs expression in lung tumorigenesis remains largely unknown.

In our earlier studies, we have found hBD-2 up-regulation in cells of some human epithelial tumors (cervical, vulval, gastric) [11–13]. The present pilot research was aimed on the study of hBDs expression patterns in human lung tumors.

In the study, the samples of surgically resected human lung tumors (squamous cell carcinoma (SCC), n = 10; adenocarcinoma (AC), n = 10) paired with conditionally normal tissue samples were studied. The tissue samples were obtained during the surgical treatment of patients with lung cancer cured in the Thoracic Department, National Cancer Institute (Kyiv, Ukraine). The patients did not receive chemo- or radiotherapy prior to the surgery. All patients provided an informed written consent to perform the study, and the present research was approved by Ethic Committee of the Institute. Immediately after surgical removal, tissue samples were frozen in liquid nitrogen and stored at –70 °C until use. The clinico-pathological characteristics of lung cancer patients and tumors are presented in Table 1.

Total RNA was isolated from tissue samples by the method of Chromzynski and Sacchi [14]. RNA concentration was evaluated at 260 nm using Beckman DU-8B spectrophotometer, its purity — by OD relation at 280 nm and 260 nm, its quality — by electrophoresis in 1% agarose gel containing 20% formaldehyde. For detection of expression of hBD-1, hBD-2, hBD-4 in tissue samples, semiquantitive RT-PCR analysis was performed using the set of specific primers (primer sequences and conditions of RT-PCR are presented in Table 2). The expression level of beta-actine served as the control. The products of RT-PCR were routinely analyzed by electrophoresis in agarose gel.

Received: April 19, 2007.

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Abbreviations used: AC – adenocarcinoma; hBD – human beta-defensin; HNP – human neutrophil peptide; SCC – squamous cell carcinoma.

The content of hBD-2 in blood serum of the patients with blood cancer was analyzed by immunoenzyme method with the use of beta-defensin-2 (human) ELISA kit (Phoenix Pharmaceuticals, Inc., USA).

Table 1. Expression of hBDs mRNA in human lung tumors and healthy lung tissue samples

Case	TNM	Differentiation grade	Expression level hBD-1 N	hBD-1 T	hBD-2 N	hBD-2 T	hBD-4 N	hBD-4 T	Content of hBD-2 in blood serum (pg/ml)
Squamous cell carcinoma									
1	$T_1N_0M_0$	LD	+	+	++	+++	+	+	520
2	$T_2N_1M_0$	LD	++	+	++	+++	+++	+++	320
3	$T_2N_0M_0$	LD	++	+++	++	++	+	+	360
4	$T_2N_0M_0$	LD	++	+++	++	+++	+	+	30
5	$T_2N_0M_0$	LD	+	++	+	+++	+	+++	0
6	$T_2N_1M_0$	LD	++	++	++	+	++	+++	0
7	$T_2N_2M_0$	LD	+	+	+++	++	+	-	1200
8	$T_2N_1M_0$	MD	++	+++	++	+++	+	++	NA
9	$T_2N_1M_0$	MD	+++	+++	+	+	+	++	NA
10	$T_3N_0M_0$	MD	+	++	–	++	+	+	NA
Adenocarcinoma									
1	$T_2N_1M_0$	MD	+	–	++	++	+++	+	NA
2	$T_2N_1M_0$	MD	++	+	–	++	+	+	800
3	$T_2N_1M_0$	MD	++	+++	++	++	+	+++	60
4	$T_2N_1M_0$	MD	+	++	–	+	+	+++	120
5	$T_2N_1M_0$	Mixed type	++	+++	++	++	++	++	40
6	$T_2N_2M_0$	MD	+	++	+	++	+	+	120
7	$T_2N_2M_0$	MD	+	++	-	+++	+	+	0
8	$T_3N_0M_0$	MD	++	+	++	+++	+++	+	840
9	$T_1N_0M_0$	HD	++	+	++	+	++	++	640
10	$T_1N_xM_x$	HD	–	+++	++	++	++	++	50

Notes: N – normal tissue; T – tumor tissue; differentiation grade: LD – low-differentiated; MD – moderately-differentiated; HD – highly-differentiated; SCC –; AC –; "+, ++, +++" – low, moderate, high expression levels respectively; "–" – expression is non-detectable; NA – blood serum was not available.

Table 2. Primers for genes of interest used in the study

Gene	Primer	Conditions of RT-PCR
hBD-1	Forward -5′-tgttgcctgccagtcgccatgag	35 cycles (67 °C, 10 s;
	Reverse 5′-tcacttgcagcacttggccttccc	72 °C, 10 s)
hBD-2	Forward -5′-gaagctcccagccatcagcc	35 cycles (59 °C, 10 s;
	Reverse -5′-gtcgcacgtctctgatgaggga	72 °C, 10 s)
hBD-4	Forward -5′-gaagctcccagccatcagcc	35 cycles (65 °C, 10 s;
	Reverse 5′-gtcgcacgtctctgatgaggga	72 °C, 10 s)
Beta-actine	Forward -5′-ctggaacggtgaaggtgaca	35 cycles (59 °C, 10 s;
	Reverse 5′-aagggacttcttgtaacaatgca	72 °C, 10 s)

With the use of RT-PCR analysis it has been revealed that in total, 85% of studied lung tumor samples are characterized by overexpression of one of three studied beta-defensin genes (hBD-1, hBD-2 or hBD-4) compared to paired control tissue specimens (see Table 1; Figure). For hBD-1 mRNA, down-regulation was detected in 1 of 10 cases of SCC and in 4 cases of AC, while its up-regulation — in 5 cases of SCC and 6 cases of AC. hBD-2 was up-regulated in 60% of SCC cases and in 50% of AC. Expression of hBD-4 was found to be a more stable parameter — its up-regulation was detected only in 4 SCC cases and in 2 AC cases, while in 11 of 20 cases studied in the present research the level of hBD-4 expression was equal in tumor tissue sample and its paired conditionally normal specimen.

We have to state that the low number of studied cases and heterogeneity of hBDs expression do not allow us to make particular conclusions on the peculiarity of defensin expression in lung tumors in dependence on the stage of the disease, differentiation grade, lymph node status etc. In a small cohort of studied lung cancer patients, no correlation between mentioned parameters was found. Also, the level of expression of hBD-2 mRNA did not correlate with the content of hBD-2 peptide in blood serum of the patients with lung cancer (Table). However, summarizing the obtained data, one may note that hBD-1 and hBD-2 expression is often up-regulated in tumor lung tissues.

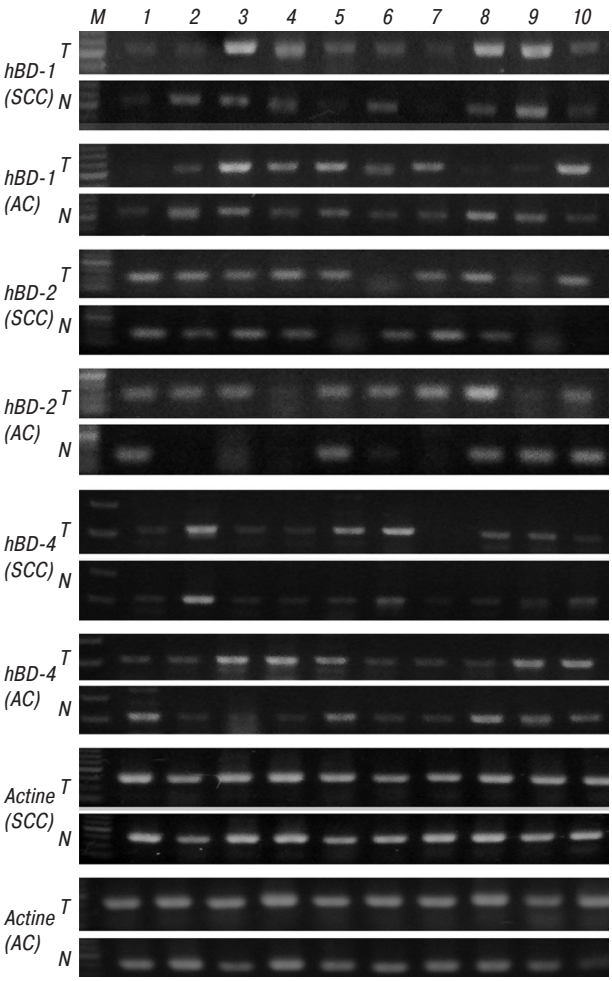

Figure. Semiquantative RT-PCR analysis of expression of mRNA for hBD-1, hBD-2 and hBD-4 in human lung squamous cell carcinomas (SCC) and adenocarcinomas (AC). Expression of beta-actine served as internal control. Molecular weight markers (Fermentas CeneRuler 1 kb DNA Ladder for hBD-4; Fermentas CeneRuler 50 bp DNA Ladder for hBD-1, hBD-2, and beta actine) are shown. T – tumor tissue; N – normal tissue

Expression patterns of beta-defensin genes in human tumors are in spite of interest of few research groups including our laboratory. Up-to-date there are reports showing that the tumors of oral cavity, colon and stomach are characterized by elevated expression of some alpha- or beta-defensins [13, 15, 16].

There are also some studies where cancer-related down-regulation of defensin genes has been revealed: it was reported that in prostate and renal cancers defensin expression is down-regulated [17]. It was demonstrated recently [18] that expression of human beta-defensin-1 is down-regulated in prostate cancer, and the authors proposed that hBD-1 may play a role of tumor suppressor gene in prostate cancer cells. To test such hypothesis, the authors have performed the cloning and ectopic expression of hBD-1 in prostate cancer cells of different lines, and demonstrated that

hBD-1 production by these cells led to their growth suppression and death [18].

However, in lung tumors the patterns of hBD-1 expression are different from these in prostate and renal cancers, and moderate down-regulation of hBD-1 was observed only in 5 of 20 studied cases, while up-regulation of hBD-1 and hBD-2 is a frequent event in both SCC and AC. These data are in accordance with the data of Arimura *et al*. [10] who found significantly elevated concentrations of hBD-1 and high concentrations of hBD-2 in blood serum of lung cancer patients compared to these of patients with pneumonia and healthy donors. Taking into account the possible chemokine activities of beta-defensin-2 and its ability to attract immature dendritic cells [2, 19], one may hypothesize that up-regulation of hBD-2 in tumor cells may result in the beneficial effect to the host; however, the prognostic value of this index has not been studied yet, and follow-up data for lung cancer patients and monitoring of hBDs expression will be useful to answer these questions.

In conclusion, our pilot investigation has revealed that in the studied cohort the large majority of lung tumors is characterized by up-regulation of hBD-1, hBD-2 or rarely - of hBD-4 mRNAs. Overexpression of hBD-2 seems not be related to differentiation grade of the tumor and its histological type, as well this parameter does not influence the content of hBD-2 peptide in blood serum of lung cancer patients.

ACKNOWLEDGEMENTS

This study was supported by the Joint Russian-Ukrainian Research Project n 0106U012537 (2006–2009) “The New Methods for Non-Invasive Diagnostics of Cancer and Pathologies of Pregnancy”.

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ЭКСПРЕССИЯ мРНК БЕТА-ДЕФЕНСИНОВ-1, 2, 4 В ТКАНИ ОПУХОЛИ ЛЕГКОГО ЧЕЛОВЕКА: ПИЛОТНОЕ ИССЛЕДОВАНИЕ

***Цель*: проанализировать особенности экспрессии мРНК бета-дефенсинов-1, 2, 4 (hBDs) в ткани опухоли легкого человека. *Материалы и методы*: с помощью метода полуколичественного RT-PCR-анализа изучали уровень экспрессии мРНК hBD-1, 2, 4 в образцах ткани хирургически удаленных опухолей легкого человека (плоскоклеточный рак — ПКР, n = 10; аденокарцинома — АК, n = 10) по сравнению с образцами условно-нормальной ткани легкого тех же пациентов. *Результаты*: в ряде исследованных образцов опухолей легкого выявлена повышенная экспрессия мРНК hBD-1 (50% ПКР и 60% АК), hBD-2 (60% ПКР и 50% АК) или hBD-4 (40% ПКР и 20% АК). Зависимости между уровнем экспрессии бета-дефенсинов и гистологическим типом опухоли, стадией заболевания и содержанием пептида hBD-2 в сыворотке крови больных не установлено. *Выводы*: в ткани опухоли легкого человека часто активирована экспрессия hBD-1 и hBD-2.**

***Ключевые слова*: бета-дефенсины человека, опухоль легкого, экспрессия.**